WATER BUFFALO IN ASIA : IV

Diseases Of the Buffalo

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Water Buffalo in Asia - Diseases
Alwis, Subasinghe, Horadagoda
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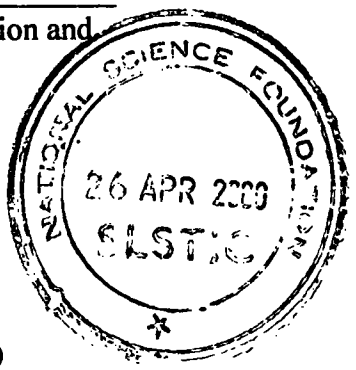
WATER BUFFALO IN ASIA

Publication of the SAREC/NARESA Buffalo Information and Dissemination Programme



Volume 4 – Diseases of the Buffalo

Edited by

M.C.L. De Alwis, D.H.A. Subasinghe and
N.U. Horadagoda

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WATER BUFFALO IN ASIA

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FOREWORD

The water buffalo has been of great economic significance to Sri Lanka and most other Asian countries. It has provided man with many of his basic needs like milk and meat for nutrition, draught power for agriculture and transport, bones and hides for fertilizer and industry. The buffalo has been preferred to dairy cattle among the Asian countries, because of its adaptability to the hot tropics and its ability to perform optimally under relatively adverse environmental conditions.

One of the major constraints to buffalo development in Sri Lanka in the past has been the lack of adequate research facilities and the paucity of published information on the water buffalo. An attempt was made in the recent past by the Natural Resources and Science Authority of Sri Lanka (NARESA) the predecessor to the National Science Foundation (NSF), under a long term buffalo research and development programme which was operative from 1980 to the present time, through Swedish funding to address this need. Scientists working on the 3rd and final phase of this programme has initiated action to fill the gap in technical knowledge by publishing a series of technical publications targeting the farmers, extension workers veterinarians and researchers. This book written on Diseases of the Buffalo is the 4th volume of the text book on Water Buffalo in Asia and is the 19th publication in this series. It has seven chapters, each dealing with one or more diseases of economic and/or zoonotic importance particularly to the Asian region. The authors have attempted to incorporate most of the recent knowledge relevant to the subject in this publication. It is known that only a healthy animal will perform and produce at optimal level. Therefore, greatest attention has to be paid to the health care of animals.

This book has been aimed at a wide readership of graduate students, research scientists, teachers, veterinarians and others interested in buffalo research and development .I have no doubt that the readers will benefit immensely from this book. As chairman of the NSF, I am happy to be associated with this programme and wish to commend Dr. H. Abeygunawardena, the chief grantee of the SAREC/NARESA Buffalo Information Dissemination Programme and the editors of this book for their genuine effort and commitment to the task undertaken by them.

Professor Kapila Dahanayake
Chairman,
National Science Foundation,
16.12.99

PREFACE

Although the buffalo plays a pivotal role in the rural economy of most Asian countries, there are only a very fews books on the diseases of the Asian buffalo. It is generally assumed that the aetiology, pathogenesis, clinical manifestations and treatment of buffalo diseases are similar to those described for cattle. On the one hand, one cannot be blamed for making such an assumption since very little research information is available on the buffalo as compared to cattle. Therefore, most buffalo diseases were treated as for cattle. However, recent research investigations in the Asian and the Mediterranean regions have produced a wealth of information on buffalo diseases caused by a wide variety of aetiological agents. Some of these studies have clearly shown differences of the same disease in the two species. The most striking examples that illustrate the latter point are *Toxocara vitulorum* infection and haemorrhagic septicaemia to which buffaloes are more susceptible than cattle.

Most books on bovine diseases are based on cattle, and these are written primarily by authors in developed countries, while only a very few books are available on cattle diseases in the tropics, and even fewer have focused on buffaloes in the Asian region. This book is the result of an effort made to compile the current knowledge on the diseases of the water buffalo with special reference to the Asian animal and is the 4th volume in a series of books on the Asian Water Buffalo published by the National Science Foundation, as a part of the information dissemination programme of the Third Phase of SAREC/NARESA Water Buffalo Research Programme

In this book, information from Sri Lanka has been used extensively and where such information is not available reports from the region and other sources have been incorporated. This book is arranged into seven chapters. The first chapter is on parasitic diseases in which the description is presented under main groups i.e. gastrointestinal, haemo-and ectoparasites. As described in chapter 2, haemorrhagic septicaemia ranks as the most important contagious bacterial disease affecting buffaloes. Other bacterial diseases included in the chapter are black quarter, mastitis as well as lesser important diseases such as anthrax, tuberculosis and paratuberculosis. Chapter 3 is focused on infectious diseases affecting the reproductive system resulting in infertility and abortion. Although a wide variety of microbes are incriminated in such infections only the descriptions related to brucellosis and leptospirosis are included. Two of the most important viral diseases in the buffalo namely, foot and mouth disease and rinderpest are described in chapter 4. Metabolic diseases are not as commonly reported in buffaloes as in cattle, but chapter 5 has reviewed the information on the buffalo. Buffalo calf mortality is a major constraint in buffalo husbandry and chapter 6 has focused on some of the important diseases affecting this age group of animals. The final chapter concentrates on healthcare practices which help to minimize the affect of disease in buffalo farming. This book is primarily intended for veterinarians, undergraduates, postgraduates, teachers and research workers in the field of animal production and health.

The editors wish to acknowledge the assistance by Ayesha and Janaka Herath in typesetting and printing of this publication. The constant guidance and co-operation extended by Dr. H. Abeygunawardena, chief grantee and Dr. J.A. de S. Siriwardene, coordinator SAREC/NARESA Buffalo Research programme is gratefully acknowledge.

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CONTRIBUTORS

R. Hettiarachchi: BVSc (Sri Lanka), MSc (Edinburgh)
Department of Animal Production and Health, Peradeniya.

N.U. Horadagoda: BVSc, MVSc (Sri Lanka), PhD (Liverpool)
Senior Lecturer in Veterinary Pathology, Department of Veterinary Paraclinical Studies, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya.

S.R. Jayasinghe: BVSc (Sri Lanka), MSc (Queensland)
Veterinary Research Officer, Veterinary Research Institute, Peradeniya.

S. N. Kodituwakku: BVSc, MSc (Sri Lanka), PhD (Bristol)
Deputy Director (Animal Health), Department of Animal Production and Health, Peradeniya.

I.D. Silva: BVSc (Sri Lanka), PhD (California)
Associate Professor, Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya.

R. Sivakanesan: BVSc (Cey), PhD (Hull)
Associate Professor, Department of Biochemistry, Faculty of Medicine, University of Peradeniya, Peradeniya.

D.H.A. Subasinghe: BVSc (Cey), MS, PhD (Hawaii)
Former Deputy Director, Department of Animal Production and Health, Peradeniya; 32/16, Sangaraja Mawatha, Kandy.

D.J. Weilgama: BVSc, MVSc (Cey), PhD (Queensland)
Senior Lecturer in Parasitology; Department of Parasitology, Faculty of Medicine, University Peradeniya, Peradeniya.

T.G. Wijewardana: BVSc, MPhil (Sri Lanka), PhD (Edinburgh)
Veterinary Research Officer, Veterinary Research Institute, Peradeniya.

B.D.R. Wijewardana: BVSc (Sri Lanka), MSc (Trop. Vet. Sc., Edinburgh)
Veterinary Investigation Officer, Veterinary Research Institute, Peradeniya.

CONTENTS

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Chapter 1

PARASITIC DISEASES

1.1 Gastrointestinal Parasites

S.R. Jayasinghe

Productivity in cattle and buffaloes is influenced by a variety of factors and indeed gastrointestinal parasitism constitutes one of the major factors influencing productivity, under both tropical and temperate climatic conditions. Gastrointestinal parasites of buffaloes include unicellular organisms (protozoa) such as coccidia and multi cellular helminths. Helminth parasites fall mainly into three common classes, nematodes trematodes and cestodes. In general, buffaloes are less affected by gastrointestinal parasites when compared to cattle. These parasites may do little or no damage to their hosts, but given an appropriate environment they may cause economic lossess by producing illness, loss of appetite, weakness, decreased feed efficiency, reduced weight gain, and may even result in death.

Gastrointestinal helminth parasites of buffaloes

Nematodes

Among the gastrointestinal parasites which parasitise buffaloes, *Toxocara vitulorum* is the most important nematode. Consequently it has been studied extensively by several research workers (Pandey *et al.*, 1990; Roberts, 1990b; Gupta, 1986). The other nematode parasites which occur in buffaloes are Strongyloids *spp, Mecistocirrus digitatus,* Haemonchus species, Cooperia species, *Oesophagostomum radiatum* and *Bunostomum plebotomum* (Perera *et al.,* 1989) Whilst the effects of these parasites on buffaloes of different age groups have not been fully documented, the pathogenic effects of *Toxocara vitulorum* has been well established (Roberts, 1990b).

Toxocara vitulorum: The life cycle and mode of infection of *Toxocara vitulorum* is still controversial. The maternal host acquires infective eggs from the environment but the sites of hatching and penetration of the intestine are not known. Both prenatal and lactogenic routes have been described. Bulk of the evidence from several studies do not support the occurrence of uterine transmision for *T.vitulorum*. There is a marked increase in the number of larvae in the mammary gland in the periods from just before parturition until five days after parturition. No larvae are present in the milk for more than eight days after parturition (Roberts, 1990b).

Visceral larval migrans is a very important phenomenon in *T.vitulorum* infection. Larvae hatch from the infective eggs and penetrate the walls of the small intestine. Most larvae migrate straight to the liver via the hepatic portal vein, but a few enter the mesenteric lymph nodes. Some larvae then migrate to the lungs and a few to muscle, brain, kidney and peripheral lymph nodes. However, many larvae remain in the liver. They lie dormant in these organs resulting in visceral larval migrans. This is very important from a zoonotic point of view, as man may become infected by eating raw liver. In a pregnant bovine host, larvae grow in liver and lungs one to eight days before parturition and migrate to the mammary gland at the time of parturition (Roberts, 1990b). In the mammry gland they grow again, pass into the milk during the first seven days after parturition. It has also been

speculated that human infection with *T.vitulorum* is possible through the consumption of raw milk (Benerjee *et al.*, 1983).

Strongyloid species: The life cycle of *Strongyloid* species is different from other nematodes in that completely free living and completely parasitic cycles occur. Eggs are passed in the faeces, and in the homogenic cycle, first stage larvae develop directly to become infective third stage larvae. In contrast, in the heterogenic cycle, first stage larvae may develop to become free living adult males and females. When environmental conditions are favourable the heterogenic cycle predominates while the homogenic cycle predominates when environmental conditions are adverse. In the heterogenic cycle, the first stage larvae are rapidly transformed, so that within 48 hours, free living males and females occur. Females produce eggs after copulation, and these eggs hatch in a few hours and larvae metamorphose to become infective larvae (Soulsby, 1982). In the homogenic cycle, first stage larvae metamorphose rapidly to become third stage or infective larvae within 24 hours at 27° C (Soulsby, 1982).

Infection of the host is mainly by skin penetration, but oral infection may also occur (Soulsby, 1982). The migration of larvae through skin is facilitated by the release of histolytic proteases which are capable of degrading cutaneous connective tissue macro molecules. The major neutral protease secreted by L3 larvae is a metalloprotease (James *et al.*, 1990). Clinical outbreaks or high levels of infection commonly occur in young calves. Occasionally, heavy infections in adults have been reported as a result of a breakdown of immunity during dry periods (Yazwinski and Gibbs, 1975). Pre-parasitic stages are highly susceptible to dryness and this restricts the percutaneous infection to wet periods or in situations where calves are kept under unhygienic conditions (Copeman, 1982). After skin penetration the larvae reach skin capillaries or venules and are carried by blood to the lungs from where they migrate to the small intestine via tracheo-oesophageal route. Infection by milk borne larvae has also been reported. However, not much work has been done on this (Kawanabe *et al.*, 1988).

Bunostomum species: The development in *Bunostomum species* is direct and simple. Eggs are passed in the faeces and larvae metamorphose to become third stage infective larvae. Infection of the host occurs through the mouth or skin (Soulsby, 1982). Following skin penetration, larvae reach the intestine via the tracheo-oesophageal route. Preparasitic stages are highly susceptible to dessication reducing the rate of infection in dry seasons.

Other Trichostrongylid nematodes: Life cycles of most of the gastrointestinal trichostrongylid nematodes such as *Haemonchus*, *Cooperia* and *Mecistocirrus* are direct and simple. Eggs are passed in the faeces and given appropriate environmental conditions they hatch to become first stage larvae. First stage larvae feed on bacteria, grow and moult to second stage larvae which in turn feed, grow and moult. The second moult is incomplete and the third or infective larvae retain the cuticle which provides protection against adverse environmental conditions. In tropical areas eggs in the dung develop to the infective stage within one to two weeks throughout the year (Copeman, 1982). Migration from dung pats to pasture is dependent on the presence of moisture. After an extended dry period when dung pats are dry and hard, more rain is required for larval migration than where regular rainfall keep pats moist and soft (Copeman, 1982).

The parasitic phase of the life cycle begins with the ingestion of infective larvae by the host. The protective sheath is shed as a result of exposure to specific stimuli such as temperature, carbon dioxide concentration and pH. The third moult takes place within a few days after exsheathment and fourth stage larvae undergo major changes in morphology, differentiate sexually and increase considerably in size. Inhibited development of early fourth stage larvae of *Haemonchus* and *Cooperia* species in calves has been reported. This occurs at the approach to and during the dry period. After the final or fourth moult to the adult stage, further growth and maturation occurs and female worms commence laying eggs to complete the life cycle (Fabiyi and Copeman, 1986).

Trematodes

Fasciola hepatica and *Fasciola gigantica* are the most important flukes which inhabit the bile duct of buffaloes. However, *Fasciola* infections have not been recorded in cattle and buffaloes in Sri Lanka. (Perera *et al.*,). *Gigantocotyle explanatum* is a conical fluke (Paramphistomatid) which occurs in the bile duct of buffaloes, especially in the dry zone of Sri Lanka. (Perera *et al.*, 1989). Other species of common conical flukes which occur in the rumen and reticulum of buffaloes and cattle are *Paramphistomum cervi, Gastrothylax cruminifer, Gastrothylax cobboldi, Gastrothylax elongatus, Calicophoron species* and *Ceylonocotyle streptocoelium*. They are red in colour, convex dorsally and concave ventrally (Perera *et al.*, 1989).

Schistosoma nasale is an elongate, unisexual blood fluke which inhabits the veins of the nasal mucosa of cattle, buffaloes, goats and horses. The incidence of this infection in Sri Lankan buffaloes is not clearly understood (Perera *et al.*, 1989).

Life cycle of trematode parasites: Eggs are passed in the faeces of the host and under suitable environmental conditions, they hatch and a larva termed 'miracidium' is produced. Hatching is influenced by factors such as temperature and light. The miracidium is roughly triangular in shape and covered with cilia. Miracidium does not feed and further development occurs after it enters the intermediate host, a snail. After penetrating the snail, the ciliated coat is lost and becomes a 'sporocyst', which is an undifferentiated mass of cells. Germinal cells of the sporocyst multiply and produce either daughter sporocysts or the next stage 'redia'. One or more generations of rediae may occur before the final stage, the 'cercaria', emerges from the redia. Cercariae, which resemble a tadpole with a tail, then escape from the snail to surrounding water and encyst on blades of leaves or grass and undergoes physiological maturation to produce the infective stage, the 'metacercaria'. The metacercaria has to enter the definitive host in order to complete the life cycle. The encysted metacercaria is swallowed by the final host and excystation occurs in the intestinal tract. However, the cercaria of *Schistosoma nasale* does not encyst and enter the host by active penetration of the skin.

Cestodes

Cestodes or tape worms are endoparasitic in the alimentary tract and associated ducts. The body of the adult tape worm is dorsoventrally flattened and often white. Tape worms are hermophrodites without a body cavity or an alimentary canal. They vary in size from a few millimeters to several metres in length. The body consists of a head or scolex with suckers and hooks and a strobila made up of a number of segments or proglottides. Anterior proglottides are the youngest and they increase in size as the development of their internal

parts progresses and they are pushed further away from the scolex by the younger segments. *Moniezia species* is the common cestodes found in the small intestine of sheep, goats, cattle and buffaloes. They may reach a length of 600 cm and a width of 1.6 cm (Perera *et al.*,1989).

Life cycles of cestode parasites: Cestode life cycles are indirect and require the development of metacestodes in intermediate hosts. Proglottides and eggs are passed in the faeces of infected hosts. Eggs hatch after ingestion by the intermediate host and embryo penetrates into the intestinal wall in order to reach a suitable part of the body for further development. Then it grows into a cyst with a cavity filled with fluid. This is known as the "bladder worm" and is now ready for ingestion by the final host to enable the parasite to attach itself to the intestine.

Gastrointestinal protozoan parasites of buffaloes

Coccidia

Coccidia, belongs to the family, *Eimeridae* and are intracellular parasites of the epithelial cells of the intestine of animals. Coccidiosis is one of the major parasitic diseases in buffalo calves and young calves less than eight months old are severely affected by the disease. In Sri-Lanka, coccidiosis has now become a problem in young buffalo calves (Bahirathan *et al.*, 1995). It is reported that nine species of *Eimeria* occur among buffalo calves in Sri Lanka. These are *E.subspherica, E.zuernii, E.ellipsoidalis, E. cylindrica, E. bovis, E. bareillyi, E. canadensis, E. auburnensis* and *E. ankarensis.* (Bahirathan *et al.*, 1995).

Life cycle of coccidial parasites : The oocyst which contains a zygote, is expelled in faeces of affected animals. These oocysts vary in size according to the species and the most common shapes are spherical, sub-spherical and ovoid or ellipsoidal. These oocysts develop into the mature infective stage under suitable environmental conditions. In the mature sporulated oocyst, there are four sporocysts and each sporocyst contains two sporozoites. The calves become infected by the ingestion of infective oocysts and the excystation releases the contained sporozoites. These sporozoites enter the epethelial cells of the intestine and become rounded up to develop into a schizont.Initially, the cytoplasm of the schizont is undivided, but later, by a process of asexual multiple fission, a number of elongate organisms, merozoites, are produced. When the schizont is matured these merozoites are released and they then enter other epethelial cells and continue the cycle of asexual development. Eventually after second or third generation of asexual reproduction, merozoites may differentiate into male and female gametocytes. They undergo sexual reproduction and oocysts resulting from the union of these are passed in the faeces.

Epidemiology of gastrointestinal helminth parasitism

In general, epidemiology is the study of factors which are responsible for incidence, prevalence and severity of a disease in a population.When applied to helminth diseases it is a study of factors affecting number of parasites, both within the host and outside the host.

Ecology of free living stages

Gastrointestinal nematodes are subjected to various environmental factors during their development as free living stages outside the host. Climate and weather are the two main factors that directly influence the survival and development of free living stages. The

distinction between climate and weather is important in relation to parasitic infections. Weather refers to day to day temperature, rainfall, humidity, wind direction and so on. Climate is the sum of weather conditions over a longer period. Climate determines the development of free living stages and it affects different species of parasites differently. In other words, climate determines which nematodes are generally found in particular area while weather determines the prevalence of nematode parasites in that area at a particular time of a particular year. The effects of environmental factors on free living stages are briefly discussed below.

Temperature: Temperature is one of the most important factors governing the process of egg hatching and subsequent development to the infective stage. It is reported that the satisfactory temperature range for the development of many of the nematode species lies between 20-35^0 C. The survival of pre-parasitic stages is highly dependent on the environmental temperature. Mortality of pre-infective larvae (L1 and L2) is very high at temperatures over 40^{0}C while at low environmental temperatures, larvae survive on pastures for longer periods. This is presumably due to the slow rate of utilisation of stored energy. This explains why the survival of larvae on pasture in tropical areas is much lower than that which occurs in temperate areas. Temperature may influence the migration of infective larvae. There is little or no migration at temperatures below 10 ^{0} C.

Moisture: Moisture plays an important role in the development and survival of free living stages. Numerous studies have been done on seasonal availability of nematode larvae on pastures. These reveal that relatively low numbers of infective larvae are available on pastures during dry periods when compared to wet periods. Wet conditions with steady soaking rains, are favourable for the development of eggs and infective larvae and their subsequent mass migration on to the pasture. However, heavy rains cause the disintegration of faecal pats and then the free living larvae would become more directly exposed to the sunlight.

Sunlight: Although, less important than both moisture and temperature, sunlight also influences the development and survival of larvae on pasture, and also the translation of larvae to the pasture (Tahir, 1981). The harmful effects of direct sunlight include increased temperature, desiccation, and ultraviolet radiation.

Oxygen: Oxygen also plays an important role in the development of eggs and larvae. Parasitic development is inhibited at low oxygen concentrations The requirement of oxygen for development varies in different parasitic species (Tahir, 1981).

The dung pat: The dung pat has been recognized as a protective shelter and a reservoir for the larvae (Michel, 1976). The pat protects larvae from adverse environmental conditions and permits them to survive longer. Whatever the weather, death rates of larvae on pasture are higher than for those remaining in the dung pat. Unlike goat faeces which can dry easily, cow pats retain sufficient moisture for the development of larvae (Okon and Akinpelu, 1982). The hard crust which generally forms on the surface of the pat prevents larvae from leaving the pat and protects the middle of the pat from desiccation. Dissemination of infective larvae from the pat to the pasture is an intermittent process. This depends on the thorough wetting of the pat and surrounding pasture by rain. A continuous film of moisture is required for larvae to leave the pat and move onto the pasture. Translation of larvae from

the fresh pat can take place even after a light rainfall, but if the outer surface of the pat has already dried out, rain in excess of 50mm during a minimum period of two days is necessary (Fabiyi, 1978).

Dung beetles: Studies revealed that the number of parasitic nematode larvae recovered from faecal pats was greatly reduced by beetle activity. Dung beetle populations on pasture cause more contaminated faeces resulting in fewer parasitic larvae being available to grazing animals (Fincher, 1975)

Pathogenesis of gastrointestinal parasitic infections

Nematode infections are generally mixed and it is very difficult to find specific details of the signs and pathological effects attributable to individual nematode species. Although these parasites in many instances produce little serious damage to the host, they are never benificial and in some instances can produce severe and even fatal disease. The most common signs of severe gastrointestinal parasitism are a mixture of anorexia, diarrhoea, submandibular oedema and emaciation (Cole, 1986). Clinical signs are more common in young calves, but adults may also be affected in severe infections. It is reported that calves are more susceptible to the effects of parasitism during the first five to eight months after being exposed to significant levels of infection (Copeman, 1982).

Pathogenesis and clinical signs

Toxocara vitulorum infections are more common in buffalo calves where they cause very heavy, even fatal, infections. Clinical signs of heavy infections are emaciation, diarrhoea with foul-smelling yellowish faeces, and breath with butyric odour (Copeman, 1982). The diarrhoea is followed by constipation. It has been reported that the mortality in calves due to toxocariasis ranges from 25-50 per cent and is particularly high in malnourished calves.

Haemonchus species, with their relatively short prepatent period and large egg laying capacity, they have the potential to build up heavy infections in a few months in susceptible stock under favourable environmental conditions in the tropical areas. These are medium sized parasites which live in the abomasum and measure 12-17 mm in length. Adult worms are readily seen on the mucosal surface, and females have a barbers pole appearence due to the spiral intertwining of a blood filled gut and white egg-filled uteri.

Infective third stage larvae reach the abomasum and can be found in the pits of the gastric glands, some penetrating to the deepest part. Neither gross nor microscopic changes in the mucosa can be detected at this stage. Development of the fourth stage larvae is accompanied by a progressive inflammatory reaction, hyperaemia and fluid exudation. They feed on hosts blood and moult to become immature adult worms. These immature adult worms attach to the mucosa for feeding, giving rise to circumscribed erosions on the surface.

Heavily infected animals are anaemic, often with submandibular oedema. Affected animals are unable to travel far for grazing and show signs of discomfort, panting or even collapse. Visible mucous membranes are pale and the coat may be rough (Hungerford, 1990). The severity of the changes and their speed of onset will be determined by the number of larvae initially ingested.

Cooperia species are not generally regarded as serious pathogens but may cause parasitic gastroenteritis in calves. They usually affect young calves 4-5 months of age and rarely affect calves older than 7 months. Fourth stage larvae are found either deep in the intervillus crypts or coiled around the tips of the villi. Adult worms lie in close contact with the mucosal surface. The main pathological changes in *cooperia* infections are destruction of the tips of the villi, intensive inflammatory reaction and exudation of plasma proteins. The clinical signs include intermittent diarrhoea, progressive loss of condition and listlessness. Death may occur if the infection is severe.

Bunostomum is a hook worm found in the small intestine attached to the mucosa by the large buccal capsule. The worms are blood suckers. The mucosa is damaged by the attachment of the worms. In heavy infections there may be enteritis and diarrhoea. Points of attachment are seen as red spots and are often visible from the serosal surface of the duodenum. Heavy infections may lead to anorexia, submandibular oedema, severe anaemia, malena and death. Young stock aged 3-18 months are more susceptible.

Strongyloides species are found in the small intestine and the effect on the host is not well understood. Animals with heavy infections show intermittent diarrhoea. Eggs are found in faeces as early as six days after birth.

Oesophagostomum radiatum is one of the more pathogenic species in buffaloes when it is present in large numbers. There is inflammation of the small and large intestine and diarrhoeic faeces are passed. Extensive nodule formation occurs (pimply gut), affecting the whole intestinal tract. It has been reported that *O. radiatum* nodules were more frequent in the caecum and colon of buffaloes than of cattle. Most severe effects are associated with the early fifth stage larvae (Bryan and Kerr, 1989).

Gigantocotyle explanatum which occurs in the bile duct, does little or no harm to the host. However, in severe infections there may be a series of haemorrhages indicating the sites of attachment. Affected liver may show thickening of the bile ducts.

Other conical flukes which occur in the rumen and reticulum are essentially non pathogenic even though large numbers are present. However, the immature stages of paramphistomes are responsible for pathological changes. This condition is known as immature paramphistomiasis. These immature forms are embedded in the intestinal mucosa, drawing pieces of the mucosa into the suckers causing inflammation and haemorrhage. The main lesions are localized in the first metre of the small intestine and the pyloric end of the abomasum. The superficial layers of the tunica are destroyed and parasites at various stages of development are present in the mucosa and desquamated cell elements are found in the mouth of the immature flukes (Panjoo *et al.*,1988). Clinical signs of immature paramphistomiasis consist of diarrhoea, anaemia, weakness and eventually death.

Buffalo calves are more vulnerable to coccidial infections very early in life. Severe diarrhoea in young calves is more frequently associated with shedding of large numbers of coccidial oocysts. Later stages of the development of the schizonts and the liberation of merozoites cause distortion and disruption of the villi, respectively. In severe infections majority of the crypts of the intestine are destroyed and the lumen of the intestine is filled with blood.

Affected calves develop diarrhoea and in acute cases diarrhoea may be haemorrhagic. Diagnosis of coccidiosis in buffalo calves is based on the clinical signs and demonstration of oocysts in the faeces.

Pathophysiology of helminth infections

Physiological changes induced by gastrointestinal helminths are less documented due to technical difficulties associated with such studies. Nevertheless, observations have been made which indicate the type of change induced, both at the site of infection and more generally. Numerous studies have demonstrated that even sub clinical levels of infection can cause considerable physiological changes in the host. Furthermore, the adverse effects of the gastrointestinal parasites may persist even after the majority of the parasite population has been eliminated (Jayasinghe, 1991).

In their general effects on the host animal, there is a remarkable similarity among gastrointestinal nematodes, despite the different changes induced at different sites of infection. These general effects are manifested by several ways such as change in body weight, decrease in the absorption of fat, protein and skeletal calcium and phosphorus together with increased body water as percentage of body weight. Although the effects are well established, the precise mechanisms involved and the relative importance of different influences are not well understood.

Decreased food intake

A common feature of gastrointestinal helminthiasis is a reduction of voluntary food intake. As a general rule, there is a direct relationship between the severity of the infection and the degree of anorexia (Symons, 1985). Despite the obvious importance of inappetance in parasitised animals, it is still not precisely known why it occurs. The general occurrence of inappetance tends to suggest a common physiological pathway, perhaps one mediated by the activity of neural and hormone secreting cells which abound at all levels of the gut. It is even conceivable that secretions released by the worms may act directly on the food intake regulatory centres within the central nervous system. Altered plasma concentrations of gastrointestinal hormones, and especially cholecystokinin (CCK) have also been implicated as a cause of inappetance in parasitised animals (Symons and Henessey, 1981). However, subsequent studies by Symons (1985) has refuted the involvement of CCK as a cause of inappetence during parasitism.

Gastrointestinal motility, digestion and absorption

Effects on gastrointestinal motility in helminth infections have not been studied extensively. From the limited studies conducted on ruminants, it can be concluded that infections of the abomasum, small intestine and possibly the large intestine may interrupt the normal pattern of gastrointestinal motility and digesta flow (Gregory *et al.*, 1985). It has been reported that the digesta flow from the rumen was reduced but the flow increased in the small and large intestines of lambs infected with *Trichostrongylus colubriformis* (Roseby, 1977). More recent studies have shown that sub clinical parasitic infections in ruminants alter the normal pattern of gastrointestinal motility in the absence of any diarrhoea and cause inhibition of abomasal and proximal small intestinal motility and digesta flow (Gregory *et al.*, 1985). However, the increased frequency of migrating myoelectric complexes (MMCs) helps to maintain digesta flow through the proximal small intestine to prevent a more severe reduction in the rate of digesta transit (Gregory *et al.*, 1985). The increased frequency of

MMCs and some, or all, of these changes in gut motility might depend upon altered secretions of gastrointestinal hormones, such as CCK and secretin, as a consequence of parasitic infections. Both of these hormones inhibit abomasal emptying (Grovum, 1981).

Attempts have been made to determine whether impaired digestion and absorption are major causes of poor utilisation of food by parasitised ruminants. Even though some studies showed a reduction in digestibility and absorption of dietary nitrogen, or other nutrients, such values are poor indicators of malabsorption. It has been shown that the decline in nitrogen digestibility in parasitised ruminants is probably due to a drop in hydrochloric acid production and an increase in permeability of the abomasal mucosa (Fox *et al.*, 1989a). Impaired protein digestion in the abomasum in monospecific abomasal parasitic infections, thought to be due to the rise in abomasal pH and is nullified by a compensatory increase in protein digestion in the small intestine. However, colonisation of the entire small intestine by parasites and associated mucosal damage prevent any compensatory response, resulting in a reduction in the apparent digestibility of crude protein in heavily parasitised animals (Parkins *et al.*, 1990). Several other studies have shown that there is a reduction in nitrogen digestibility by parasitised ruminants (Parkins *et al.*, 1990; Entracasso, 1988; Entracasso, *et al.*, 1986a; Entracasso *et al.*, 1986b; Randall and Gibbs, 1981; Van Adrichen and Shaw, 1977).

Energy metabolism

There is a dearth of information on energy metabolism in parasitised ruminants. Anorexia is obviously a major factor reducing the availability of energy for maintenance and growth. The low energy balance is manifested by lower growth rates. In addition to a reduction of appetite and a small reduction in energy digestibility, a major factor in reducing animal performance is a marked reduction in efficiency of utilisation of metabolisable energy. It has also been shown that sub clinical parasitism also causes deleterious effects on nitrogen and energy utilisation. (Randall and Gibbs, 1981). The reasons for low energy retention in parasitised ruminants have not been accurately determined. Increased methane production along with urinary urea secretion in subclinical haemonchosis, could play a role in reduction of retention of digestible energy.

Protein metabolism

Endogenous protein loss: Gastrointestinal parasitism causes substantial loss of proteins into the gastrointestinal tract from the sites of infection. These proteins are composed of plasma and red cells, exfoliated epithelial cells and mucous. Decreased plasma protein levels or hypoproteinaemia is a common feature of gastrointestinal nematodiasis and is caused by a loss of plasma protein into the gastrointestinal tract. Mean daily flow of about one litre of plasma into the gut lumen has been recorded for at least five weeks after parasites' fourth moult (Bremner, 1982). There is a relationship between the magnitude and duration of gastro-enteric plasma loss and the relative effects of the parasites on performance of the host. Most marked depressions in live weight gains in parasitised ruminants are recorded during the periods of increased gastro-enteric plasma loss (Steel *et al.*, 1980).

The precise mechanism involved in plasma loss is not clearly understood. However, it is generally believed that invasive activities of the parasites and/or inflammatory reactions result in separation of the junctions between the cells of the mucosal epithelium at the site of

infection, resulting in increased permeability of capillaries and venules (Steel and Symons, 1982).

The haemorrhage caused by Haemonchus and Oesophagostomum species leads to loss of blood cells and proteins, notably haemoglobin into the gastrointestinal lumen. The iron bound to haemoglobin cannot be reabsorbed once in the intestinal lumen, therefore haemorrhage into the gut lumen results in a depletion of iron reserves. Both adult and the fourth larval stage of haemonchus spp. suck blood. In addition, they move and leave wounds which bleed into the abomasum.

An elevation of desquamation of mucosal epithelial cells which may explain the typical villus atrophy at the sites of infection may also contribute to endogenous nitrogen loss. Proliferation of goblet cells at the site of infection and in turn, increased mucous production by the gut, is a generalised response to parasitic infections. Unfortunately, methods to evaluate losses from desquamated epithelial cells and mucous are not yet available. Therefore, their contribution to endogenous nitrogen loss cannot be assessed.

Protein digestion and absorption: In parasitised animals, malabsorption of proteins at the sites of infection is attributable to deficiencies in digestive enzymes, proliferation of undifferentiated non-absorptive cells on villi, and a reduction in surface area due to villus atrophy. However, increased absorptive capacity in uninfected areas compensates for the impaired function of the infected areas.

An important function of the abomasum is to break down proteins and polypeptides by enzymatic action. The enzyme responsible for this breakdown pepsin, is derived from a precurser, pepsinogen produced by mucous neck cells and chief cells. Pepsinogen, in the presence of an acid medium, is transformed into pepsin. Gastrin is produced by G-cells, which are mainly found in the antrum of the stomach, and controls the flow of digesta and the pH in the abomasum. Gastrin is released in response to increased abomasal pH. It not only regulates the hydrochloric acid secretion, but also influence the motility and may affect trophic growth of abomasal mucosa. It has been reported that hypergastrinaemia in cattle infected with Haemonchus species, is probably due to the increase in abomasal pH. Such a change may stimulate hormone secretion by gastrin cells in the pyloric antrum and, in turn, increase hydrochloric acid production (Fox *et al.*, 1986). However, restricted feed intake has no effect on gastrin levels in infected calves. Gastrin levels increase markedly before the depression in appetite. Many workers have reported a correlation between worm burden and levels of pepsinogen (Sinder *et al.*, 1981; Chiejina, 1978; Selmoan *et al.*, 1977; Ford, 1976). When newly moulted adults emerge from the gastric glands, they cause mechanical damage to the glands, and consequently, to the parietal and chief cells. As a result gastric pH increases and consequently transformation of pepsinogen to pepsin is decreased, resulting in impaired protein digestion. No clear understanding of tissue protein metabolism in parasitised cattle is still available.

It can be concluded that, due to inappetance, gastrointestinal losses of proteins and increased rates of gastrointestinal tissue protein metabolism, there may be a net movement of amino acid nitrogen from muscle and skin to the liver and gastrointestinal tract which decreases the availability for growth and production.

Water and electrolyte balance

Diarrhoea is a common clinical sign in parasitised ruminants, especially in those at pasture. Diarrhoea may occur in almost all gastrointestinal nematode infections. The onset of diarrhoea occurs at the time of maturation of larvae into young adults. This is also the time that many other pathophysiological changes, such as inappetance, leakage of plasma into the gut and negative nitrogen balance occur. Diarrhoea is an important clinical sign because it disturbs the overall regulation of body fluids. However, contrary to expectation, water retention in parasitised animals is normally higher than in non infected animals (Abott *et al.*, 1986).

Parasitised calves excrete less sodium (Bremner, 1982) but the potassium loss is high when compared with uninfected animals. This loss occurs in the faeces. The source and the consequence of potassium loss has not been clearly understood. However, it may be due to the massive sloughing of intestinal epithelial cells from the infected gastrointestinal epithelium (Holmes, 1986).

Mineral metabolism

It has been demonstrated that chronic subclinical parasitism of the small intestine impaired the skeletal growth and mineralisation (Coop *et al.*, 1981; Sykes *et al.*, 1979; Sykes and Coop, 1976). There is an increase in numbers in bone resorption sites in parasitised animals. However, the blood calcium and phosphorus levels are normal in these animals (Sykes and Coop, 1977). Therefore, the major cause of impaired skeletal growth is impaired mineral absorption. There is also an increased endogenous loss of calcium and phosphorus in intestinal parasitism, but not in abomasal parasitism (Wilson and Field, 1975).

Impaired productivity due to gastrointestinal parasitism

Gastrointestinal parasites cause impaired productivity in ruminants. These production losses may occur with or without any clinical signs. Furthermore, the adverse effects of gastrointestinal parasitism may persist even after the withdrawal of parasites from the host. The adverse effects on productivity are manifested in several ways ranging from body weight loss to death. In any given form of animal production the effect of gastrointestinal parasitism is influenced by several factors such as the level of infection, the species of parasite, and the age, nutrition and physiological status of the host, or even by management practices.

The most common parasites involved in production losses are Haemonchus and Trichostrongylus in abomasum, Cooperia in the small intestine, and Oesophagostomum in the large intestine. In addition, *T.vitulorum* and *M.digitatus* cause production losses in tropical areas.

The precise mechanisms involved in production losses are still unknown. Reduction in voluntary feed intake is undoubtedly the major factor affecting the production loss. However, reduced feed intake due to impaired appetite is not only an effect of gastrointestinal parasitism, but also occurs when there is a reduction in quality and quantity of feed available. Therefore, it may be difficult to differentiate simple malnutrition from malnutrition resulting from parasitism. The difficulty is even more pronounced when parasitic disease is subclinical and there is no accurate method to diagnose it.

Weight gain

The immediate consequence of impaired food intake in ruminants due to gastrointestinal parasitism is a reduction in live weight gain or a loss of body weight. The degree of reduction in body weight is related to the severity of infection (Van Adrichen *et al.*, 1977). However, other factors such as age of the animal and season of the year may also be involved.

The information available on weight loss in cattle and buffaloes is limited due to practical difficulties. In many studies, weight gain in parasitised animals have been compared with treated animals. Some studies revealed that the difference in weight gain between treated animals and the controls was not statistically significant. (Corlis, 1980). However, some improvements in daily weight gains were recorded in treated or uninfected animals by several other studies (Bell *et al.*, 1990; Fisher and Macneil, 1982; Coop *et al.*, 1979a; Coop *et al.*, 1979b; Copeman and Hutchinson, 1979).

Milk production

The anthelmintic treatment of lactating cows to increase their milk production is still controversial. It is well known that lactating cows carry very low helminth burdens. Therefore, any gain in milk production after treatment is probably due to elimination of sub clinical infection.

If there is a significant increase in milk production after anthelmintic treatment, then the question arises, how does the relaively low worm burden in adult animals cause significant losses? Does leakage of plasma proteins into the gut occur even in sub clinical infections? Or is energy for milk production diverted to maintain an immunity against infection? However, more information is needed to comprehend the precise mechanism (Jayasinghe, 1991). The effect of anthelmintic treatment on milk production is still controversial as a consequence of contradictory results obtained (Kloosterman *et al.*, 1984; Fisher and Macneil, 1982; Barger and Lisle, 1982; Michel *et al.*, 1982).

Mortality

In heavy infections mortality may arise as an important cause of economic loss. In other words, it is an extreme form of reduced production. In most cases not only the current years production is lost, but also the capital cost for replacing the dead animal. In some countries 40% of deaths in untreated buffalo calves up to 12 weeks of age is due to *T.vitulorum* infections (Copeman, 1982).

Reproductive performance

There is some evidence to show that gastrointestinal parasitism may adversely affect different phases of the reproductive cycle. Infections with *O.radiatum*, Cooperia species and *Haemonchus placei* increase the age of puberty in heifers grazing in tropical areas (O'Kelly *et al.*, 1988). Helminth parasites can reduce fertility of cows (Mackay, 1980; Oakley *et al.*, 1979; Hope-Cawder, 1976).

Increased cost of production

Helminth parasites are responsible for increasing the cost of producing many animal products. The most obvious example is the cost of helminth control. As well as causing reductions in production by ruminants, helminth parasites may also cause reduction in the unit value of such products. For instance, reduced weight gain caused by parasites may

lower the quality of carcass and its value, or infected animals may have to be retained on the farm longer for finishing.

Factors affecting helminth burdens

There are several factors affecting the prevalence and severity of helminth parasitism. These include host factors such as age, nutrition, sex, reproductive status, genetic aspects, and environmental factors of climate, weather and type of pasture.

Host factors

Age and sex of the host: It is well known that young animals are more susceptible to gastrointestinal parasitism and they develop resistance as they get older. Calves usually are exposed to infective larvae as soon as they start grazing when they are a few weeks old. As a result, the burden of adult worms increase, faecal out put of worm eggs rise and the infection remains until the animal acquires resistance or immunity to the infection. Generally, acquired resistance develops within 5-8 months of weaning, if animals are exposed to significant infection during that period (Copeman and Hutchinson, 1979).

The precise relationship between age and worm burdens is not well understood, even though several suggestions have been made. Some suggested that as animals get older, their tissues become tougher forming a greater physiological barrier to the parasites. Some others reported that acquired resistance develop more rapidly as the hosts get older (Herlich, 1980).

The effect of sex of the host on parasitic burdens has been investigated in many host-parasitic systems. Generally males and females are equally susceptible until, usually but not invariably, the age of puberty. After puberty, males may become more susceptible than females (Tahir, 1981). However, sex related resistance in cattle is less studied than in other host parasite systems.

Reproductive status of the host: Reproductive status of the host, especially pregnancy and lactation, has long been known as factors affecting worm populations in a wide variety of hosts. During late pregnancy and lactation, the resistance to parasitic infections is relaxed. As a consequence, parasites that would be rejected normally are able to develop (Jayasinghe, 1991). Increased numbers of mast cells, eosinopils and globule leucocytes are found in the gut mucosa of animals immunologically responding to the parasites, but very few globular leucocytes are present in lactating and pregnant animals (Tarigan, 1982). Cows in their first lactation have the highest larvae per gram of faeces compared with their second or third lactations.

The origin of the rise in faecal egg count during pregnancy and lactation has been widely debated. This may be due to increased fecundity of already present adult worms, and also, there is some evidence to suggest that heifers in early lactation are not able to limit the establishment of worms as effectively as non-pregnant or mid-pregnant heifers (Borgsteede, 1978). Furthermore, this rise may be due to the maturation of dormant larvae accumulated in the host some months previously. Fewer arrested larvae are found in lactating heifers and the estimated rates of development is faster. A greater population of adult worms are present as a result (Barger and Southcott, 1975).

Nutrition of the host: It has been known very clearly that nutrition of the host plays an important role in influencing parasitic burdens. Well nourished animals are more resistant to worm burdens when compared with malnourished animals. Type of pasture and the composition of the diet, mainly protein can also influence the worm burden in grazing cattle. Grazing calves under conditions of impaired protein intake can be markedly affected by nematode infections even after anthelmintic treatment (Parkins *et al.*, 1982).

Different types of pastures vary in their nutritive value. Calves grazing on a pasture with a low nutritive value may be more liable to parasitic infections than calves grazing on pastures with high nutritive value. Thus, pasture type may also influence intestinal parasitism either directly by improving the nutrition of animals or indirectly by altering the environment of free living stages (Tarigan, 1982).

Malnutrition therefore, is an important factor predisposing animals to helminth infections. However, a high plane of nutrition is only a minor factor affecting worm burdens when larval prevalence is high or where haemonchus and fasciola are involved (Johnstone *et al.*, 1979).

Environmental factors

Climate and weather: Gastrointestinal nematodes are subjected to various environmental factors during their development as free living stages outside the host. Climate and weather are major environmental factors which affect the development of free living stages. The population of free living nematodes fluctuates with the changes of temperature, rainfall and humidity.

Grazing management and type of pasture: Certain grazing management systems and type of pasture may affect the transmission of nematode infections. Management decisions, perhaps made for reasons unrelated to helminth control may modify the extent and severity of the infection. The severity of parasitic infection increases with the intensity of grazing. Increased stocking rates will increase the contamination of pasture, make infective stages more accessible and render conditions less favourable for survival and development of free living stages (Morley and Donald, 1980). Indirectly it may affect the severity of parasitism by decreasing consumption of pasture per animal and hence lowering the nutritional status of the animal. Pasture resting and rotational grazing are intended to reduce the infectivity of pastures. Even though pasture resting is costly, it helps to control the gastrointestinal parasitism to a certain extent (Morley *et al.*, 1978). There is limited evidence available that type of pasture has any influence on parasitism.

Control of gastrointestinal parasitism in buffaloes

Use of anthelmintic drugs

The treatment of parasitic diseases has been going on for as long as such diseases have been known and was primarily concerned with parasites that caused disease in man. Chemotheraphy has played a role in livestock production only relatively recently. The origins of many effective drugs are found in traditional treatments. Modern broad spectrum anthelmintics were discovered and developed by the pharmaceutical companies.

Anthelmintics are extensively used to maintain levels of production and to prevent mortalities due to parasitism. Control measures which rely entirely on anthelmintics are

however no longer sustainable. Parasites progressively become resistant to anthelmintics and the development of resistance to anthelmintic drugs is a major threat to parasite control, worldwide.

Accumulation of residues of anti-parasitic chemicals in the tissues of host animals may have adverse consequences. Consumers around the world are also demanding lower levels of chemical residues in their foodstuff. Therefore, alternatives to anthelmintic control of helminth parasites are essential.

Vaccines

The development of effective vaccines against helminth infections has now been identified as a major need. The outstanding success achieved in the control of bacterial and viral infections through vaccination have so far not been transfered to helminth infections. The major steps in the development of helminth vaccine include the identification and purification of helminth antigens which are responsible for inducing the protective immune response during natural infection and the induction of the appropriate type of immune response through vaccination which will result in rejection of the parasite.

Resistant hosts

Resistance has been defined as the "ability of a host to initiate and maintain responses to suppress the establishment of parasites and eliminate the parasite load". Therefore, resistant animals harbour fewer parasites than susceptible animals. Including "parasite resistance" in livestock breeding programmes has now been identified as an alternative control strategy. Sheep bred for worm resistance have showed impressive results from experimental challenges.

Biological control.

Biological control is defined as the action of natural enemies which maintain a host population at levels lower than would occur in the absence of the enemies (Peter *et al.*, 1996). Such organisms could attack either the parasitic or the free living stages of the parasites. All nematode parasites of livestock have a life cycle which involves a parasitic phase within the host and a free living stage on pasture. Free living stages are more vulnerable to attack by biological control agents. There is good evidence to show that dung beetle activity is directly correlated with reduction in infective nematode larvae recovered from faeces and surrounding herbage. Earthworms also play an important role in the structural decomposition and disappearence of cattle dung. Nematode destroying fungi, characterized by their ability to capture and exploit nematodes, either as the main source of nutrient or supplimentary for their exsistence have been described (Peter *et al.*, 1996).

Grazing management

There is much more field evidence available for the effectiveness of various forms of grazing management in controlling helminth parasites of livestock. Alternative grazing of pastures by sheep and cattle is a way of preparing "clean pasture" (Barger, 1996).

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NA-218-21

1.2 Haemoparasites

D.J. Weilgama

Buffaloes too are affected by blood parasites though to a lesser degree than the cattle. Almost all parasites that invade the circulatory system of cattle have been recorded from buffaloes. A large majority of these are protozoans and those reported from buffaloes include members of the genera *Babesia*, *Theileria* and *Trypanosoma*. The first two are intracellular parasites whilst the third, a haemoflagellate is usually extracellular, though in some species an intracellular stage is present. These belong to the sub kingdom Protozoa in the kingdom Protista (Levine, 1985). Another 'parasite' that affects buffaloes is Anaplasma belonging to the order Rickettsiales. In buffaloes, another parasite often encountered are the microfilariae of the filaroid nematodes such as *Setaria spp*. This chapter discusses two parasites that are encountered commonly among buffaloes in Sri Lanka namely, *Theileria* and *Trypanosoma*. Brief mention is also made regarding two other parasites *Babesia* and *Anaplasma* that are responsible for clinical disease among buffaloes in the neighbouring countries.

Theileria

This haemoprotozoan parasite affects cattle, buffaloes and other ruminants. The organisms are found in erythrocytes, lymphocytes and histiocytes. They are highly pleomorphic and in erythrocytes, round, ovoid, rods, dots and cigar shaped forms could be observed. In the lymphocytes another stage schizonts, could be found. About 8 species of theileria have been recorded in cattle and in buffaloes. The most pathogenic of these are the organisms of the *Theileria parva* complex found in Africa. Mortality rates of 90-100 % among affected animals are not uncommon and the survivors remain solidly immune. The species of importance in the South Asian region are *T.annulata* and *T.orientalis* (syn. *T.buffeli*). The former is pathogenic while the latter usually cause a mild disease. Another species, *T.sergenti*, has been reported from Japan and Korea.

Transmission

Theileria spp. are transmitted by ticks in nature. Ticks of the genera *Hyalomma, Amblyomma, Rhipicephalus and Haemaphysalis* have been incriminated as vectors in different parts of the world. Transmission is by nymphal and adult stages and transovarian transmission does not occur. Mechanical transmission by blood inoculation and by inoculation of tissue suspensions of spleen, lymph nodes or liver is possible.

Life cycle

The feeding tick injects infective sporozoites into the host. These enter lymphocytes and grow to become macroschizonts. These divide and the host cell divides synchronously. Some macroschizonts give rise to microschizonts, which produce the merozoites. These invade the erythrocytes where multiplication takes place by division into 2 or 4 (Maltese-cross forms). Parasitized erythrocytes that are ingested by feeding ticks initiate the cycle in the tick development which begins in the lumen of the tick, continues in the epithelial cells of the gut to produce kinetes. The kinetes enter the salivary glands to finally produce the infective sporozoites.

Clinical signs

The affected animals usually show high temperature (40- 41.7°C); about 8-14 days following infective tick bites. There may be laboured respiration, watery lachrimation and a serous nasal discharge. The lymph nodes enlarge and the animal would become erythropaenic and icteric. Haemoglobinuria is seen rarely. Animals infected with *T.annulata* may become weak and later succumb to the disease. Cerebral form of the disease has also been noted with nervous signs such as paddling movements, muscle twitching and pressing of head against hard objects.

Post mortem lesions

The carcass is usually emaciated with pale mucosae and enlarged superficial lymph glands. The spleen and liver are often enlarged and congested. Gall bladder appears distended with thick granular bile. In *T. annulata* splashy haemorrhages in serous surface of the rumen, abomassum and intestine may be present. The kidneys are congested and show petechial haemorrhages on the external surface. The lungs too may be congested and oedematous.

Diagnosis

The disease could be diagnosed on clinical signs and by demonstration of the parasite in blood smears and in lymph node biopsy smears stained with Giemsa. In lymph node smears schizonts known, as Koch's blue bodies are characteristic. In carrier animals' specific diagnosis could be made by the complement fixation test and the indirect fluorescent antibody tests. In recent times highly specific and sensitive tests such as ELISA and DNA probes have been developed and are in use.

Treatment and control

Many chemical compounds have been used in the treatment of theileriosis in cattle and buffaloes. Tetracycline (Oxytetracycline) either alone or in combination with anti-malarials such as camaquin, or chloroquine or nivaquine has given varying results (Rao *et al.*, 1973; Sharma and Gautum, 1971; Gautum *et al.*,1970). A new chemical parvaquone (Clexon R) has shown to be effective specifically against *theileria.*

The disease could be controlled by regular treatment of animals against ticks. Immunisation of susceptible animals with a strain of low virulence or with tissue culture vaccine has also been successful.

Theileriosis in buffaloes

Two species of *theileria* namely, *T.annulata* and *T.orientalis* have been identified from buffaloes in Sri Lanka (Weilgama, *et al.*, 1989a). The latter is the most common parasite seen in buffaloes with the prevalence rate reaching 100% in certain areas. *T.orientalis* is transmitted by *Haemaphysalis bispinosa* in Sri Lanka (Weilgama *et al.*, 1989a). *T.annulata* was detected only in Polonnaruwa and Ridiyagama areas (Weilgama, unpublished). Seneviratna and Kumaraswamy (1960) recorded high mortality among cattle due to *T.annulata* (*Gonderia annulata*).

In India theileriosis due to *T.annulata* is a serious disease among bovines (Gautum and Dhar, 1980). This parasite has been reported from the ox, zebu and the water buffalo in North Africa, Southern Europe, Southern USSR and Asia causing 10 –90 % mortality depending on the area (Levine, 1985).

Trypanosoma

Trypanosomosis is an important disease among livestock and also in man. The members of the genus *Trypanosoma* occur in all classes of vertebrates (Levine, 1985). They are extracellular and are found in the circulatory system and tissue fluids. Some species such as *T.cruzi* however, also invade cells. Almost all are transmitted by blood sucking invertebrates and most species are nonpathogenic. These are haemoflagellates that belong to the family Trypanosomatidae in the Order Kinetoplastorida, of the class Zoomastigophorasida. It possesses a leaf like body with one end been pointed and the other more rounded. There is a nucleus, a kinetoplast and an undulating membrane. A single flagellum arises from a kinetosome or basal granule, which traverses the entire length of the organism to end beyond the anterior end.

Two species of trypanosomes have been detected in buffaloes namely, *T.evansi* and *T.theileri.* The later is a relatively large and nonpathogenic organism that occurs in the blood of cattle and buffaloes. It is about 60-70 μm long but could reach up to 120 μm. *T.evansi* on the contrary is more pathogenic and infect many other animals. The disease caused by this organism is widely referred to as Surra, although different names are in use in different localities. It has been reported in horses, dogs and in camels as well. The typical forms are about 15-34 μm in length. Most are slender or intermediate in shape, but stumpy forms have been reported.

Transmission

T.evansi is transmitted mechanically by biting flies. The common vectors are flies of the genera, *Tabanus* (horse flies), *Stomoxys* (stable flies), *Haematopota* and *Haematobia* (buffalo flies). No cyclic development takes place in these vectors. In *T.theileri,* cyclical transmission occur in various Tabanid flies including *Tabanus* and *Haematopota.* Intrauterine transmission is also believed to occur (Levine, 1985).

Clinical findings

In buffaloes, the disease may appear as peracute, subacute or as chronic. In peracute form the buffaloes may show sudden death. There could be convulsions, ataxia, blindness and frenzy. In other forms of the disease there may be a transient rise in temperature with enlargement of superficial lymph nodes. Anaemia, abortions, progressive weakness and recumbency at terminal stages are the other signs recorded.

Post mortem lesions

In per acute cases no specific lesions are seen. In others the findings include enlargement of lymph nodes, kidneys and congestion of liver. There could also be pulmonary emphysema, muscular atrophy and dark tarry blood.

Diagnosis

The disease may be diagnosed by detecting trypanosomes in the blood. Thick blood smears are preferable to thin ones and could be stained with Giemsa. A fresh drop of blood also give useful results. Microhaematocrit centrifugation is also suitable for detection of trypanosomes and these could be seen at the buffy coat interface under the microscope. Various serologic tests such as passive haemaglutination, gel diffusion and indirect fluorescent antibody test

have been used. Another method of diagnosis is by culturing blood or tissues in laboratory animals or in artificial media.

Treatment and control

Many different drugs have been used against trypanosomiasis. The ones used with efficacy in more recent times are diminazene aceturate (Berenil) and isometamidium chloride (Samorin). Control of this condition in the Asian region is based on treatment of infected animals and on vector control. Treatment against vector would include clearing of breeding places and the use of repellants on animals.

Trypanosomiasis in buffaloes

In Sri Lanka both *T.evansi* and *T.theileri* have been recorded from buffaloes (Weilgama *et al.,* 1989a). Although parasites are rare in blood these authors reported a 50% positivity for *T.evansi* among buffaloes in the dry zone using an indirect fluorescent antibody test. Clinical infections in buffaloes are very rare but heavy mortality among Murrah buffaloes in a farm in the south has been recorded. In India, Verma and Gautum (1978) recorded mortality up to 80% among infected buffalo calves.

Babesia

These are protozoan parasites infecting erythrocytes of vertebrate hosts. Two species *Babesia bigemina* and *B.bovis* have been reported from buffaloes. Although there are no records of their presence among buffaloes in Sri Lanka they have been recorded from many countries including India, Vietnam, China and Turkey (Miranpuri,1988; Sharma *et al.*,1985; Ma *et al.*,1989; Celep, 1984). These organisms are transmitted in nature by ticks. Both transovarian and transtadial transmission has been reported and ticks of the genera *Boophilus, Rhipicephalus* and *Haemaphysalis* are incriminated as vectors.

Infected animals develop high temperature (40-41.5°C), become listless and anorexic. The animals become anaemic and icteric. Haemoglobinuria is a common sign and in animals affected with *B.bovis*, nervous signs appear. They may show muscle twitching, paddling of limbs and ataxia.

At post mortem, the urinary bladder frequently contains red urine. The spleen and liver are usually enlarged. Gall bladder contains thick granular bile and kidneys are often congested. Lungs become oedematous and may contain blood tinged fluid.

The disease is diagnosed on clinical signs and by demonstration of parasites in Giemsa stained thin and thick blood smears. Serological techniques such as Immunofluorescent antibody technique (IFAT), Complement fixation test (CFT), Radioimmuno assay (RIA), Enzyme immuno assay (EIA) and slide agglutination test are used in epidemiological surveys.

Control of babesiosis depend on treatment of affected animals, control of vector and preimmunisation of susceptible animals. Live attenuated vaccines are available and have been used successfully in cattle in many countries including Sri Lanka (Weilgama *et al .,* 1989 b).

Anaplasma

Anaplasma belong to the Family Anaplasmatacea in the Order Rickettsiales. They are small spherical bodies about 0.2-1 μm in diameter and are found in the erythrocytes. Two species, *A.centrale* and *A.marginale* are found in cattle. In buffaloes *A.marginale* has been recorded from many countries such as India, Vietnam, China and Turkey (Miranpuri,1988; Sharma *et al.*,1985; Ma *et al.*,1989; Celep,1984).There are however no records of its presence among buffaloes in Sri Lanka although in cattle it is considered an important organism (Jorgenson *et al.*, 1992).

In stained smears, *A.marginale* appear as dots on or close to the periphery of erythrocytes. Each dot is in effect an aggregation of 4 to 8 subunits. *Anaplasma* are transmitted mainly by ticks of the genera *Boophilus* and *Rhipicephalus* and by biting flies such as *Stomoxys* and *Tabanus*. This disease is also transmitted by contaminated needles. Prenatal transmission has also been recorded in cattle. The organisms multiply in the erythrocytes by binary fission. In infected animals the temperature rises to about 41^{0}C. Clinical signs seen include inappetance, depression, weakness, anaemia, and muscular tremors.

The common post mortem lesions seen are enlarged liver and spleen. The gall bladder too may be enlarged and contain thick granular bile. The carcass would be pale and jaundiced with watery blood. The disease could be diagnosed on clinical signs and by the demonstration of the organisms in Giemsa stained thin blood smears. Serological techniques available include CFT, IFAT, EIA and capillary tube agglutination tests.

The disease is controlled by treatment of affected animals, elimination of vectors and by vaccination of susceptible animals. Vaccines containing attenuated strains of *A.marginale* and *A.centrale* are used in control programmes against cattle in most countries.

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NA-218-27

1.3 Ectoparasites

D.J.Weilgama

Ectoparasites are organisms which live either temporarily or permanently on the outside of the body of an animal or man. Some spend the entire life cycle on the host (eg. lice) while in others only a stage of the life cycle is parasitic (e.g. fleas). Those that are forced to remain on a host during their entire life cycle are termed obligatory parasites while those that are able to exist as free-living organisms and as parasites are termed facultative parasites. Most ectoparasites are arthropods which are closely associated with the hosts. It is estimated that nearly three fourths of earth's living organisms are made up of arthropods. These are classified under the Phylum Arthropoda which is further divided into a number of Classes. Over a million species are found in this phylum. Arthropods of only two classes are of veterinary importance. A simplified classification of the phylum giving only those arthropods of veterinary significance is shown in Figs.1.3.1 and 1.3.2.

The ectoparasites, as members of the phylum Arthropoda, possess characters such as a segmented body with paired segmented appendages. The body could be divided into a head, thorax and an abdomen. In some, the head and thorax are fused to form a cephalothorax. The life cycle consists usually of an egg stage, one or many larval stages,one or many nymphal stages and an adult stage. Some, however, lay larvae in place of eggs. In the development of these arthropods the young may undergo morphological changes to varying degrees before reaching adult stage. In some insects like lice these changes are less marked and the young resemble the adult closely except in size. This is known as hemimetabolous development. In some others (e.g flies), the young are markedly different from their adults in size and form and is said to undergo holometabolous development. Most ectoparasites live on the body surface of the host whilst few like the mites may burrow into the skin. They feed on blood, lymph or tissue fluids and tissue debris. They cause harm to the hosts in many ways. Heavy infestations *per se* cause annoyance, blood loss, damage to hides and skins, loss in weight and loss in production. But the greatest danger comes in the form of these acting as vectors or as intermediate hosts of disease causing pathogens. The transmission of such pathogens may be cyclical or mechanical. In the latter, the ectoparasites may accidently transfer the pathogens from excreta or filth due to their feeding habits and the pathogens do not undergo any changes in the vector. In cyclical transmission the pathogen undergoes changes in form and also multiply in the vector The transmission to the host in most situations is through the bite or *via* faeces.

Control of ectoparasites has always been a formidable one. The financial losses to the animal industry due to ectoparasites become substantial when expenses for the control of these are added. Many methods had been in use for control of ectoparasites. These include the use of chemicals, manipulation of the environment (mico-environment) of the parsites, use of tolerant breeds of animals (genetic manipulation), genetic manipuation of the vector and the use of biological control methods. The use of any one of these methods or a combination though simple in the early years has now become complicated and in most situations require the services of a specialist in the discipline. The reasons for such a situation is the development of novel chemicals, means and methods of application, environmental changes, increasing knowledge of vector bionomics and the cost of operations.

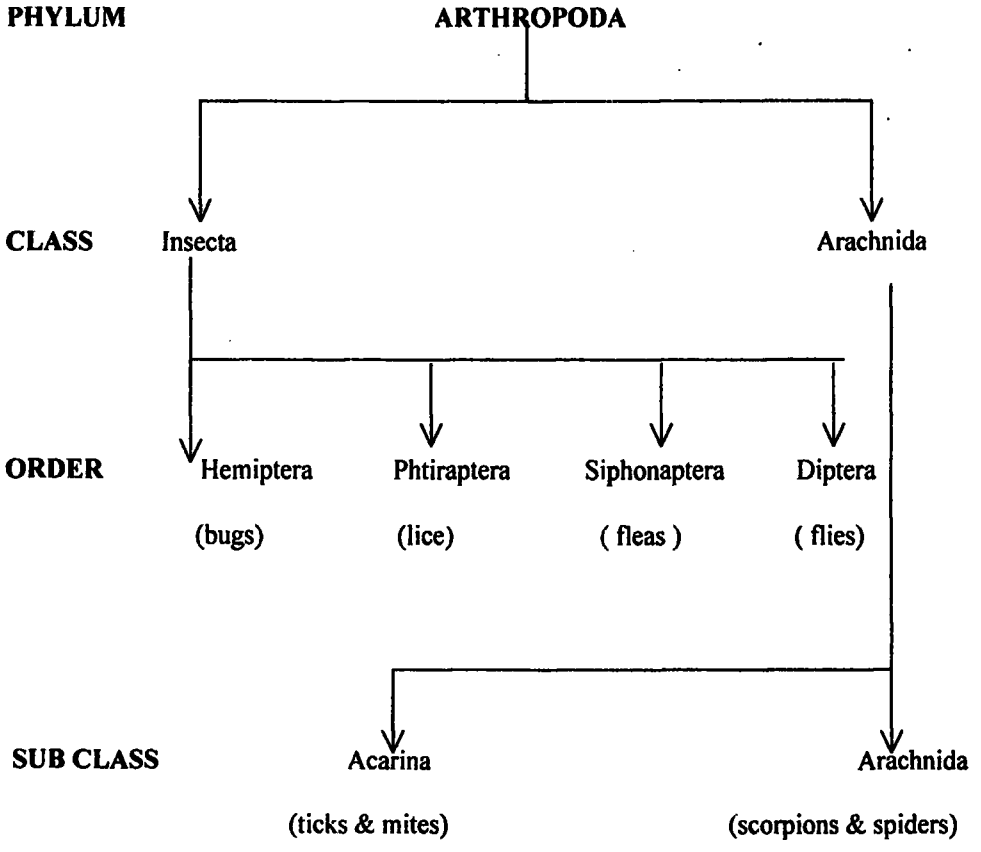


Fig. 1.3.1 Classification of Arthropoda

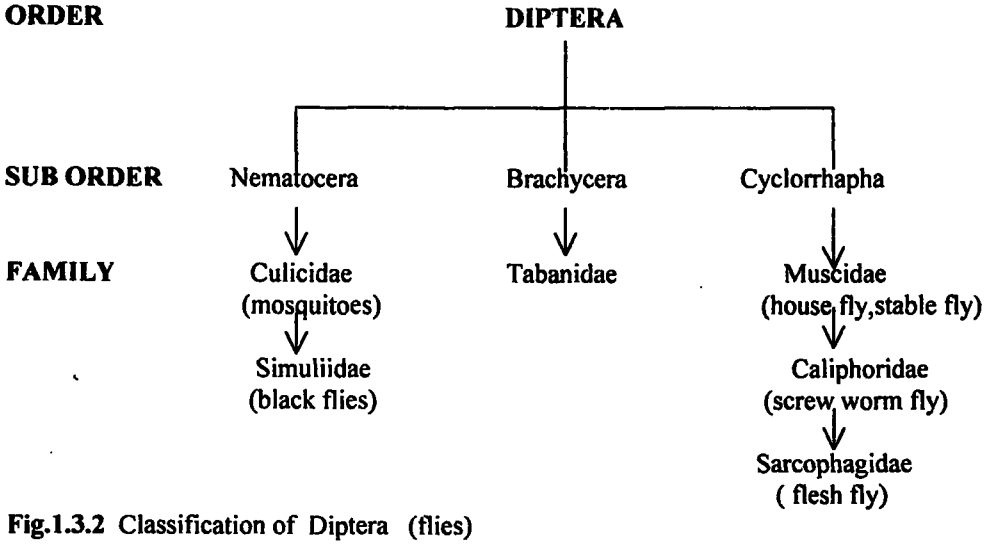


Fig.1.3.2 Classification of Diptera (flies)

The extent of damage to buffaloes due to ectoparasites is relatively of a low magnitude in comparison to other livestock. The common ectoparasites encountered in buffaloes in the tropics and sub-tropics are lice, ticks, mites and some flies of muscidae, sarcophagidae and calliphoridae. Although prevalence rates of ectoparasites in buffaloes are at times equal to that in cattle the intensity of infestations with most is quite low. This section deals with some common ectoparasites that infest buffaloes in the tropics specially in Sri Lanka and other Asian countries.

Lice

These are relatively small, wingless insects with dorsoventrally flattened bodies which belong to the order *Pthiraptera*. Lice are generally host specific and are permanently parasitic. Most species are unable to survive off the host for more than a few days. Lice belong to two types; the sucking lice (Sub order - Anoplura) and the biting lice (Sub order - Mallophaga). Sucking lice occur only on mammals while biting lice are found on both birds and mammals.

Morphologically, the body of louse could be divided into a head, thorax and an abdomen. The head contains two short antennae and mouth parts that are adapted either for biting or sucking. Most species have no eyes while primitive eyes are present in some. The head of biting louse (Fig. 1. 3.3) is relatively much larger than that of the sucking louse, is as wide as the thorax and is rounded anteriorly. The head of sucking louse (Fig. 1.3.4), on the contrary, is much narrower than the thorax and is more or less pointed anteriorly. The thorax is small with three segments fused together. There are three pairs of legs and these terminate in claws. The species parasitic on birds have two claws and those on mammals only one claw. The third pair of legs is usually the largest with strong claws.

The biting lice feed on epithelial debris of skin of host and even on feathers of birds whilst some species could also suck blood off their hosts. The sucking lice feed on blood and tissue fluid of the host.

Life cycle

Both sucking and biting lice have similar life cycles. The female lays whitish, operculate eggs ('nits') that remain glued to the hair or feathers. Each female on an average would lay about 200 – 300 eggs during her life span which is about a month. The eggs hatch into nymphs which though smaller, resemble the adults. There are three nymphal stages before they become adults. The whole life cycle from egg to adult would take about 2 – 3 weeks.

Louse infestation in buffaloes

Louse infestations in buffaloes have been reported from many countries. The following sucking lice have been recorded from buffaloes *viz: Haematopinus eurysternus, H. bufali* and *H. tuberculatus* and *Linognathus vituli*. In cattle, in comparison, sucking lice belonging to the three genera *Haematopinus, Linognathus* and *Solenoptes* have been reported.The lice of the genus *Haematopinus* show preferential sites on the host and *H.eurysternus* occur on the poll, base of horns, ears and around the eyes. The biting lice reported from buffaloes belong to the genus *Damalinia*. The human pubic louse *Pthirus pubis* has also been reported from buffaloes (Joseph *et al.*, 1986; Alwar and Raja, 1972). Calves harbour more lice than adults. Although there is no seasonality in infestations, in warm countries, heavy infestations have been noted in winter in buffaloes in countries with marked seasons. Damalinia could

also easily build up its numbers in winter because of its parthenogenetic facility. Lice are common on buffaloes in Sri Lanka but little or no work has been conducted on these.

Effects of Louse infestation

Lice are normal inhabitants of cattle and buffaloes and light infestations do not usually cause harm. Heavy infestations with biting lice, however, cause pruritus associated with heavy licking and rubbing with resultant damage to hide. Alopecia is common. Sucking lice cause anaemia and weakness in heavy infestations. Lau et al., (1980) reported gangrene in severely affected animals which at times lead to death. In buffaloes, heavy infestations have been noted in winter.

Control

A number of chemical compounds (insecticides) are effective against lice. These include the organochlorines, organophosphates, carbamates and synthetic pyrethroids. With organophosphates a second treatment is usually recommended one week later, to kill the newly emergent lice. Many workers have reported that wallowing helps to control louse infestation in buffaloes (Lau *et al.*, 1980, Cameons, 1976). It is claimed that when the mud dries up after wallowing, the lice too fall off along with the mud.

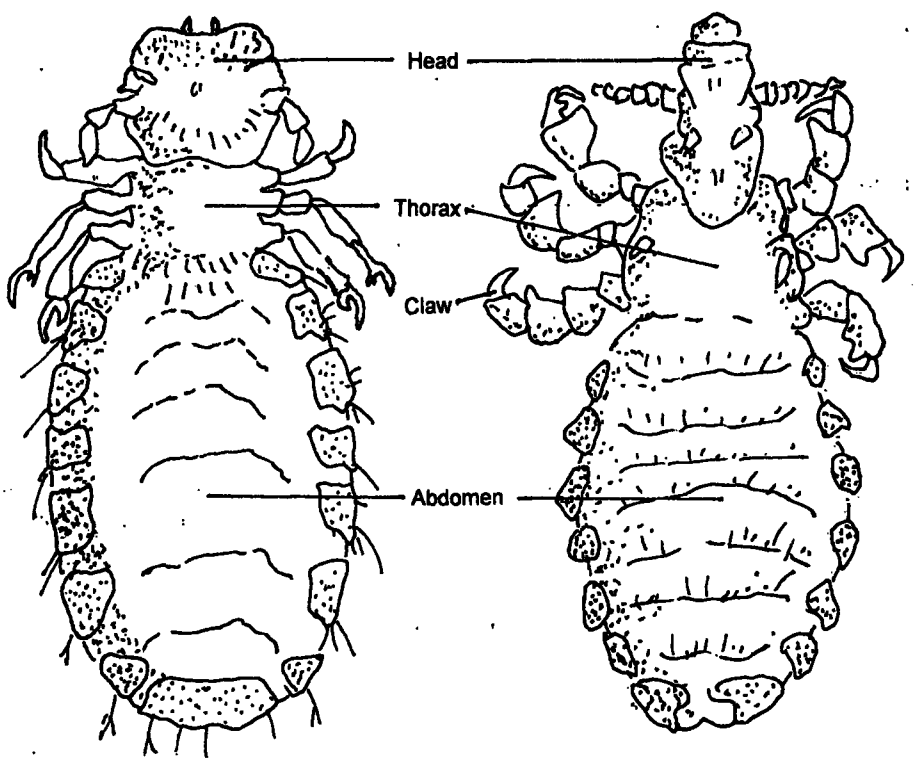


Fig. 1.3.3 Biting Louse

Fig. 1.3.4 Sucking Louse

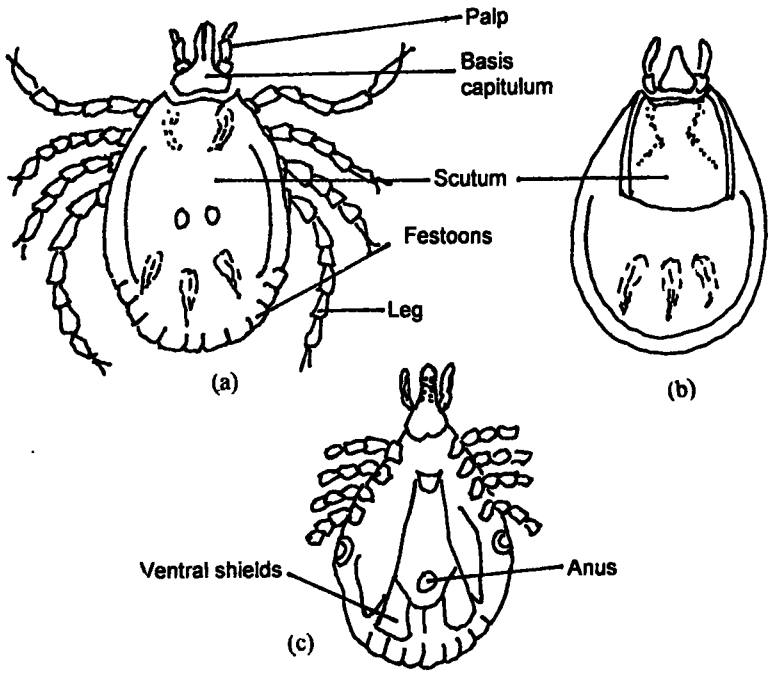
Ticks

Ticks parasitise all groups of animals except fish. They belong to the Order Acarina, Class Arachnida in the Phylum Arthropoda. Three families of ticks are recognized of which two are important namely, Ixodidae and Argasidae. There are about 800 species of ticks of which about 650 belong to Ixodidae or ' hard ticks' and about 150 to Argasidae or ' soft ticks'.

Hard ticks contain a chitinous scutum, which covers the entire dorsal surface in the male and only the anterior portion in the female, nymph and larva. Soft ticks lack a scutum and in addition the mouth-parts are situated on the ventral side in adults and nymphs. Hard ticks are common on animals including buffaloes whilst the soft ticks are frequently associated with birds. Ixodidae (hard ticks) consists of about 12 genera of medical and veterinary importance and are widespread throughout the world.

Morphology

The tick is composed of a 'head' and an unsegmented body. The head or *capitulum* consists of a *basis capitulum* to which is attached the palpi and the mouth-parts. The *basis capitulum* may be of different shapes such as being rectangular, hexagonal or sub-triangular, depending on species. The palpi, which are sensory structures, may appear as long or short; stout or slender. Each palp consists of four segments or articles. Mouth- parts are made up of a hypostome, which bears rows of teeth and a pair of chelicerae that are sheathed (Fig. 1.3.5).



(a) Male, dorsal (b) Male, ventral (c) Female, dorsal

Fig. 1.3.5 Morphology of hard tick

The body of the tick is flat when unfed and is somewhat oval in shape. Hairs are present which are either long or short. Adults of some species are brightly coloured (ornate) while in others they are inornate. The posterior border of the body in many species is divided into rectangular areas or festoons. The ventral side of the body contains the legs, genital aperture, anus, spiracular plates and in the male the ventral shields. There are three pairs of legs in larvae and four in adults and nymphs. In the first pair of legs there is a sense organ, Haller's organ, which carry sensillae.

Life cycle of hard tick

There are four stages in the life cycle; the egg, larva, nymph and adult. Except the egg stage all other stages are parasitic and feed uninterruptedly until fully engorged. The male in some species, however, is an intermittent feeder whilst in others it may not feed at all. The female tick when fully fed drop to the ground to lay eggs after which she dies. The number of eggs laid by a single female may vary from about 2000 to about 18000 according to the species. The eggs hatch, after a period of days, depending on temperature and humidity, into larvae, which then commence to feed. From this point onwards the life cycle differs depending on the species. They could thus be classified into one-host, two-host or three-host ticks.

One-Host tick: These make use of only one host to complete its life cycle (eg. *Boophilus microplus*). The larvae moult into nymphs which later feed and moult into adults on the same host. These leave the host only as fully engorged females. The males continue to be on the host till death.

Two-Host tick: Here two hosts are involved in the completion of the life cycle. The larva after feeding on the host moult into a nymph, on the host itself, which later drops to the ground when fed. This then moults into an adult, which attaches to another host to feed.

Three-Host tick: In these the larva which attaches to a host drop to ground when fed to moult into the nymphal stage. The nymph then attaches to the second host to feed and drop to moult into an adult. The adult attaches to the third host from which it finally drops when fully engorged.

Effects of ticks

Ticks produce many pathogenic effects in animals on which they feed on. They cause irritation and damage to skin due to bite wounds and also predispose the animals to attack by flies (myiasis). Heavy infestations lead to anaemia specially in the young due to blood loss. Many tick species also transmit pathogenic protozoa, viruses, spirochaetes and bacteria. Most of these diseases cause high morbidity and mortality. Some ticks are also capable of causing paralysis in animals and man through a toxin injected in the saliva. Tick toxicosis, a condition of different entity, is also brought about by some ticks, especially in ruminants and pigs.

Tick infestation in buffaloes

Ticks have been reported in buffaloes from most tropical and sub tropical countries in the world. In India ticks of 5 genera (and 18 species) namely *Haemaphysalis, Hyalomma, Amblyomma, Boophilus* and *Nosomma* have been recorded (Miranpuri,1988). Studies conducted in India have shown that the tick load in buffaloes could be equal to that in cattle in some areas (Rajagopalan and Sreenivasan,1988). In Sri Lanka ticks belonging to 5 genera and 7 species namely, *Boophilus annulatus (sensu lato), Amblyomma integrum,*

A.testudinarium, A.clypleolatum, Hyaloma marginatum isaaci, Hy. brevipunctata, Rhipicephalus sanguineous, R.haemaphysaloides and Nosomma monstrosum. are reported (Seneviratna, 1965; Weilgama *et al,* 1989).

Ticks are not considered as major ectoparasites in buffaloes in Sri Lanka. Heavy infestations, however, due to *B.annulatus (sensu lato)* are at times encountered in buffalo calves reared in large farms. Infestations in adults are minimal, but in the dry zone it is not uncommon to observe buffaloes with necrotic tails due to tick bites. Majority that cling to tail and tail base are of the genera Hyalomma and Amblyomma. *H.bispinosa* was found to transmit *Theileria orientalis* to buffaloes in Sri Lanka (Weilgama *et al.*, 1989).

Hy. Marginatum isaaci and *R. haemaphysaloides*, two other species common on buffaloes are not involved in transmission. Liu *et al.*, (1987) reported *R.haemaphysaloides haemaphysaloides* as a transmitter of babesiosis to buffaloes in China. Another haemprotozoan disease transmitted by ticks to buffaloes is anaplasmosis and has been reported in India.

Control

Control of ticks on buffaloes are mainly by the use of acaricides (chemicals). Most compounds available in the market are efficacious and control of most species could be attained by following the manufacturer's recommendation. Wallowing too helps in keeping the tick burden to a minimum. The methods used with control of ticks in cattle such as rotation of pasture, burning of pasture etc. are not practised with buffaloes.

Flies

Flies of the genera *Musca, Stomoxys* and *Haematobia* in the family *Muscidae* are known to worry buffaloes as with other animals. Except *Musca* the other two are blood sucking flies.The genus *Haematobia*, however, is the more important and occur in many countries and is a serious nuisance in buffaloes.

Haematobia

Three species are known namely; *Haematobia* (syn. *Liperosia*) *irritans* (horn fly), *H.exigua* (buffalo fly) and *Haematobia.* (syn. *Haematobosca*) *stimulans*. The adult fly may reach about 4 mm. in length and are grey in colour with several dark stripes on the thorax. At rest the wings overlap to about 3/4ths of their width. The flies suck blood and usually remain on the host for several days. They feed at intervals and move to lay eggs when the animal defaecates. They are seen in large numbers on the underside of the abdomen and around the shoulder areas. When disturbed swarms of these flies could be seen to leave the host only to settle back again quickly.

Life cycle: The females lay eggs in fresh dung. These hatch into larvae in about 4-5 days under suitable conditions of temperature (28^0 C) and humidity (80%). Most eggs dry at low temperatures and at low humidity. The larvae burrow into the dung and feed on it and become pupae.The pupae are found under the dung heaps or in the surrounding soil. Adults are formed in 6 to 8 days under suitable conditions of temperature and humidity. Adult flies immediately seek a host, usually buffalo or cattle, and continue the cycle.

Effects on hosts: Haematobia cause severe irritation in affected hosts. The animals may rub irritated parts which result in wounds that later become septic. Heavy infestations interfere with feeding and cause loss of condition and production. Some species of Haematobia are known to transmit Stephanofilaria, a skin filaroid to cattle.

Infestations in buffaloes: Haematobia spp. have been reported from many countries in Asia, Europe ,United States and also Australia. They are found in most tropical and sub tropical countries and in Australia even survives severe winters. Preliminary studies show that only *H.exigua* is present in Sri Lanka. Heavy infestations in buffaloes have been observed in the dry zone. It is absent in mid and hill country regions of the country (Dr. A. Naguleswaran, personal communication).

Control: Infestations in animals are controlled mainly by the use of insecticides. Organophosphates and synthetic pyrethroids are commonly used and good control has been achieved. Cyhalothrin as a spray has given good protection for nearly a month against the buffalo fly (Stubbs *et al.*, 1982). In Australia, a back-rubber was used effectively to control *Haematobia* sp. This method consisted of a rubber charged with an insecticide which was suspended between two posts allowing the animals to rub themselves (Waters,1978). Biological control has also been attempted with *Haematobia*. In Australia dung beetles have been shown to cause mortality of immature *Haematobia* and also stunting of adults. The use of correct fauna is of value in such operations otherwise the desired results cannot be obtained.

Other flies

Stomoxys

The flies of this genus are members of the family Muscidae. *Stomoxys calcitrans* or the (biting) stable fly is the commonest species encountered. It resembles the house fly being similar in size and colour (grey) with four stripes on the thorax. The proboscis of the fly, however, is conspicuous and project forwards. Both male and female flies feed on blood. They inflict a painful bite and are a source of annoyance to animals and would cause loss in production when heavy.

The female flies lays on decaying vegetable matter such as hay and straw contaminated with urine. The life cycle from egg to adult may take about 12 to 60 days depending on temperature.

Chrysomyia

Flies of this genus are important parasites in the warm countries. *Chrysomyia bezziana,* the common fly, is termed the 'old world screw worm' fly. These are bluish green flies with stripes in the thorax and orange brown eyes. They are swift fliers and are known to fly up to 50 km. The fly is attracted to wounds in animals and man. The eggs are laid on the edges of wounds which hatch into larvae that feed on tissues creating a large foul-smelling lesion. This condition called myiasis could even cause death of the host if left untreated. There are three larval stages and one pupal stage in the life cycle of the fly. Pupae are formed outside the body which then moult into adults.

Mites

These acarines too are important ectoparasites of animals. These however, are not comparatively significant parasites of buffaloes. The species frequently encountered are *Sarcoptes scabiei* and Psoroptes spp. The former is a minute parasite about 0.2 – 0.5 mm long and are circular in outline. They burrow into the skin and spend their entire life cycle on the host. Transmission is by close contact with other animals. Hence in overcrowded stalls the infection spreads rapidly. The parasites cause marked irritation which causes intense itching and scratching. Alopecia is a common sign on affeted animals. Sarcoptes mites are host specific. However, Sarcoptes of buffaloes have been reported to have infected humans in certain parts of India.

The Psoroptes mites reported from India are *Psoroptes natalensis* and *Psoroptes communis ovis*. These are non-burrowing mites measuring about 0.75 mm and causes direct damage to the skin.

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Chapter 2

BACTERIAL DISEASES

2.1 Haemorrhagic Septicaemia

T.G. Wijewardana

Haemorrhagic septicaemia is an acute septicaemic disease of cattle and buffaloes caused by specific serotypes of *Pasteurella multocida*. It is a primary pasteurellosis which is reproducible with pure cultures of the causative organism. It is endemic in many countries of Asia and Africa. In Asian countries where the staple diet is rice, it is economically significant, as buffaloes and cattle are a source of draught power in rice fields. The enormity of the problem could be gauged by the fact that Asian and the Pacific region (except Australia, New Zealand and Japan) is estimated to hold approximately 30 % of cattle and 95 % of the buffalo population in the world.

Aetiology

Pasteurella multocida serotypes B:2 and E:2 have been found to cause haemorrhagic septicaemia (HS). In Asia only serotype B:2 has been associated with the disease, while in Africa E:2 has been the only serotype recorded. A few countries such as Egypt and Sudan have recorded both serotypes (Farid *et al.*, 1980, Shigidi and Mustafa, 1979). Few sporadic outbreaks in the United States of America has been ascribed to B:2, but HS is not considered to be an endemic disease (Rimler and Wilson, 1994).

The organism *P. multocida* is a Gram negative, non - motile rod or coccobacillus with sizes ranging from 0.3-1.25 μm in length (Carter, 1984a) and they display a characteristic bipolar staining with either Leishman or methylene blue stains (Carter, 1984b). The organisms are facultatively anaerobic and non-sporing. They do not grow on MacConkey's agar and they are non-haemolytic on blood agar (Carter, 1984a). On nutrient agar they produce fine, translucent colonies with a characteristic musty odour. *P.multocida* organisms are oxidase positive (Carter, 1984b), catalase positive (Cowan, 1974) and indole positive (Namioka, 1978). They ferment sugars such as glucose, sucrose, sorbitol and mannitol with acid, but no gas production. Lactose, maltose and salicin are not fermented, while variable fermentation results are obtained with arabinose, trehalose and xylose (Cowan, 1974). Gelatin is not liquefied (Carter, 1984a).

Clinical Signs

Inhalation and ingestion are the accepted modes of transmission. The typical clinical syndrome and pathological lesions have been produced by experimental infection through these routes. Clinical signs appear after an incubation period which ranges from 1-2 days, being somewhat shorter in experimental infection as compared with natural exposure. The apparent clinical course also ranges from a few hours to 3 - 4 days. Broadly the clinical syndrome consists of three phases, firstly a phase of inappetance and temperature elevation next a phase of respiratory distress and finally recumbency and death. Sub-mandibular oedema which may extend to the brisket region and in some instances down the forelimbs

may occur at any stage. It is possible in the field cases, the first phase escapes undetected. Occasionally, a protracted pneumonic form has been reported (De Alwis *et al.*, 1975), whilst in most field cases the disease is peracute and is frequently associated with sudden death. Syndromes described in the literature as septicaemic form, respiratory form, cutaneous, pectoral oedematous forms are presumably based on which of the clinical signs are dominant.

Bacteriological findings

Septicaemia in HS is essentially terminal and is detected only on blood cultures made a few hours prior to death. It is possible that initial multiplication occurs elsewhere prior to rapid invasion into the blood stream. At death viable bacterial counts in blood are in the region of 10^5-10^6 cfu per ml. Rapid proliferation occurs after death, and counts of 10^{11}- 10^{12} cfu per ml have been observed around 20h after death (De Alwis, unpublished data). The appearance of the organism in nasal secretion is inconsistent and the chances of isolation increases with progress of the disease (De Alwis, unpublished data; Hordagoda *et al.*, 1991). The only significant haematological change observed during the course of the disease was a progressive leucytopenia (Hordagoda, 1991).

Pathology

At necropsy the gross findings are usually limited to generalised petechial haemorrhages, particularly under the serosae, and oedema of the lymph nodes. Subcutaneous infiltration of gelatinous fluid specially in the submandibular, throat, pharyngeal and brisket region may be present and in some animals there are lesions of early pneumonia and a haemorrhagic gastroenteritis (Blood *et al.*, 1983). The spleen may show a few haemorrhages but is not swollen as in the case of anthrax (Belschner, 1967). An experimental study in Sri Lanka showed that animals dying within 24-36h of infection had only widespread petechial haemorrhages, particularly on the base of the heart, abdominal wall and to a lesser extent on the intestines, while lungs showed only congestion. When the illness lasted over 72h, there was extensive consolidation of the lungs, with lobulation due to marked thickening of the interlobular septa. Pleurisy and pericarditis were the other necropsy lesions observed when the disease ran through a longer course (De Alwis *et al.*, 1975).

Epidemiology

Buffaloes are more susceptible to the disease, when compared to cattle. The incidence is directly related to animal husbandry practices, the incidence being higher in large nomadic herds compared to small intensively- reared herds (De Alwis and Vipulasiri 1980). The animals aged between six months to two years have been observed to be the most susceptible group (De Alwis *et al.*, 1976). Generally the case fatality rate is 100 % (De Alwis, 1981, De Alwis and Vipulasiri, 1980)

In endemic areas the outbreaks are seasonal and the link between successive outbreaks is believed to be the carrier animal. The presence of the organism in the nasopharynx of healthy animals is well established (De Alwis *et al.*, 1986; Hiramune and De Alwis, 1982). The nasopharyngeal carrier state is followed by an immune state which persists for a long period (De Alwis *et al.*, 1986). The HS causing organism has been readily isolated from

such sites as tonsils and the lymph nodes associated with the head and neck (De Alwis *et al.*, 1990). The intermittent recovery of the organism from the nasopharynx is suggestive of the carrier animal appearing in two forms ie; a "latent" form when the organism persisted in the tonsils only and an "active" form when the organism presumably multiplied and was shed in the nasal secretions. It has been further observed that treatment with antibiotics does not affect carriage in the tonsils (De Alwis *et al.*, 1990). Experimentally-induced carriers have been found to harbour the organism in the crypts of the tonsils (Horadagoda and Belak 1990). This may be the reason why the organisms in the carrier escape the body's immune mechanism and are also unaffected by antibiotics.

The factors that lead to latent carriers becoming active carriers are not very clear. In the endemic areas outbreaks usually coincide with onset of rains, which is normally preceded by a long dry period with limited availability of forage. These adverse climatic factors and accompanying nutritional limitations which cause stress may well precipitate the activation of the carrier to become a source of infection to susceptible animals.

Diagnosis

Clinical diagnosis

A clinical field diagnosis is based on the history, clinical signs and necropsy findings. The history includes the pattern of mortality in relation to the surrounding circumstances, such as the previous occurrence of HS in the herd, whether it is an enzootic area, the age group and species affected, and whether the animals had been vaccinated against HS.

Laboratory diagnosis

As the septicaemic phase in HS is essentially terminal, blood samples taken from sick animals before death usually do not contain the organisms. The nasal secretions also do not show the organism consistently. The only significant haematological change that had been observed during the course of the disease was a progressive leucocytopenia (Horadagoda *et al.*, 1991). Therefore at postmortem examination, heart blood or, if it is an 'old' carcass, as usually happens under field conditions, a long bone (e.g. femur or humerus) is the preferred specimen. These should be transported to the laboratory on ice and well packed to prevent leakage. Blood samples, or swabs eluted into 2-3 ml sterile normal saline, or marrow extracted from the long bone, are cultured. A suitable medium for the growth of the organism is casein-sucrose-yeast (CSY) agar containing 5% blood. Alternatively, the specimen in normal saline is inoculated into mice (0.2ml) subcutaneously or intramuscularly. The mouse acts as a screen and pure cultures of the organism can be obtained in the heart blood of the mouse. This is preferred over the direct culture as the specimens would invariably be over grown with postmortem invaders and contaminants thus obscuring *P.muldocida*.

The organism is identified by morphological and cultural characteristics, biochemical reactions and serological tests. Biochemically, strains of *P.multocida* causing HS are no different from those producing various other diseases, and they can be differentiated only by serology. A rapid slide agglutination test (Namioka and Murata, 1961a) requiring a single colony is used initially. This is followed by an indirect haemagglutination test for capsular typing (Carter, 1955) and an agglutination test with hydrochloric acid- treated cells for somatic typing (Namioka and Murata, 1961b). An agar gel immunodiffusion test is also

available for both capsular and somatic typing (Wijewardana *et al.*, 1982; Heddleston *et al.*, 1972).

Treatment

HS under field conditions is always fatal with the majority of affected animals dying without showing any previous signs. Therefore treatment is of little value in the field, especially as the sudden onset and rapid course of the disease makes early detection difficult. Treatment is effective only in the very early stages. The only practical procedure is to check the rectal temperature at least twice daily of all in-contact animals following the detection of the first case and, to commence antibiotic therapy immediately (De Alwis, 1984). The existing field practice of using sulphonamides in the treatment of clinically affected animals is discouraged in the light of the finding of a high proportion of isolates (15/27) being resistant to sulphamethoxazole (Abeynayake *et al.*, 1993). Preparations of this drug in which the route of administering is intramuscular are preferred over those that have to be administered intravenously, as the latter route is difficult in animals having HS. Streptomycin resistance has been shown by a strain originated from Thailand (De Alwis 1984; Abeynayake *et al.*, 1993). Of the drugs recommended to be used in cattle and buffalo, *P.multocida* is highly sensitive to penicillin, ampicillin, cephalothin, enrofloxacin and oxytetracycline (Abeynayake *et al.*, 1993). Treatment with hyperimmune serum is of no practical value.

Prevention and Control

Vaccination is the accepted method of control for HS. Currently two vaccines are in use. The alum precipitated vaccine is used in the face of outbreaks and had been found to induce an immunity of 4-6 months duration. The oil adjuvant vaccine (OAV) which is known to confer immunity up to one year is the choice in prophylactic programmes. The recommendations are to immunise calves over 4 months of age using the OAV, a booster given 3 months later and repeat vaccinations annually (De Alwis *et al.*, 1978).

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NA-218-43

2.2 Black Quarter

T.G Wijewardana

Introduction

Black quarter (BQ) is an acute, infectious disease characterized by inflammation of muscles, severe toxaemia and a high mortality (Blood *et al.*, 1989). Cattle and buffaloes are mainly affected (Jha *et al.*, 1991), however other animals are prone to infection initiated through trauma (Blood *et al.*, 1989). It is also described as quarter ill, symptomatic anthrax, black leg and emphysematous gangrene (Merchant and Basher 1964). BQ is distributed worldwide and especially found in low-lying and swampy grounds (Merchant and Basher 1964). The disease was first identified and differentiated from anthrax by Bollinger in 1875 as cited by Carter and Chengappa (1991).

Aetiology

BQ is caused by the bacterium *Clostridium chauvoei* (Iyer, 1952, Nilakanthan and Dhanda, 1964). It is a Gram positive, anaerobic, spore forming rod (Carter, 1973).

Epidemiology and Pathogenesis

Black Quarter has a world wide distribution with reports of occurrence in India (Iyer, 1952), Egypt (Gadalla *et al.*, 1974), Sudan (Bagadi, 1974), Canada (Barnes *et al.*, 1975), the United States of America (Brown *et al.*, 1976), Nigeria (Bagadi, 1978), Nepal (Jha *et al.*, 1991) and Sri Lanka (Gamage and Wijewardana, 1995). In Sri Lanka, it has been found that it is confined to certain regions of the island, namely the North, East, North Central, North Western, Uva and Central (Anon, 1994). An epidemiological survey of BQ among cattle and buffaloes in Sri Lanka revealed that cattle and buffaloes are equally susceptible (Gamage and Wijewardana, 1995). It was further observed that the peak mortality occurred during the driest period of the year; conversely in Nigeria, the highest incidence have been found to occur during the rainy season (Bagadi, 1978).

Generally BQ is regarded as an infection of the young, but at times it could also affect older animals. In Sri Lanka, the local and Indian breeds of cattle were found to be more susceptible to the disease when they are between 6 months to 2 years of age. In European breeds, the susceptible age extends from 6 months to 4 years (Gamage and Wijewardana 1995). Apart from cattle, the condition had been observed among buffaloes, sheep and goats (Anon, 1992). It is reported to occur occasionally among deer, horses and swine (Merchant and Basher, 1964).

The organism exists in the soil (Gillespie and Timoney, 1981, Wijewantha, 1961). The portal of entry has been reported to be by ingestion in cattle and through external wounds in sheep (Carter and Chengappa, 1991). Bagadi (1974) has forwarded the theory that the natural habitat of the organism is the intestines of the animals and that the surroundings get contaminated via faecal matter, leading to a build up of the bacterium in the soil under favourable conditions, such as in soils with high organic matter, resulting in the animals being consistently exposed to the organism. From the intestines the organisms enter the

lymphatics and the blood circulation and thence to the muscle tissues and the liver (Gillespie and Timoney, 1981). They have further stated that a stimulus such as muscle damage is required for the clinical manifestation of the infection. Selman (1981) suggested that in the pathogenesis of BQ the metabolic activities of various muscle groups play a vital role.

Clinical Signs

The commonest muscle groups to be affected are femoral, lumbar, gluteal and those in the head and the neck regions. These would be swollen with resultant lameness of the limb. The skin over the area would become bluish black and upon 0palpation crepitations could be felt. The course of the infection is 12 to 24 hours ending usually in death. In recently calved animals, at times, the vulva, vagina and perineal areas may also be affected (Merchant and Basher, 1964; Selman, 1981).

Diagnosis

Usually BQ is diagnosed on the basis of history, clinical signs and postmortem lesions (Merchant and Basher, 1964). A confirmation of the diagnosis could be made only by laboratory examination. A sample of muscle tissue has to be collected aseptically and submitted under refrigeration. An impression smear over the affected tissue is also useful. Samples from old carcasses may complicate diagnosis as postmortem invaders could mask the presence of *Clostridium chauvoei.* In the laboratory, stained smears are examined for the presence of the organisms. Guinea pigs are inoculated with 1 ml of a 10 % suspension of the muscle tissue in 2.5 % Calcium chloride by the intra muscular route. If the animal is found dead the following day, an impression smear is stained with Grams' stain and examined for the presence of the bacterium. Heart blood of the dead guinea pig is subjected to anaerobic and aerobic culture procedures for 48 hours at 37^0C and, finally the organisms in the anaerobic culture system are identified using cultural, morphological and biochemical characteristics (Carter, 1973).

Treatment

Penicillin at the rate of 17,000 IU/kg for sheep and 13.2 to 17.6 x 10^5 IU/kg body weight for bovines had been recommended. This dose is reported to effect a complete cure even after the manifestations of clinical lesions of BQ (Nilakantan, 1965).

Prophylaxis

As stated earlier, in an endemic area the soil is heavily contaminated with the organisms. Drainage and cultivation of the land or burning of pasture after allowing it to grow to a height has been recommended as practices to free the soil from the bacterium (Seiden, 1961). These practices however have to be carried out repeatedly over several years to rid the soil of the bacterium. In the event of an outbreak, further spread of the infection could be minimised by deep burial of carcasses and removal of animals at risk to another location (Selman, 1981). Due to the practical difficulties in eliminating the presence of the organisms in the soil, annual vaccination is undertaken to prevent new outbreaks, while old cases would gradually phase out (Gamage, 1997).

According to Gadalla and Farrag (1967) first attempts at vaccination were made in 1887 and 1888 in France. One of the earliest was a pellet vaccine incorporating heat inactivated organisms in the muscle tissue and implanting under the skin (Roberts, 1946). Inactivation of the organisms by the use of formalin was also adopted and, was reported to be superior to the former method in terms of simplicity of the process and high virulence of the organisms maintained by continuous passage in cattle (Piercy, 1951). Presently an adjuvant is incorporated to enhance the degree of immunity.

In Sri Lanka a vaccine is produced against BQ using a local isolate of *Clostridium chauvoei* (Gamage, 1997). This could be stored at 4^0 C up to 12 months. It is recommended that animals within the age group of 4 months to 2 years be vaccinated with a dose of 3 ml by the intramuscular route. The period of duration of immunity is 9 months.

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NA-217-47

2.3 Mastitis

I.D Silva

The buffalo has been described as "Asia's beast of burden and the key to progress". In some Asian countries buffaloes have made a very substantial contribution to the total food supply as milk producers (Guzman and Allo, 1975). The buffalo is gaining popularity as a dairy animal in Asia and the Middle East, because of their resistance to udder infections compared to cattle (Wanasinghe, 1985). In spite of their relatively high resistance to mastitis, udder infections in buffaloes can present a serious problem in the large herds due to poor hygiene (Uppal, *et al.*, 1994).

Inflammation of the mammary gland is designated "Mastitis". The term is derived from the greek word *mastos* meaning mammary, and the suffix *itis* meaning inflammation of (Schalm, *et al.*, 1971). Mastitis is a complex disease because of the numerous causative agents and the varied management and environmental practices that influence the severity of the disease.

Incidence of mastitis in buffaloes

Mastitis is a major cause of economic loss in the dairy industry. The economic loss due to mastitis in India is estimated to be several thousand millions of rupees, and the loss is approximately twice as high when mastitis occurs at sub clinical level (Singh and Singh, 1994). A daily loss of 178 kg of milk (average yield of 2.66 kg per day) in buffaloes due to mastitis had been speculated by Indian scientists (Singh, *et al.*, 1982). The overall total economic loss due to mastitis has been recorded as 16,072 million rupees in India (Singh and Singh, 1994). Although the buffalo is a popular dairy animal in Asia, little information is available, on mastitis in buffaloes. The incidence of mastitis is lesser in buffaloes than in cattle (Singh and Singh, 1994; Saini, *et al.*, 1994; Uppal *et al*, 1994; Shukla and Supekar, 1987; Rasool, *et al.*, 1985; Wanasinghe, 1985). This lesser incidence in buffaloes (43%) compared with cattle (66%), has been noted even when they are reared together (Wanasinghe, 1985). The effect of seasons on mastitis is not distinct (Paranjape and Das, 1986; Rasool *et al.*, 1985; Sharma, 1983). The incidence of mastitis increases with age and lactation number, and is highest in early lactation (Mitra, *et al.*, 1995; Joshi and Shrestha, 1995; Saini *et al.*, 1994; Rasool *et al.*, 1985; Rahman, *et al.*, 1984; Khalaf, 1983; Kapur and Singh, 1978). The incidence is significantly higher in domesticated buffaloes maintained exclusively for milk production in large organized farms, relative to buffaloes in "field conditions" (Fenizia, *et al.*, 1990; Sharma, 1983; Cockrill, 1974). This is due to overcrowding, extreme selection, unsuitable sanitary conditions and management practices related to overproduction in such farms. In contrast, the incidence is very low in buffaloes managed under extensive management systems (Joshi and Shrestha, 1995). The incidence could be high in machine milked buffaloes since they do not always adapt to mechanical milking systems and this may result in traumatization of teats (Fenizia *et al*, 1990). The incidence has been observed to be greater in the hind quarters of the udder in buffaloes (Mitra, *et al*, 1995; Saini, *et al*, 1994; Rahman, *et al*, 1984; Kapur and Singh 1978).

The incidence of Subclinical mastitis in buffaloes is lower than in cattle (Singh and Singh 1994). Reports in literature suggests that approximately one third of the milking buffaloes

seem to be affected subclinically; 20-35% in India (Mitra, *et al.*, 1995; Singh and Singh, 1994; Kalorey, *et al.*, 1983; Chander and Baxi 1975), 32% in Iraq (Khalaf, 1983), 34.6% in Egypt (Zaitoun, *et al.*, 1991), with a very low prevalence reported (0.5%) from Bulgaria (Tsonev, *et al.*, 1975). The incidence increases with age, and is highest in seventh and eighth lactations (Chander and Baxi, 1975). Frequently, only one quarter is subclinically infected (Chander and Baxi, 1975). Staphylococci and streptococci are the bacteria predominantly isolated from subclinically mastitic samples (Rahman *et al*, 1984; Chander and Baxi, 1975). Non-bacterial causes, such as, irritation due to improper milking or management practices has been suggested as a cause of subclinical mastitis in buffaloes (Silva *et al.*, 1995).

The reported incidence of clinical mastitis vary from 4% in India (Singh and Singh, 1994) with considerable variations between states (Kapur *et al.*, 1990; Sharma, 1983), 20% in Pakistan (Rasool, *et al.*, 1985), 25% in Iraq (Khalaf, 1983), 9-17% in Nepal (Joshi and Shrestha, 1995) and 5-16% in Egypt (Zaitoun and Eissa, 1994; Zaitoun, *et al.*, 1991; Badran, 1985). Although various agents have been associated with mastitis, bacteria are the major cause of udder infections. Knowledge on the type and distribution of bacteria are important to study the epidemiology of infections (Kapur *et al.*, 1990). The organisms isolated from buffalo milk are shown in Table 2.3.1.

Table 2.3.1. Microorganisms isolated from buffalo milk

Organism	Incidence (%)
Staphylococcus aurues	8,16, 27, 25 [1,3,10,11,18]
Staphylococcus epidermidis	4,10,19 [1,3,11]
Staphylococcus	4,10,19 [1,3,13,18,19]
Streptococcus agalactiae	8, 25, 61 [1,10,11,15]
Streptococcus dysgalactiae	2,17, 18, 24 [1,11,15,18]
Streptococcus uberis	0.5, 3,7 [1,11,15]
Streptococci	14, 27, 28, 30 [1,2,3,11,13,15, 19]
Escherichia coli	6.5, 4.5, 4,13,15,24 [1,2,3, 4,11,13]
Klebsiella	2 [1,2]
Mycoplasma spp. (*M. bovis, M. bovigenitalium*)	2 [7,9,14, 16, 17]
Ureaplasma	2 [7]
Pseudomonas	0.7, 5 [1,2,3]
Bacillus	6.3 [2,3]
Corynebacterium pyogenes	2,6 [1,11]
Other Corynebacteria	10, 13 [1,3,13]
Mycotic (Candida, Aspergillus, Cryptococcus, Cladosporium, Trichosporum, Rhizopus, Penicillium, Norcardia, Saccharomyces, Rhodotorulla qhutenes, Geotrichum candidum, Alternaria spp., Torulopsis spp., Cladosporium olivacium,)	1.4, 6,8 [1,5,6,8,12]

[1]-Kapur, *et al.*, 1990; [2]-Paranjape and Das, 1986; [3]-Char, *et al.*, Rao and Rao, 1983; [4] -Varshney and Joshi, 1986; [5]-Sharma, 1983; [6]-Pal and Mehrotra, 1983; [7]-Ashwani and Garg, 1990; [8]-Singh, Thakur, Sudhan and Verma, 1992; [9]- Zaitoun, *et al.*, 1991; [10]- Bansal, *et al.*, 1990; [11]-Kapur and Singh, 1978; [12]-Mahapatra, *et al.*, 1996; [13]-Mitra, *et al.*, 1995; [14]- Kumar and Garg, 1996; [15]-Yass, *et al.*, 1983; [16]-Zaitoun and Eissa, 1994; [17]- Pal, *et al.*, 1982; [18]-Singh, *et al.*, 1982; [19]- Silva, *et al.*, 1996b.

The chief pathogens reported were staphylococci, followed by streptococci, escherichia and corynebacteria (Mitra *et al.*, 1995; Bansal, *et al.*, 1990; Kapur *et al*, 1990; Paranjape and Das, 1986; Khalaf, 1983; Char, *et al.*, 1983; Kapur and Singh, 1978). Staphylococcal infections in buffaloes in Sri Lanka are less than in cattle and this is attributed to the efficiency of the buffalo leukocytes in removing the invading *Staphylococcus aureus* (Silva, 1993a; Wanasinghe, 1985). The role of the leukocytes in mastitis is discussed in detail, later. The use of vaccines have been recommended and justified as a very effective therapy for staphylococcal mastitis relative to the high cost of intramammary antibiotic preparations (Fenizia, *et al*, 1990).

Allergic mammitis had been described as the cause of udder inflammation with reddening and sloughing of the teats in Andhra pradesh, India (Piedy and Sreeramulu, 1993).

Intermittent Hypogalactia

An inhibition in the let down of milk in buffaloes causing economic loss had been reported from India, and has been termed "Palaserlu" and "Dokas" (Bhaskarsingh, *et al.*, 1976; Kesavamurthy and Kotayya, 1973). Earlier, this condition was thought to be due to a digestive disturbance, hypocalcemia, mastitis, oedema or venous engorgement of the udder. The incidence had been observed to be relatively higher during winter (Kotayya *et al*, 1976). The veterinarians have claimed that the condition subsided following treatment with stomachic powders, mineral mixtures or intramammary calcium gluconate or antibiotics. However, some claimed that the condition subsided even without treatment.

A similar syndrome had been observed in large organized buffalo farms in Sri Lanka (Silva, *et al.*, 1996a). The daily milk yield fluctuate substantially after the 75th day of lactation when compared to cattle. This fluctuation may vary from hypogalactia to even a temporary cessation of lactation (agalactia). The weighted milk production for 10 day periods (the difference between the minimum and maximum daily yield divided by the yield for the period) could be as high as 1.5 or even more. The persistency of the milk yield in a majority of the buffaloes, after the 75th day of lactation, was 10% of the previous month's yield, which was similar to cattle. A few cows can produce a yield of >100% of the previous month's yield which could be attributed to hypogalactia which had occurred in the previous month. However, there could be other factors contributing to an increase in the monthly yield, especially during the final days of the lactation. Some cows can even produce an increase in the yield of ≥10% of their peak yield during a 15 day period. Such unusual patterns seem to be unique to the buffalo since it has not been reported in cattle.

Structure of the buffalo mammary gland

The anatomical factors which are responsible for the relatively higher resistance of buffaloes to mastitis than cattle could be the thicker and more compact epithelium; thicker keratin layer; and the thicker and better organized muscle sphincter. The second line of defence is provided by the leukocytes which infiltrate the intraepithelial and subepithelial tissue and the lymphoid nodules (Uppal *et al* , 1994). Buffalo udder is relatively pendulous with long teats, and thereby could be more liable to injury and infection than cow's udder (Mohan, 1968). However, the teat sphincter contains more smooth muscle fibres and blood vessels, and might therefore function as a better barrier against infection than in cows

(Krishnaswamy, *et al.*, 1965). A description of various parts of the buffalo udder is given below (Uppal *et al*, 1994, Mohan, 1968, Krishnaswamy, *et al*, 1965).

Teat

The teat has rich muscle fibres and vascular tissue (Krishnaswamy, *et al.*, 1965). Other than the higher amount of melanin pigment in the epidermis, the histomorphology of skin is almost identical to cattle (Uppal, *et al.*, 1994). The extra melanin in the buffalo teat is probably responsible for protection against severe environmental conditions. However, there is no relationship between pigmentation and soreness (Thomas, *et al.*, 1981). The teat of the cow is more susceptible to irritation from harsh environment, to chapping and soring.

Duct System

The duct system is divided into streak canal, Furstenberg's rosette and teat cistern, as in cattle.

Streak canal: The length is similar to cattle with an average of 9.8 mm ranging from 7.5 to 13 mm. The length in cows varied from 7.0 -14 mm (Uppal, *et al.*, 1994, Murphy and Stuart, 1955). There is no relationship between the length and the incidence of mastitis (McDonald, 1975). The stratified squamous epithelium is comparatively compact and thicker in buffaloes than in cows (357.2 ± 4.8 μ and 327.3 ± 5.9 μ, respectively). This extra thickness may provide resistance against the invasion of pathogens. The mitotic activity in stratum germinativum is more frequent than cattle. The buffalo stratum granulosum had extra keratohyaline granules containing neutral and acidic mucopolysaccharide. As in cattle, neutrophils and lymphocyte, the cells which are responsible for cell mediated immunity were found in the epithelium. The keratin layer is comparatively thicker than in cattle (154.7 ± 6.3μ and 101.3 ± 4.1μ, respectively) and this may be due to the extra keratohyaline granules. Cattle keratin contain bactericidal and bacteriostatic lipids and cationic proteins. The cationic proteins prevent bacterial movement and inhibit the growth of *Streptococcus agalactiae* and *Staphylococcus aureus* by altering the osmoregulatory mechanism of the bacteria (Nickerson, 1985). The excess keratin in buffalo may be an important factor in the higher resistance of buffalo to mastitis.

Buffaloes have a thicker muscle sphincter than cattle (382.5 ± 5.9μ and 334.4 ± 6.8μ, respectively) and it is richer in blood vessels and nerve fibres. This ensures a tight closure of the teat canal, thus preventing the entry of pathogens. The diameter of the lumen of the streak canal is smaller than in cattle (1.7 ± 0.07 mm and 1.83 ± 0.11 mm, respectively.

Furstenberg's rosette: This acts as a defence mechanism against invading pathogens, and structurally similar to cattle. It is lined by either stratified cuboidal or columnar epithelial cells with infiltrated lymphocytes and monocytes. The subepithelial connective tissue consist of 10 to 14 folds rich in blood vessels and nerve fibres. Neutrophils, monocytes, plasma cells and mast cells infiltrate this connective tissue, and these folds provide more surface area for leukocyte infiltration. The subepithelial neutrophils are the first defence against the pathogens which penetrate the teat duct. The lymphoid nodules in the rosette are the sites for antibody production.

Teat cistern: This is lined by two layers of stratified cuboidal to columnar epithelium (Nigam and Tyagi, 1970). The lamina propria has lymphoid nodules and is highly infiltrated

with neutrophils, mast cells, monocytes, macrophages, plasma cells and lymphocytes, particularly at the junction of the rosette and the cistern. These cells are the defence mechanisms responsible for defending the tissue against ascending infections. The role of neutrophils as a defence mechanism is discussed later.

Composition of normal buffalo milk

Monitoring individual cow milk, bucket or tank milk plays an important role in health programmes in dairy cattle farms. It is, therefore, important to know the constituents of normal buffalo milk. The pH of buffalo milk range from 6.1 to 7.0 (mean=6.5) and contain a cellular and a non-cellular component (Silva *et al.*, 1995; Silva and Silva, 1994).

Cellular components

Normal buffalo milk gives a CMT score of 0 and the Somatic Cell Count (SCC) range from 50,000 to 375,000/ml (Silva *et al*, 1995; Silva and Silva, 1994). The variation among buffaloes could be attributed to the level of management in different agro-climatic zones. In comparison, normal cattle milk can carry up to 500,000 cells/ml and an excellent sample would have <200,000 SCC/ml (Jasper, 1963). The buffalo and cattle milk seem to be similar in their SCC. However, it has been shown that the concentration and functional efficiency of leukocytes in milk in the buffalo is superior to that of cattle (Silva, 1993b). The adenosine triphosphate (ATP) generated by the somatic cells in milk is an indirect measurement of the SCC in milk (Silva *et al*, 1996b). Normal buffalo milk has a mean of $5x10^{-10}$ ATP moles/ml.

The different types of cells in buffalo milk is shown in Table 2. As in cattle, the neutrophil is the most frequently observed cell although its concentration is higher in buffaloes than in cattle (Silva and Silva, 1994). The lymphocyte is the next most commonly observed cell in milk. However, in blood, the lymphocytes are in a higher concentration than the neutrophil, both in buffalo and cattle. The neutrophil, therefore, appears to be selectively migrating from blood in to the lacteal secretion.

Table 2.3.2. The SCC, differential cell counts, electrical conductivity, chloride and acidity percentages, and pH of normal milk of buffaloes.

Parameter	Mean	Range
Somatic cell counts (SCC)	140,000/ml	50,000-375,000/ml
Neutrophils	56%	22-88%
Lymphocytes	28%	10-54%
Macrophages	8%	0-28%
Epithelial	5%	0-15%
Eosinophils	1%	0-6%
Necrotic*	2%	0-6%
pH	6.5%	6.1-7.0
Electrical conductivity	3.86 mS/cm	3.4-4.7 mS/cm
Chloride	3.86 mS/cm	0.08-0.21%
Acidity	0.15%	0.08-0.22%

Source: (Silva *et al.*, 1995; Silva and Silva, 1994).

* Nuclear morphology of these cells show pyknosis, karyorrhexis and karyolysis.

A small percentage of cells in buffalo milk show nuclear morphology varying from pyknosis, karyorrhexis to karyolysis indicating that these cells were undergoing necrosis. The presence of necrotic cells implies that the cells in the normal mammary secretion of buffaloes may be functionally more active and therefore, undergo the process of cell death much faster than in cattle.

Somatic cell counting has become one of the recommended tests in bulk milk analysis as an indicator of mastitis because a major factor responsible for high SCC in udder infections due to pathogenic organisms (Schulz, 1977). The analysis of milk SCC requires simple laboratory equipment such as a manual haemocytometer or an electronic blood cell counter.

The non-cellular component (Physical and Chemical parameters)

This comprises of proteins, fats, electrolytes and enzymes, etc. The electrolyte concentration could be measured by the electrical conductivity. The major ions responsible for the EC in milk from a normal gland are sodium, potassium and chloride (Linzell and Peaker, 1975). The relatively high K and low Na concentrations are maintained by the integrity and active metabolism of the cells. The electrical conductivity, chloride and acidity percentages of normal buffalo milk are shown in Table 2. Mastitis could be detected using the electrical conductivity, long before other clinical signs ecome evident. The electrical conductivity of milk could be detected at the time of milking. With advanced age and lactation, the chloride and fat percentage increase and the lactose content decrease (Badran, *et al.,* 1986). An elevated chloride and reduced lactose will reduce the specific gravity.

Changes in composition in mastitic buffalo milk

In response to an insult, the bovine mammary cells release soluble factors, such as, prostaglandins, leukotriene B_4, serotonin, histamine, complement components and cytokines (Silva, *et al.*, 1996b, Silva *et al.*, 1995). These mediators induce an increase in capillary permeability, hyperalgaesia and haemodynamic derangement resulting in the breakdown of the blood-milk barrier. This, in turn, lead to marked oedema, swelling, tenderness of the mammary gland and transudation of serum proteins and recruitment of leukocytes, mainly neutrophils, in to the lacteal secretion. These responses are critical to the cow's ability to survive a microbial challenge and to return to normal production. The serum factors and cells act together to remove the invading pathogens. The serum factors, leukocytes, and leukocyte factors are therefore, indirect measures or markers of udder inflammation and infection. The leukocyte factors include plasmin activator, lipase and phospholipase, leukotriene B_4, proteolytic enzymes; while serum factors consist of proteins and proteases such as plasmin (Rose, *et al.*, 1989; Andrews, 1983).

Changes in the cellular component

The increased vascular permeability during early inflammation of the buffalo udder is followed by an elevation in the SCC in milk. A large number of circulating neutrophils enter the mammary gland to provide resistance against most mammary pathogens (Jain, 1976). The buffaloes tend to release exponentially increasing numbers of somatic cells into milk (Silva, *et al.*, 1997). Other than the defence mechanisms in the teat, neutrophils are the most important defence mechanism against ascending infections of the mammary gland (Paape, *et al.*, 1979). Subclinical mastitis can elevate the SCC up to several millions/ml. Experimentally induced aseptic subclinical mastitis in *Bubalus bubalis* elevate the SCC from

50,000/ml to 75 million/ml, and 90% of those cells will be neutrophils (Silva, *et al.*, 1996b). After the inflammation subsides, the milk SCC reach the pre-induced levels around the third post-induction day. Neutrophils are the most active phagocytes in the body. The functional activity of neutrophils in milk in the buffalo is similar to their neutrophils in blood (Silva *et al*, 1996b, Silva, 1993b). The role of the neutrophil in mastitis will be discussed separately. In subclinical mastitis, the entry of serum into milk precede the entry of cells and the SCC would increase with increasing degree of inflammation (Silva *et al*, 1995).

Application of the California Mastitis Test (CMT) on buffalo milk

The CMT was first reported in 1957 as a procedure for estimating the SCC in cattle milk (Schalm and Noorlander, 1957). In assigning a score to a CMT reaction, the operator becomes involved in making a decision which is partly subjective, although it is possible to acquire skill to score the reaction with a high degree of repeatability.

The CMT has been validated for buffalo milk (Silva, *et al.*, 1997). A progressive elevation in the SCC was positively associated with elevated CMT scores (Table 3). A positive CMT reading (CMT 1,2 or 3) reflects a SCC above 350,000/ml. The SCC of CMT 3 reading was markedly higher than that of all CMT scores, as has been reported for cattle. The highest percentage of organisms had been isolated from milk showing a CMT score of 3 (El-Sagheer, *et al.*, 1992).

Table 2.3.3. The range of the SCC and the respective CMT scores obtained for buffalo milk.

SCC/ml	CMT score
0-350,000	Negative
200,000 - $2x10^6$	1
$1.5x10^6$ - $8x10^6$	2
$>8x10^6$	3

The overlapping of SCC distributions between the CMT scores has also been demonstrated in cattle. When the CMT score is 1 or above 1 in buffalo milk, the SCC are relatively higher than the SCC for the respective CMT scores in cattle milk. Since the CMT reaction interprets the DNA content of somatic cells in milk (Schalm *et al.*, 1971), the above finding may be a reflection of the possible differences in the DNA content in buffalo somatic cells. The fact that the chromosome number in buffaloes (48 or 50) is smaller than in cattle (60) could justify the above statement. Accordingly, it could be hypothesized that relatively more buffalo somatic cells are needed to produce a CMT score similar to cattle.

Changes in the Non-cellular components (physical and chemical parameters)

The serum and leukocyte factors change the chemical parameters of milk during mastitis. The occurrence of an increased vascular permeability is the initial event in the chain of reactions involved in mastitis (Rose *et al.*, 1989; Giri *et al.*, 1984). As a result of this altered permeability, NaCl and bicarbonate ions enter the lacteal secretion thus elevating the electrical conductivity and chloride ion concentration in milk. It has been shown that subclinical mastitis in buffaloes can be detected relatively early by a significant increase in the electrical conductivity and chloride ion concentration, in addition to, elevation in the cellular component (Silva *et al.*, 1995). The pH does not reduce significantly and ranges

from 6.2 to 6.8 (mean=6.4). In *Bubalus bubalis* an elevation of the electrical conductivity in milk above the mean plus twice the standard deviation of the normal levels (3.87 + {0.3x2} = 4.47), when measured individually for Silva *et al.*, 1995). The increase in electrical conductivity in milk is a direct effect of an elevation in chloride ion concentration (Linzell and Peaker, 1975). The chloride percentage is an efficient indicator of inflammation and is not related to leukocyte count or the total bacterial count (Badran *et al.*, 1986). The chloride ion concentration in milk of *Bubalus bubalis* significantly elevates from 0.108% to 0.142% during subclinical mastitis (Silva *et al.*, 1995). There are no apparent differences in these parameters either in natural or in experimentally induced subclinical mastitis. The serum albumin content in milk determined by a radial immunodiffusion test could also be used to diagnose subclinical mastitis (Dahiya and Kapur, 1990).

The catalase and adenosine triphosphate (ATP) appear in milk as a consequence of the presence of neutrophils in milk. The catalase content of buffalo milk is each buffalo, can be used as an indicator of subclinical mastitis (measured by the time taken by a paper disk impregnated with milk (and 0.02% ethanol) to float to the surface of a H_2O_2 containing EDTA. This assay is sensitive in detecting subclinical mastitis (Sharabi, *et al.*, 1986). The ATP concentration in milk is measured by a luminometer. An increase from $5x10^{-10}$ ATP moles/ml to $33x10^{-10}$ ATP moles/ml has been observed in buffalo milk with CMT scores above 2 with an average SCC of $4.25x10^6$/ml (Silva *et al*, 1996b). The presence of bacteria in milk was associated with an elevation of lysozyme concentration to a mean value of 550 µg/ml, in comparison to 178 µg/ml in cattle (Farid, *et al.*, 1984). A maximum lysozyme content in buffalo milk from bacteriologically negative quarters has been recorded to be 100 µg/ml.

In summary, the SCC, electrical conductivity and concentrations of chloride, albumin, catalase, lysozyme and ATP are markers of subclinical mastitis in the buffalo.

Pathogens involved in mastitis

Unlike other diseases which are caused by specific organisms, mastitis can be caused by a variety of pathogens. The relative importance of different mastitogenic agents is likely to vary in different areas and countries (Kalra and Dhanda, 1964). The pathogens isolated from buffalo milk are shown in Table 2.3.1. The chief mastitis pathogens reported were Staphylococci, followed by Streptococcus, Escherichia and Corynebacteria (Mitra *et al.*, 1995; Bansal *et al.*, 1990; Kapur *et al*, 1990; Paranjape and Das, 1986; Khalaf, 1983; Char *et al.*, 1983; Kapur and Singh, 1978). The biochemical characteristics of *Staphylococcus aureus* of buffalo origin is similar to those of cattle origin, and 39% of strains belonged to Group A (Varshney, *et al.*, 1993; Nag and Ray, 1986). Out of the O-serotypes of *Escherichia coli* in India, O28, O29, O68 and O74 are common to cows and buffaloes (Kapur *et al*, 1990). Immunization of dairy cows with the *Escherichia coli* (O111:B4) J5 bacterin at drying off had reduced the incidence of clinical mastitis caused by Gram-negative bacteria by a third (Hogan, *et al.*, 1992a).

Prevalence of mixed bacterial infections vary from 10% to 25% of cases (Kapur *et al*, 1990; Paranjape and Das, 1986; Kapur and Singh, 1978). Streptococcus and staphylococcus were the commonest combination (Paranjape and Das, 1986). A low incidence (4%) of fungi with bacteria (Candida/*Staphylococcus aureus* and penicillium/streptococci) also has been

reported (Mahapatra, *et al*, 1996). *Listeria monocytogenes* with mycoplasma had been isolated from one single case (Zaitoun and Eissa, 1994). The efficiency of neutrophils in removing some bacteria may be hampered in mixed infections (Silva and Thattil, 1995).

A summary of abnormalities detected in buffalo milk collected aseptically

In general, the samples can be divided as follows:

a) Clinical samples or samples from non-responsive quarters
b) Herd investigations - samples from non-responsive problem herds. These would be primarily subclinical cases
c) Samples from individual cows for examination for normality.

The examinations should be carried out as shown in Table 2.3.4.

Table 2.3.4. Selection procedure used to examine milk according to the reason for investigation.

Somatic cell count	Examination of stained smears	Bacteriological examination	Test for inhibitory substances
No	Yes	(a) Clinical samples 25 or 50 µl inoculum	Yes, if no growth
Yes	No	(b) Herd investigations 10 µl inoculum	Only if recent antibiotic therapy was given
Yes	No	(c) Normality check 25 or 50 µl inoculum	No

Extracted from Bovine Mastitis: Australian Standard Diagnostic Techniques for Animal Diseases (1993).

1. *Bacteriological examination for the presence of mastitis causing pathogens:* In the laboratory, the bacteriological procedures should be kept as simple as possible. It is recommended that primary isolation be attempted by direct culture onto thin blood agar plates. Very thin plates are necessary to correctly determine the haemolytic patterns of streptococcal isolates. Plates should be examined at 18 hours and again at 42 hours.

2. *Measurement of the concentration of somatic cells in milk* :
   2.1 Indirect measurement by the California Mastitis Test (Table 2.3.3).Normal milk would show a CMT negative score. The CMT scores1, 2 or 3 reflects the presence of high SCC.
   2.2 Direct measurement by counting the somatic cells in milk, using a haemocytometer or an electronic cell counter. A sample with ≥400,000 cells/ml of milk should be considered as mastitic.

3. *Measurement of the electrical conductivity in milk*: In the laboratory, this can be measured individually for cows by using a conductometer. An elevation above the mean plus two standard deviations of the normal values can be considered as mastitic. A hand-held conductometer with sophisticated electronic technology is

used in developed countries for cow-side measurement of electrical conductivity of cattle milk to detect subclinical mastitis ("Mas-D-Tec", Wescor Inc., Logan Utah, USA).

4. *The concentration of adenosine triphosphate (ATP) concentration in milk*: The ATP concentration can be measured using a luminometer. Levels over $5x10^{-10}$ ATP moles/ml is indicative of mastitis.

5. *Other tests to detect subclinical mastitis are*:
   a. Chloride ion concentration in milk
   b. Catalase reaction
   c. Serum albumin in milk which could be measured by the radial immunodiffusion test
   d. Lysozymal content in milk.

The role of the neutrophil in the udder defence mechanism

The efficiency of the defence mechanisms of the mammary gland, namely, the conformational changes in the streak canal, immunoglobulins (Ig), phagocytes and their products (lactoperoxidase/thiocyanate/hydrogen peroxide complex, lysozymes. lactoferrin) are critical to the cow's ability to survive mastitis (Silva *et al*, 1996b). Neutrophils, the most active phagocytes in the body, are the most prominent cells in buffalo milk (Silva and Silva, 1994). The phagocytic activity of buffalo neutrophils is different for various mastitis causing bacteria and individual variation among buffaloes is common (Silva, 1993a; Silva *et al*, 1996b). The phagocytic activity of buffalo neutrophils is highest for *Staphylococcus aureus* (86%), followed by *Streptococcus agalactiae* (77%), although the activity for *Escherichia coli* (73%) is relatively lower. The phagocytic activity of cattle neutrophils also vary for different pathogens and is highest for *Escherichia coli* (81.3%), followed by *Streptococcus agalactiae* (77%) and intermediate for *Staphylococcus aureus* (64%) (Silva and Jain, 1988). Accordingly, the phagocytic activity of buffalo neutrophils, relative to cattle neutrophils, is 22.5% higher for *Staphylococcus aureus*, 8% lower for *Escherichia coli*, and similar for *Streptococcus agalactiae*. This explains the predominance of streptococcal mastitis and the resistance to staphylococcal mastitis in buffaloes (Wanasinghe, 1985; Kapur and Singh, 1978).

Lymphokines secreted by primed lymphocytes in the cattle mammary mucosae attract blood leukocytes (90-95% of total milk cells), irrespective of the cause of the inflammation (Sears, 1984; Jain, 1976; Schalm *et al*, 1971). Similarly in sub clinical mastitis, an average of $5.6x10^6$ cells/ml will appear in buffalo milk and 90% of these cells will be neutrophils and their viability is high (94-100%) as in cattle (Silva *et al*, 1996b).

The invading bacteria will be opsonized by immunoglobulins in milk and phagocytized by these neutrophils (Niemialtowsky, *et al.*, 1988; Watson, 1976). The efficiency of the phagocytic efficiency of cattle neutrophils is relatively low when they are in milk (Russell, *et al.*, 1977; Jain, 1976). In contrast, the phagocytic activity of neutrophils which appear in milk in buffaloes, during acute mastitis, are as efficient as blood neutrophils (Silva, 1993b; Silva *et al.*, 1996b). However, the phagocytic activity is relatively delayed for *Streptococcus agalactiae*. Incidentally, streptococcal species are a major mastitis causing

bacteria isolated from buffalo milk (Silva *et al.*, 1996b). The bactericidal activity following phagocytosis has been demonstrated in all phagocytically active buffalo neutrophils (Silva *et al.*, 1996b). Most neutrophils kill all the ingested bacteria whereas, a few kill only some of the ingested bacteria.

The reduced phagocytic activity of cattle milk neutrophils has been ascribed to the ingestion of fat globules, reduced glycogen content, and surface coating of casein which makes the cells less mobile and less active (Dulin, *et al.*, 1988; Jain and Lasmanis, 1978). However, since the extravasation of blood neutrophils begins soon after the initiation of an inflammatory process, the neutrophils which appear in milk in acute mastitis may be devoid of ingested fat globules. The most efficient bactericidal effect was observed at or near the peak of the somatic cell count (Daley, *et al.*, 1991). This reveals the potential of reducing bacterial mastitis by improving the phagocytic activity of milk neutrophils. This may be achieved by enhancing the opsonin concentration in milk perhaps by vaccination. An experimental challenge trial with *Escherichia coli* J5 vaccine had revealed a higher milk IgG titre to *Escherichia coli* J5 bacterin in vaccinated than in control dairy cows (Hogan, *et al.*, 1992). The mean serum IgG titre to whole cell *Escherichia coli* J5 had been significantly greater in vaccinated than in unvaccinated cows. The vaccinated cows had lower bacterial counts in milk and lower rectal temperatures than unvaccinated controls, following intramammary challenge with a heterologous strain of *Escherichia coli*. Cattle neutrophils has as much as 10,000-fold variation in the bactericidal failure rate for staphylococci during cell cycling (Daley *et al.*, 1991). A major virulence factor of *Staphylococcus aureus* is the development of an exopolysaccharide capsule *in vivo* which inhibits the cell-wall opsonization of *Staphylococcus aureus* (Guidry, *et al.*, 1991). Bovine antibodies to *Staphylococcus aureus* capsule have been shown to be opsonic for bovine neutrophils.

Although the phagocytic efficiency of neutrophils encountering either pure or mixed bacterial cultures is similar, the neutrophils do not always phagocytize all species of bacteria in mixed cultures (Silva and Thattil, 1995). A lesser preference was shown for *Escherichia coli*. The affinity for *Escherichia coli* was much less in the presence of *Staphylococcus aureus* than *Streptococcus agalactiae*. It should be noted that microorganisms or their metabolic products can inhibit the phagocytic activity of neutrophils.

Treatment and control of mastitis

Mastitis is a preventable disease. The prognosis of mastitis would be better when treated at the earliest possible instance. A delay in treatment even by a few hours can cause permanent damage to the glandular tissue. The milk production can be permanently reduced in such cows and it would have dramatic effects on the economic gains to the farmer. In majority of glands, intramammary infection and clinical mastitis occur at different times (McDonald, 1984). Most infections occur during the dry period and all clinical mastitis occur during lactation. The aims of any treatment programme are (Gill and Robertson, 1985),

- early detection,
- rapid clinical response,
- to restore the structure and function of the gland,
- to achieve a high bacteriological cure rate,
- to treat all clinical cases as they occur, and
- to plan a minimum milk withholding period.

Treatment of clinical mastitis

Since the clinical signs are caused by endotoxins and other toxic microbial products, the aim of treatment should be to eliminate the toxins and to neutralize their effects (Eberhart, 1984; McDonald, 1984). Frequent milking of the gland, under hygienic conditions, will remove bacteria and their toxins. The milk in the affected quarter would enhance bacterial multiplication and also cause stress to the gland due to increased secretion. Therefore, the udder should be evacuated frequently; minimum of twice a day. If it is difficult to milk when the udder is swollen, intravenous oxytocin can be given to stimulate milk let down (Sudershan and Bhat, 1995; Eberhart, 1984). Instruct the farmer to begin to milk out the infected gland, immediately, especially in high yielding cows.

Preferably, a milk sample should be taken prior to treatment, so that antibiotic sensitivity test results could be used to modify treatment, if the initial treatment has not been effective. Prompt systemic therapy in acute mastitis will prevent gangrene formation and will supplement the intramammary preparation to bring about an early cure of clinical mastitis. This includes intravenous electrolyte fluids, corticosteroids or non steroidal anti-inflammatory drugs (Eberhart, 1984). This is because the intramammary preparations may not be distributed throughout the mammary tissue due to the swelling in the tissue and blockage of duct system by inflammatory products. Administration of treatment should always be done under direct veterinary supervision. The selection of the parenteral antibiotic depends on the (Eberhart, 1984),

- bioavailability of the active compound in serum
- ability of the drug to cross the blood-to-milk barrier
- expected minimum inhibitory concentration (MIC) against the causative agent.

The clinical mastitis cases of buffaloes with no evidence of infection and not responding to antibiotics have been treated with parenteral antihistaminics and corticosteroids (Sreeramulu and Sreeramulu, 1993). These conditions considered to be allergic mammitis may respond to such treatment.

Administer intramammary preparations after complete evacuation of the udder. Frequent milking could be preceded by infusing sterile distilled water into the quarter which will increase the concentration of leukocytes in the gland. This high count of leukocytes (majority neutrophils) will enhance removal of the causal pathogens (refer section on the Role of the Neutrophil in the Udder Defence Mechanism). Sterile saline may be used to wash out the quarter. Intramammary therapy is always required in any case of mastitis (McDonald, 1984). Intramammary preparations may be administered during the night time when it is not possible to evacuate the udder. Administer the full intramammary antibiotic dosage at 12-24 hour intervals for at least three times. A full course of most intramammary preparations require three treatments, and it is important to follow manufacturers instructions. The following factors should be considered when selecting an intramammary preparation (Gill and Robertson, 1985) -

1. It must be non-irritant.
2. The causal organism must be sensitive to the antibiotic.
3. Consideration should be given to the type of base used in the intramammary preparation. Slow releasing bases give prolonged release but lead to antibiotic residues in milk. Quick releasing bases necessitates frequent treatment to maintain the minimum inhibitory concentration of antibiotic in milk.
4. The concentration of the antibiotic on which depends the frequency of treatment.
5. The time period of antibiotic persistency in milk.
6. Distribution and absorption of the antibiotic throughout the udder tissue.

Preferably, the cow should continue to be milked twice daily. The recommended length of time for milk withholding should be followed for each intramammary preparation. It is a good practice to test milk samples from treated cows for antibiotic residues, prior to adding the milk to the collecting tank.

Antibiotics used

Parenteral: Chloramphenicol and Gentamycin has consistent *in vitro* activity against coliforms causing mastitis. However, they are of low bioavailability, poorly cross the blood-to-milk barrier, expensive since large doses are required for the systemic route, and chloramphenicol is avoided on food producing animals. Therefore Oxytetracycline, Ampicillin, and sulphas are used parenterally. Gentamycin is recommended for intramammary use, although distribution in the udder is relatively poor (Eberhart, 1984). Intravenous Cefaloridine has been reported to be effective against Staphylococcus, *E.coli* (Baghaerwal and Shukla, 1991).

Lactational (intramammary) therapy: Lactational therapy will eliminate *Streptococcus agalactiae, Streptococcus dysgalactiae* and a majority of coagulase negative staphylococci. Treatment of other types of infection is not recommended in lactating cows, unless the cow shows clinical mastitis, because therapy at this time is not effective in eliminating those infections. In a majority of cases, lactational therapy will not eliminate infection from treated glands (McDonald, 1984). Treatment usually allows only clinical recovery and return of the milk to a saleable product. Dry cow therapy must be relied upon to eliminate infection.

A wide range of antibiotics are used in intramammary preparations for the buffalo (Zaitoun and Eissa, 1994, Saini, *et al.*, 1994; Baghaerwal and Shukla, 1991; Ashwani and Garg, 1990; Pal, *at al.*, 1988; Prasad and Khahra, 1986; Hussain, Naeem *et al.*, and Iqbal, 1984; Char, *et al*, 1983, Rahman and Baxi, 1983). Some causative pathogens and the reported antibiotic sensitivity are given below.

Staph. aureus and other Staphylococci - Chloramphenicol, Gentamycin, Neomycin, Nitrofurantoin, Cotrimaxazole, Oxytetracycline, Erythromycin, Sulphonamide, Kanamycin, Cloxacillin, Ampicillin and Cefaloridine.

E. coli - Chloramphenicol, Gentamycin, Oxytetracycline, Erythromycin, Sulphonamide, Strepto-Penicillin and Cefaloridine.

Streptococcal infections - Chloramphenicol, Oxytetracycline, Erythromycin, Sulphonamide, Strepto-penicillin, Kanamycin, Cloxacillin and Ampicillin,

Mycoplasma and Acholplasma infections - Chloramphenicol, Thiamphenicol, Spiramycin, Vibramycin, Lincomycin, Tylosine, Ledermycin, Tetracycline, Neomycin and Erythromycin.

Chloramphenicol appears to be the most widely reported intramammary antibiotic used in buffaloes. Chloramphenicol in food producing animals should be used with care due to its side effects. The use of intramammary antibiotics, without direct veterinary supervision, may lead to residues in milk (Sudershan and Bhat, 1995).

A combination of antibiotics also has been reported to be successful in the treatment of clinical mastitis. The Formula 17900-Forte containing 20mg hydrocortisone acetate,

12.5mg hydrocortisone sodium succinate, 100,000 IU procaine benzyl penicillin, 150 mg novobiocin, 50,000 IU polymyxin B sulphate, 100mg dihydro streptomycin base and 50mg chlorbutanol (anhydrous), had been effective against *Strep. dysgalactiae, Strep. agalactiae*, other streptococci, *Corynebacterium pyogenes, Staph. aureus. Staph. epidermidis* and diphtheroid bacilli (Kapur, *et al.,* 1985). A preparation containing 250mg ampicillin and 10mg prednisolone had shown partial response to Streptococci, Staphylococci and *E.coli* (Varshney and Joshi, 1986).

Staph. aureus and *Staph. epidermidis* had reported to be resistant to penicillin and streptomycin (Dahiya and Kapur, 1984; Rahman and Baxi, 1983). Mycoplasma strains had been reported to be resistant to penicillin, ampicillin, cephalosporins and oxytetracycline (Zaitoun and Eissa, 1994; Ashwani and Garg, 1990).

Treatment of subclinical mastitis (Dry Cow therapy)

Treatment of subclinical mastitis during lactation is not recommended, due to poor cure rate. Since most intramammary infections occur during the dry period, treatment at drying off gives better cure rates, especially for *Staph. aureus* infections (McDonald, 1984). Treatment at this time is efficient at eliminating infections due to the slow release of antibiotic formulations and the absence of continuing milk production, uses less antibiotic than lactational therapy, lead to minimal milk loss, and also prevents new infections in the early dry period. The regeneration of glandular tissue which were damaged due to infection will be much better with Dry Cow therapy and the possibility of antibiotic residues in milk will be decreased since the antibiotic will not be present in the secretion at the time of calving.

Blanket therapy of the herd with Dry Cow treatment is recommended when, a reliable method is not used for detecting cows with subclinical mastitis, an average of more than 2 clinical cases are observed /100 cows/month, and the bulk SCC is >400,000/ml. Selective therapy for all four quarters at drying off is recommended for any cow, with clinical mastitis during lactation or with more than normal SCC during lactation.

Prevention and control of mastitis

The main goals in a mastitis control programme should be aimed at the milk, the cow and the economics. The success of a control programme in a farm can be assessed by the following parameters (Gill and Robertson, 1985).

- The bulk tank or bulk milk will carry a SCC of less than 400,000/ml and a preincubation bacterial count of less than 10,000.ml, with no antibiotic residues.
- Over 75% of the cows in the herd would be free of clinical mastitis. The incidence of clinical mastitis would drop to 50% after the first year with less than 3% each month. The milk of over 85% of cows will have a CMT score of negative or trace (Table 2.3.3). The culling rate due to mastitis would be less than 6% per year.
- At least a 300% return will be observed on the investment in the programme. The production and the quality of milk will increase while the drug and veterinary cost will decrease after the first year.

Because mastitis is a complex disease there is no one factor that can prevent it. The most logical control programme is known as the "3x3 programme" and is based on three simple philosophies - the prevention of new infections, elimination of existing infections and monitoring of the progress (Gill and Robertson, 1985; McDonald, 1984). Prevention of new

infections could be achieved by proper pre-milking hygiene, post-milking hygiene, testing of new cows before introducing to the herd and servicing and maintaining the milking machine, if available. The existing infections can be eliminated by treating the clinical and subclinical cases, culling of cows with chronically infected glands, and segregation of large herds with confirmed *Staph. aureus* into an infected and non-infected groups. The progress of the control programme could be monitored by recording the SCC of bulk milk, correctly identifying and recording the subclinically infected cows, and maintaining proper records on treatment, antibiotic sensitivity, rate of clinical cases, etc.

An *Escherichia coli* (O111:B4) J5 vaccine given subcutaneously had been successful in preventing mastitis in dairy cows (Hogan, *et al*, 1992a). Enhanced opsonic activity of colostrum and milk (IgM and IgG) and higher serum IgM titre to *E. coli* J5 was observed in vaccinated cows (Hogan, *et al.*, 1992b). Numbers of intracellular bacteria per phagocytizing neutrophil correlated positively with IgM titres in both serum and colostrum. Similar studies on the buffalo will be worthwhile in large scale buffalo farming operations.

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NA-218-67

2.4 Anthrax

T.G. Wijewardena

Introduction

Anthrax is an infectious disease of a highly acute nature, with a worldwide distribution. It is a zoonotic disease and it is speculated that the fifth plague causing a murrain upon the livestock of Egypt, a biblical account as recorded in the book of Exodus, was anthrax. The European 'black bain', which in 1613 killed nearly 60,000 humans is believed to be anthrax. Other synonyms are 'charbon', 'malignant pustule', 'malignant carbuncle', 'mizbrand', 'splenic fever' and 'woolsorter's disease' (Anon, 1996).

All warm blooded animals including humans are affected to a varying degrees of severity. Anthrax has been reported among domesticated and wild animals including hippopotamus, elephants, cape buffalo (*Syncerus caffer*) (Turnbull *et al.*, 1991), lion (Young, 1975) and, bison (Broughton, 1992). The infection had been reported among buffaloes in India (Sharma *et al.*, 1996) and Italy (Galiero and Consalvo, 1993). The last reported outbreak of anthrax in Sri Lanka was in 1969 among sheep.

Aetiology

Anthrax is caused by *Bacillus anthracis*. It is a spore forming, Gram-positive, rod shaped, encapsulated bacterium of the genus *Bacillus*.

Epidemiology and Pathogenesis

The organisms when exposed to air, form spores that are very resistant to environmental influences. These had been found to remain viable in a rubber stoppered bottle for 60 years (Wilson and Russel, 1964). Undrained alkaline soil rich in organic matter is believed to form a favourable environment for the survival of these spores. (Radostits, *et al.*, 1994). Major climatic changes, such as heavy rains following a long period of severe drought, have been linked to outbreaks of anthrax, in areas where the infection has been last recorded over 30 years ago (Fox, *et al.*, 1977).

Infection occurs through ingestion of contaminated material such as feed, water, carcasses or through the skin or inhalation of the spores. Damage to the oral mucosa could act as a predisposing factor, however entry of the organisms could occur even through the intact mucosa. Following entry these are moved to local lymph nodes by motile phagocytes where they proliferate and, enter the blood stream via the lymphatics causing septicaemia. The bacterium produces an exotoxin which is very lethal causing oedema and tissue damage resulting in death from shock and renal failure and, terminal anoxia (Radostits, *et al.*, 1994).

Clinical signs

The infection could occur as peracute, acute or chronic forms. The usual signs associated are sudden deaths with oozing of unclotted blood from the natural orifices. In an outbreak among buffaloes in India (Sharma *et al.*, 1996), it has been reported that initially no clinical

signs were observed prior to sudden deaths. Few animals had shown fever, ruminal stasis, aggresive behaviour of a short duration followed by depression, muscle tremors, respiratory distress, distension of jugular veins, convulsions followed by death within 2 to 3 hours. A frothy sero-sanguineous exudate from the nostrils had been a common feature. Despite heavy antibiotic treatment the case fatality rate had been very high. Absence of rigor mortis, ecchymotic haemorrhages throughout the body tissues, blood stained serous fluid in all the body cavities with an enlarged and soft spleen were the postmortem findings that had been observed.

Diagnosis

It is the practice not to open up a carcass of an animal suspected to have died of anthrax, as the organisms would sporulate to form highly resistant spores which could contaminate the environment. These spores are known to remain viable for decades. Anthrax could be suspected on the basis sudden deaths accompanied by oozing of unclotted blood from natural orifices and incomplete rigor mortis. There are no pathognomonic postmortem lesions for the diagnosis of anthrax and, differential diagnosis include lightning strike, peracute black leg, acute leptospirosis, other clostridial infections, acute lead poisoning, hypomagnesaemia and snake bite (Anon, 1996).

In the case of ruminants such as buffaloes the recommended practice is to collect blood samples from a vein, such as the caudal vein (ear vein is not preferred) and, submit the blood and prepared blood smears to a laboratory for isolation and identification of the organism. The blood smear is stained with Poly-chrome methylene blue (Mac Fadyeans' reaction), where the capsule is stained pink and the bacterial cell, a dark blue (Anon, 1996).

Treatment

Recovery could be anticipated if treatment is given in the early stages when only fever is detected. Penicillin at the rate of 10,000 IU/kg of body weight twice daily has been the practice in the early years (Riggs and Tew, 1947). Streptomycin, 8-10 grammes in 2 doses by the intramuscular route (Flynn, 1968/69) and oxytetracycline, 5 mg/kg of bodyweight daily (Bailey, 1954) has been found to be more effective than penicillin. Antibiotics in combination with anthrax antiserum has been recommended, but the treatment regime is too expensive and not practicable (Radostits, *et al.*, 1994).

Control

Implementation of strict, but basic hygienic measures such as deep burial of affected carcasses covered with quicklime, along with beddings and contaminated soil, disinfection of the premises with a strong disinfectant and, segregation of all in-contact animals and placing the farm or the village under quarantine with prevention of movement of animals to and from the farm or the village would greatly aid in the prevention of spread of the infection, once an outbreak has occurred.

An avirulent variant of *Bacillus anthracis*, termed $34F_2$ Sterne, discovered by Sterne in 1937 (as cited in Anon, 1996) is the most widely used strain of the organism used in the manufacturers of vaccines. In fact 31 out of 36 manufactures use this strain. Other types

available are those incorporating live attenuated strains of the organisms and vaccines containing a cell free filtrate of a culture (Radostits, *et al.*, 1994). In Sri Lanka a vaccine incorporating the Sterne strain was produced in the past, but it had been discontinued as the country has not experienced an anthrax infection since 1969.

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NA-218-71

2.5 Infectious Keratoconjunctivitis

T.G. Wijewardena

Introduction

The occurrence of "Keratitis contagious" was first reported by Billings in 1889 (cited in Blood *et al.,* 1989). Due to the failure of the investigators to correctly identify the aetiological agent causing the specific clinical signs, any condition with infectious conjunctivitis and/or keratitis was regarded as a syndrome and, were given many synonyms such as pink eye, new forest disease, infectious ophthalmia, blight and infectious keratitis.

Aetiology

Infectious agents incriminated, in the past, included various bacterial organisms, viruses, mycoplasmas, rickettsia and a nematode Thelazia. Eventually the condition was recognised as a specific disease and termed infectious bovine keratoconjunctivitis (IBK) caused by *Moraxella bovis* employing the Koch's postulates (Punch and Slatter, 1984). In Sri Lanka, IBK has been recorded among cattle (Wijewardana *et al.*,1984; Bandaranayake, 1955). A similar condition with the same clinical signs is observed among buffaloes in Sri Lanka and, empirical treatment based on the recommendations for bovine had been successful for buffaloes.

Epidemiology and Pathogenesis

It is reported that factors such as ultra violet light (Pugh *et al.,* 1968), dust, tall grasses and flies (Dodt, 1977) are essential as predisposing factors for the manifestation of the clinical infection. The face flies have been found to be mechanical carriers of the organism (Hugh and Pugh, 1970). Pedersen *et al.,* (1972) have demonstrated that fimbriae of virulent strains of *M.bovis* are used to adhere to the conjunctiva for colonisation. A purified haemolytic and cytotoxic fraction of haemolytic *M.bovis*_ had been used to reproduce IBK (Beard and Moore, 1994), indicating that these toxins are responsible for the expression of the infection.

The incidence had been found to be high during the hot season (Slatter *et al.,* 1982b,c). During these months the fly population is on the increase and has a positive correlation with the incidence of IBK (Gerhardt *et al.,* 1982). The carrier animals are reported to be very significant epidemiologically (Pugh and Hughes, 1975 and Wijewardena *et al.,* 1984).

Clinical signs

Initially only one eye would be affected and subsequently the other eye could be crossinfected. Ocular discomfort, blepharospasm (spasmodic winking), photophobia and epiphora (overflow of tears) are followed by the conjunctiva becoming hyperaemic and oedematous with copious serous discharge from the eye which soon become purulent. The cornea becomes markedly oedematous and may slough off after rapid enlargement, thus forming an ulcer. Usually the condition is self limiting with the ulcer healing within 4-6 weeks. In serious cases the animal may become blind thereby causing production losses. In young animals it had been reported that it may progress to form a descemetocoele (hernia of the Descemet's membrane), which if bursts will lead to panopthalamitis (inflammation of all

parts of the eye) with the accompanying risk of an ascending infection resulting in the rare possibilities of meningitis and death. (Punch and Slatter, 1984).

Treatment

An opthalmic preparation containing a suitable antibiotic and a cortisone had been recommended by Scott (1957). In Sri Lanka, a commercially available eye ointment containing neomycin and hydrocortisone, instilled twice daily into conjunctival sacs had been successful in that the clinical signs disappeared within 15-20 days (Wijewardana *et al.,* 1984). It is always recommended that an antibiotic sensitivity test be carried out in order to determine the most suitable antibiotic in the light of the report of Punch and Slatter (1984) where it is stated that, the sensitivity pattern of the organism, represented by different strains vary from strain to strain. It has been recently reported that early treatment with long acting parenteral oxytetracyclines is effective (Odeon *et al.,* 1996).

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2.6 Tuberculosis

N.U. Horadagoda

Definition

Tuberculosis is a chronic disease manifested by progressive development of tubercles in any organ of the body.

Aetiology

The causative bacterium is *Mycobacterium bovis*. Previously it was customary to refer to the cattle strain, the avian strain and the human strain of *Mycobacterium tuberculosis* but those pathogenic to cattle are now referred to as *Mycobacterium bovis*. Studies in India (Mohan, 1968) have demonstrated that all cultures of tubercle bacilli from buffaloes are *Mycobacterium bovis* but Australian workers (Hein and Tomasovik, 1981) have isolated *M.aviuam*, *M. fortui* and *M. flavescens* from lesions in buffaloes in addition to *Mycobacterium bovis* which was frequently present. *M.bovis* is a long slender, rod shaped organism which belongs to the acid-fast group of bacilli. The organism is moderately resistant to heat, desiccation and certain disinfectants such as alkalis and phenols but is readily destroyed by sunlight. *M.bovis* is able to survive for long periods in warm, moist covered places.

Occurrence

The earliest records of tuberculosis in buffaloes dates back to the Nineteenth century when the disease was observed among animals slaughtered in East European countries. At the turn of the century, the disease was widely reported among buffaloes at meat inspection in Eastern Europe and Mediterranean countries. Tuberculosis still remains to be a disease of great importance although some countries such as Australia has been successful in eradicating the disease to a very large extent. The incidence of the disease in India vary between cattle and buffaloes in the different regions of the country, but in general the incidence in buffaloes is higher than that in cattle. Moreover, buffaloes are known to suffer more than cattle. Tuberculosis is seldom seen in Sri Lanka. Comparative tuberculin sensitivity studies carried out in two large buffaloes farms in Sri Lanka have demonstrated increased sensitisation of animals to mammalian tuberculin as compared to avian tuberculin (Pinto *et al.*, 1973). The difference in response is thought to be a non-specific reaction which is attributed to the exposure of buffaloes to wide variety of non-tuberculous mycobacteria known to inhabit wallowing mud pools.

Public health

Tuberculosis is a disease of great public health concern as man is susceptible to infection with *M. bovis* and *M. tuberculosis*. Man usually gets infected with *M. bovis* by the aerosol route or by drinking tuberculous milk. Pasteurisation effectively kills mycobacteria. Meat inspection and the condemnation of affected organs or parts are important measures to prevent human infection.

Epidemiology

The infected animal is the main source of the infection, as the organism is excreted in the exhaled air, sputum, faeces, milk, urine, vaginal and uterine discharges. Buffaloes could get infected by ingestion or inhalation of the organism; the latter being more common. Drinking of infected milk is the most common method of spread to young animal while the respiratory route of infection may occur in all age groups. The organism may remain viable in stagnant water up to 18 days after being contaminated by an infected animal.

Clinical signs

In the early stage there is general apathy and malaise. In the pulmonary form of the disease which is most common, infected animals have fluctuating temperature, capricious appetite, dry husky cough and gradual emaciation while in the intestinal form of the disease there is persistent diarrhoea. Infection of the mammary gland results in a hard painless enlargement of the gland that may extend to the supramammary lymph nodes. The milk from an infected udder becomes watery and contains a large numbers of organisms. Despite the clinical changes mentioned, the disease in most instances is detected only at slaughter or death from other causes.

Necropsy findings

The lesions in tuberculosis are largely confined to the bronchial and mediastinal lymph nodes and lungs. When compared to cattle, the lesions in buffaloes are less calcified and resemble abscesses with caseous material arranged in layers. The distribution of lesions may vary from a large, solitary abscess to widespread smaller lesions, the so called miliary tuberculosis affecting the peritoneum, pleura and other organs. In the lungs, these miliarly abscesses may extend to cause suppurative bronchopneumonia.

Diagnosis

In live animals, tuberculosis is diagnosed by demonstrating hypersensitivity to tuberculo-protein in the tuberculin test. Tuberculin is a purified protein derivative (PPD) produced by growing mycobacteria in a protein-free medium and precipitating a cell-free filtrate. The test is usually carried out by intradermal inoculation of tuberculin on one occasion, i.e. the single intradermal test. The site of inoculation of the skin is on the side of the back or the caudal fold. The reaction is read after 72 hours and the presence of any oedema is regarded positive. The oedema following the tuberculin test is more pronounced and extensive in the buffalo compared to cattle. It has also been noted that the skin reaction in the buffaloes persists longer than in cattle and may last up to 10 days. A small circumscribed swelling of 2-3 mm without oedema is considered negative. False positive reactions occur relatively frequently and may be caused following infection with non-tuberculous mycobacteria. Animals that have been infect under 4 weeks of the test been performed may appear negative in the tuberculin test.

At postmortem, the diagnosis is confirmed by the demonstration of the organism. Suspected material should be submitted for culture or animal inoculation; mycobacteria grow very slowly, hence the results will not be available for 4-8 weeks. Ziehl-Neelsen staining may

reveal acid-fast organisms, but is not a sensitive technique. Tuberculosis should be differentiated from norcardiosis, actinomycosis and parasitic granulomata which present similar lesions.

Treatment

Treatment is of little practical value. Attempts to treat livestock have been made on a small scale in the field, but the cost of such treatment has been found to be high and not all animal treated were cured. Moreover reinfection was common. In very valuable animals, a combination of streptomycin and para-aminosalicylic acid may have some beneficial effect as long term treatment.

Control

Effective eradication can be achieved by slaughtering all tuberculin positive animals, if the economics of the livestock industry permits. However such a policy however is beyond most Asian countries where over 96 per cent of the buffalo population exists. The policy may however be modified by slaughtering all clinical cases, testing the rest of the herd and dividing them as reactors and non-reactors. The reactors could be tested repeatedly every six months and the number of animals in the reactors could be reduced by rearing the off-springs in "clean" premises. All attempts should be made to prevent spread of the infection by isolating infected animals and by avoiding the introduction of infected animals to "clean" herds.

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2.7 Paratuberculosis

N.U. Horadagoda

Definition

Paratuberculosis (Johne's disease) is an infectious, contagious and almost invariably fatal enteritis caused by *Mycobacterium paratuberculosis*. The disease is characterised by wasting, chronic diarrhoea and there is thickening and corrugation of the wall of the intestine on postmortem examination.

Aetiology

Paratuberculosis is caused by *M. paratuberculosis* (*M .johnei*), a small acid fast bacillus which is relatively slow to grow in the laboratory. The organism is relatively resistant to light and disinfectants and may survive for many months in faeces, water and soil.

Occurrence

Johne's disease is widespread in buffaloes in many countries. It is known to occur in all breeds of buffalo and cattle in India and is endemic, since it was first detected in the early part of this century (Mohan, 1968). Johne's disease is a chronic infection where the mortality rate is usually less than 1% in infected herds, but ill health and reduced productivity due to the disease causes heavy economic losses.

Epidemiology

Young animals are very susceptible and usually become infected at an early stage but develop the clinical disease only as adults. Stress, parturition and nutritional deficiencies influence the precipitation of the disease.

Transmission

The infection is acquired by ingestion food and water contaminated by faeces of infected animals. Some clinically infected animals excrete *M. paratuberculosis* in the milk and the organism has been isolated from semen of infected bulls. Although the disease may be transmitted to young calves through infected milk, venereal transmission is not considered important.

Clinical findings

Clinical signs usually appear when animals are between 3 and 6 years of age. The signs in paratuberculosis are not characteristic, but may be suspected if adult animals show chronic diarrhoea, submandibular oedema associated with progressive emaciation and wasting. The disease runs a long course and terminates in death.

Necropsy findings

Information on the clinical and pathology of Johne's disease in buffaloes is scanty but in general it appears to closely follow that reported for cattle. The lesions are confined to the terminal part of the small intestine and extending into the large intestine. The mucosa is thickened and corrugated and has a characteristic "morocco leather" like appearance.

Diagnosis

Diagnosis is difficult owing to the lack of reliable tests. Clinical signs may give an indication of the infection. The organism may be demonstrated by microscopic examination of the of faeces or rectal mucosa but several examination need to be carried out before a definite diagnosis is made. Johnin test is an aid to detection of the disease but is found to be unreliable.

Treatment and control

Treatment is not effective even with drugs which have a high *in vitro* activity against *M. paratuberculosis*. Infected animals should be segregated and faeces properly disposed off. In endemic areas it is advisable to vaccinate young animals with a vaccine in which live bacilli are suspended in lanolin but a major complication of vaccination is that vaccinated animals become positive to both the Johnin and the tuberculin tests.

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Chapter 3

BACTERIAL DISEASES OF THE REPRODUCTIVE SYSTEM

3.1 Brucellosis

B.D.R. Wijewardana

Introduction

Brucellosis is a condition manifested by abortions and infertility. It is also known as Bang's disease and Contagious Abortion. It is a zoonotic disease and the human form, called Malta fever came into prominence during the second world war where congregating troops were infected through consuming raw goat milk and milk products. It usually causes an acute febrile illness commonly termed as undulant fever, but the chronic form may involve the musculo-skeletal, cardiovascular and central nervous systems with serious complications (Anon, 1996). Economically, brucellosis is a disease of major importance in countries where it exist, both in terms of veterinary and public health aspects. In the Mediterranean and middle eastern countries bordering the Mediterranean sea leading up to southern parts of USSR, Mongolia and northern China and, countries in Latin America where consumption of raw goat milk and milk products are common, the incidence among humans is alarming (Alton,1985). Brucellosis results in loss of calves, reduction in the milk yield and other infertility problems. The estimated losses due to Brucellosis in bovines in India was Indian Rs. 311.47 million (1 USD = Rs 40) (Mathur and Sharma, 1974) and in Sri Lanka the loss in around Sri Lankan Rs. 145 million (1 USD = Rs 70) (Gajanayake personal communication).

Aetiology

The Genus *Brucella* comprises six species. They are *B.abortus*, *B.melitensis*, *B.suis*, *B.neotomae*, *B.ovis* and *B.canis*. Bovine brucellosis is caused by *B.abortus*, but infection due to *B.melitensis* could also occur, while *B.suis* could cause the infection on very rare occasions. *B.abortus* has 9 biovars while the other two have 3 each. The clinical manifestations however are similar (Verger and Grayon, 1985).

Epidemiology

Brucellosis is endemic in most developing countries, while countries in the developed world the infection has been eliminated through eradication programmes. The disease is known to occur in cattle, buffaloes (*Bubalus bubalis*), African buffaloes (*Syncerus caffe*), goats and sheep, dogs, humans and other wild and domesticated ruminants (Radostits, *et al.*, 1994). In buffaloes, bison, yaks and elk the course of infection due to *Brucella abortus* is similar to that in cattle (OIE,1995).

Brucellosis was first recognised in Sri Lanka in 1953 in a state sector livestock farm (Perumal Pillai and Kumaraswamy 1957). Since then the disease has been detected in all parts of the island, other than in the up country areas (De Alwis *et al*, 1993). Brucellosis in buffaloes have been reported from Sri Lanka, Egypt, Iraq, Vietnam, India and Africa in recent times.

Brucellosis is not a sexually transmitted infection. The infective organisms are present in massive numbers in the aborted foetuses, placental tissues and vaginal discharges. Organisms are also excreted in the milk. Infection occurs through the nasopharynx, conjunctiva and abraded skin (Alexander *et al.*, 1981). Contamination of the environment takes place during and after calving, whereby the bacterium could survive for long periods in pasture, soil and the ground in the temperate countries. Direct sunlight and heat destroys the organism (Quinn, 1984). Infected animals are considered life-long carriers. Calves born to infected cows could acquire the infection either congenitally or through the milk. These animals remain sero-negative until the time of abortion or parturition, during which period they would commence shedding the organisms in large numbers in the aborted foetus, fetal membranes and vaginal discharges (Crawford *et al,* 1986). Non-pregnant animals which become infected also remain sero-negative until the time of parturition or abortions. In these animals the organisms become localised in the udder and in the uterus if they become pregnant (Lapraik and Moffat, 1982).

Infected bulls usually do not transmit the infection venereally. However, the chances of spread are greater if the semen is used for artificial insemination (Salman and Meyer, 1984).

Pathogenesis and clinical signs

Initially, following infection the organism localises in the lymph nodes draining the predilection sites, which are the pregnant uterus, udder, testicles and accessory male sex glands, joint capsules and the bursae. In the non-pregnant adults and congenitally infected animals the organisms localise in the udder and infect the uterus periodically if the animal becomes pregnant. Erythritol is a substance known to stimulate the growth of *B.abortus* . This occurs in significant amounts in the placental and foetal fluids, which explains why these sites are localised during parturition. Following the invasion of the uterine wall the organisms enter the lumen causing endometritis. The allantochorion, foetal fluids and placental cotyledons are next invaded. Abortions usually occur in late pregnancy. The second pregnancy could be a normal one. The resultant calf could be an infected animal, yet sero-negative, but would commence shedding the organisms during and after parturition (Radostits et al., 1994).

Abortions usually occur after the fifth month of pregnancy, with retention of placenta and metritis being the common sequelae. In infected bulls, orchitis and epididymitis may occur occasionally.

Diagnosis

Bacteriological examinations

The procedures followed are according to those described by the OIE (Anon,1996). The required samples are aborted foetus, stomach contents, vaginal discharges and vaginal swabs or paired sera collected 1-2 weeks after abortion and again 2-3 weeks later. The samples have to be dispatched at the very earliest under refrigeration conditions. If a postmortem examination is carried out mammary gland, uterus, supramammary and internal illiac lymphnodes should be collected from females and; from males testes, epididymes, seminal vesicles, accessory glands, external inguinal and internal illiac lymph nodes should be collected. Colostrum and milk are good sources of the organisms.

Smears of placental cotyledons, foetal lungs, liver and abomasal contents and vaginal discharges should be made and fixed by heat or with ethanol. These could be stained by either modified Zeihl-Neelsen, Kosters',Gram or Machiavello methods. Fluorescence antibody technique also could be used. The organisms are intracellular and are stained weakly with the acid fast stains.

The samples are cultured on serum-dextrose agar and serum dextrose agar supplemented with bacitracin (25 μu/ml), cycloheximide (100 μu/ml), nalidixic acid (5 μu/ml), nystatin (100 units/ml), polymixin B (μu/ml) and vancomycin (20μu/ml).

Milk, colostrum and tissues contain lesser number of the organisms and could be contaminated. Therefore a liquid medium of either serum dextrose broth or tryptone soya broth supplemented with amphotericin B (1 μu/ml), bacitracin (25 μu/ml), polymixin B (6 μu/ml) and vancomycin (25 μu/ml). The enrichment medium is incubated at 37^0 C in air supplemented with 10 % (v/v) CO_2 for up to 6 weeks with weekly subcultures onto solid selective medium. A biphasic medium could also be used and is reported to be more efficient than the solid medium in the ability to isolate Brucella organisms.

Brucella colonies are convex, round, transparent with a smooth surface and are slow growing. The organisms are gram negative cocco-bacilli or rods with rounded ends slightly convex sides.

A presumptive identification could be made by agglutination with a Brucella antiserum. Confirmation of the species and determination of the biovars are usually carried out at reference laboratories. These include growth characteristics with regard to urea, hydrogen sulphide, carbon dioxide, basic fuchsin and thionin and, agglutination with mono specific antisera and phage typing.

Serological Tests

The serological tests are according to procedure practised at the Central Veterinary Laboratory, UK which is the OIE, FAO, WHO Reference Centre for Brucellosis (Morgan *et al,* 1987).

Rose Bengal Plate Agglutination Test (RBPT): Killed whole cells of *Brucella abortus* strain 99 (Weybridge) stained with Rose Bengal, are used in the RBPT at a cell concentration of 1g per 22.5. ml of phenol saline adjusted to a final pH of 3.65. Equal volumes of test serum and the stained antigen are mixed together on a glass plate by agitation and observed for agglutination after 4 minutes.

Complement Fixation Test: The recommended confirmatory test is the complement fixation test (CFT). *Brucella abortus* strain 99 is used as the antigen which is standardised against the international standard *Brucella* antiserum.

Milk Ring Test: Killed whole cells of *Brucella abortus* strain 99 are used as the antigen in a solution of haematoxylin stain. It is used to screen herds of lactating cows. The test is very useful in that bulk milk originating from herds could be examined. A positive reaction is indicated by the formation of a dark blue ring at the top of the column of milk.

Control and eradication

Brucellosis in animals is a herd problem, whereas in humans it is regarded as an individual problem. Even if a single animal is infected the whole herd is assumed to be infected.

The first step is strict adherence to basic hygienic principles so as to contain the infection within the premises and prevent infection in humans. The infective organisms are shed during and after parturition. These are found in aborted foetuses, fetal membranes, vaginal discharges, colostrum and milk. An infected pregnant animal should be kept in isolation at least for 4 days before and 4 weeks after parturition. After parturition or abortion the fetal membranes should be handled safely and incinerated. The area should be thoroughly disinfected using any common disinfectant. If a normal calving occurs, the calf should be separated at the earliest opportunity and fed with milk which is well boiled. This is to prevent infection through ingestion of contaminated milk.

In an eradication programme a test and slaughter policy is adopted. Animals are tested at six monthly intervals and those found positive by the CFT are slaughtered. Once a herd is found negative by the CFT in two consecutive examinations it could be declared free from Brucellosis. This has been successfully adopted in most of the developed countries.

Due to social and financial factors, vaccination is preferred over the test and slaughter policy in the developing countries. The S19 vaccine is largely used. Vaccination of adults are discouraged due to the persistence of antibodies which interferes with serological tests. Vaccination of calves aged 4-8 months with a reduced dose is practised where the complement fixation antibodies become absent after 1 year. Vaccination reduces the incidence of abortions but a corresponding reduction in the level of infections cannot be achieved. Approximately 65-75% of the vaccinated animals would be resistant to exposure, while the remaining would be susceptible to infection but would not abort. Continuous exposure to the infective agent would result in the vaccinated animals becoming infected and subsequently developing into carriers. Vaccination is usually terminated when the prevalence is 0.2% or below and eradication of the infection through test and slaughter policy is recommended (Anon,1986).

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3.2 Leptospirosis

T.G. Wijewardana

Introduction

Leptospirosis occurs among most mammals including humans and, could cause septicaemia, interstitial nephritis, haemolytic anaemia and abortions (Rhadostits, *et al* 1994). Transmission occurs from animals to man and is therefore a zoonotic disease. It is a zoonotic disease which has a world-wide distribution. The organisms of the genus *Leptospira* are believed to be among the most widespread organisms occurring in nature (Amatredjo *et al.*, 1975). Economically, leptospirosis is a very important disease in livestock causing abortions, still births, infertility, decreased milk production, and even death. Prenatal losses due to serovar *hardjo* has been estimated to be 2.2 %. The enormity of annual calf losses and the gravity of the problem could be envisaged in countries like South and Central America with a cattle population of 220 million (Ellis, 1984). It is an occupational disease in man, the risk groups being the agricultural farmers, workers in mines, abattoir and laboratory workers and wool workers. The human form of the infection was first termed as Weils' disease, while in China it was known as rice harvest disease, and in Japan as autumn fever. It was also known as cane cutters disease, swine-herds' disease and mud fever (Faine, 1993). In Sri Lanka the disease has been reported among farmers in paddy fields, gem miners, livestock farmers and laboratory personnel handling contaminated material.

Aetiology

The term leptospirosis refers to a range of disease syndromes in man and animals associated with infection by spirochetes of the genus Leptospira. There are two species within the genus *L.biflexa* which is considered non-pathogenic and *L.interrogans* which is pathogenic. The latter comprises 26 serogroups with over 200 serovars within serogroups which are distributed world-wide (Faine, 1993).

In Sri Lanka, serovar *Weerasingha* had been the commonest among buffaloes with a prevalence of 30.2%, followed by *pomona* (26.6%), *hardjo* (24%), *pyrogenes* (11.3%) and *canicola* (5.2%) (Wijewardana *et al.*, 1995). In the same study three isolations were made. One belonged to serogroup *Javanica* serovar *ceylonica*. A bovine isolate of serogroup *Javanica* serovar *ceylonica* has also been detected in Sri Lanka (Anon, 1996).

Leptospirosis among buffaloes had been reported from India (Ratnam *et al.*, 1995), Malaysia (Bahaman *et al*, 1987), Philippines (cited by Ciceroni *et al.*, 1995), Macedonia and Italy (Ciceroni *et al.*, 1995). In Tamil Nadu State of India serogroup *Autumnalis* followed by *Pomona* were the prominent serogroups in buffaloes with an overall prevalence of 39.3% (Ratnam *et al.*, 1995). In Italy, Macedonia, Malaysia and the Philippines the dominant serogroup among buffaloes was *Sejroe* (Ciceroni *et al.*, 1995).

Epidemiology

The organism is excreted via urine and milk. Direct contact with the organisms could lead to an infection through abrasions in the skin or the mucosa and, it could also be transmitted by inhalation (Mazzonelly, 1984), or through the conjunctiva (Amatredjo *et al.*, 1975). Infected tissues, body fluids, urine and, virulent laboratory cultures act as sources of direct infection,

while contaminated food and water are considered as indirect sources of infection (Kingscote, 1986). Following an incubation period of 4-10 days the organisms spread throughout the body via the blood stream, lasting for periods ranging from few hours to 7 days. Finally, though these are cleared from most of the tissues, localisation occurs in the proximal renal tubules and in the female reproductive tract resulting in a carrier stage, which is considered as the foci of infection for animals and man (Ellis, 1984). Infection usually leads to a carrier stage in the renal tubules with shedding of the organisms in urine for over a period of an year (Leonard *et al.*, 1992). The leptospires are able to survive outside the host in environs having warm, wet conditions with a pH close to neutral (Faine, 1993). Such conditions are abundant in Sri Lanka, where the paddy fields, gem pits, stagnant water pools, water reservoirs and irrigation canals which are frequented by domesticated as well as wild animals when contaminated provide an ideal environment for spread of the infection, to other animals as well as humans.

In Sri Lanka an epidemiological survey had revealed a sero-prevalence of 41.9% among buffaloes in regions where human leptospirosis had been recorded. (Wijewardana *et al.*, 1995). It has also been recorded among cattle (Peiris and Wettimuny, 1972; Anon, 1996), dogs and goats (Babudieri and Jagels, 1962; Anon, 1997) and, humans (Babudieri and Jagels, 1962; Rajasuriya *et al.*, 1964; Walloopillai *et al.*, 1966).

Clinical Signs

The disease could manifest in subacute, acute or chronic forms. The clinical signs are not specific for any particular serovar and they are not pathognomonic for leptospirosis. In water buffalo, deer and cattle, the clinical signs are similar (Flint *et al*, 1986; Bahaman *et al*, 1988). In the subacute and the acute form the animals may be weak, depressed, anorexic, with anaemia, diarrhoea, injected conjunctival blood vessels, high fever and diarrhoea. Lactating animals may show discoloured and clotted milk with the production becoming very low. The udder would be flaccid (Higgins *et al*, 1980). In severe cases haemoglobinuria would be the first sign. Death usually follows due to kidney and liver degeneration 3 to 10 days after onset of the clinical signs. Some animals could be jaundiced and show signs of encephalitis. In pregnant animals abortions or still births could occur during the last third of the gestation period or result in birth of weak calves (Ellis *et al*, 1976a; Ellis *et al*, 1985b; Prescott *et al.* 1988).

Diagnosis

Clinical signs are not pathognomonic and therefore, laboratory confirmation is required for a conclusive diagnosis. Serology or bacteriological examinations could be employed.

Serum samples collected 2-3 weeks apart are examined by the Microscopic Agglutination Test (MAT) for the presence of antibodies and identification of the specific sero group and the serovar of the leptospira organism. A rising titre indicates a current infection while in unvaccinated animals the presence of antibodies indicates exposure to the organisms.

Bacteriological isolation is costly, slow and laborious. Samples required are blood, urine, cerebrospinal fluid and tissues such as brain, kidney and eye. However, after 5-7 days of the onset of the infection it is difficult to isolate the organism from these samples other than from brain, anterior chamber of the eye and the renal tubules.

In acute cases, blood (uncoagulated), peritoneal fluid or pleural exudate and urine could be examined by direct microscopy within the first 7 to 10 days. Samples should not be frozen and must be despatched to the laboratory without any delay. In chronic cases and carrier animals the organisms would be found only in the brain, anterior chamber of the eye, genital tract and the kidneys.

The enriched medium used for bacteriological isolation is available commercially. Volumes of 0.1 to 0.2 ml of blood are inoculated into each of 4-5 bottles containing 5 or 10 ml of Ellinghausen, McCullough, Johnson and Harris (EMJH) medium (Difco) and incubated at 30 ^{0}C and examined after 1, 3 and 5 days. Cerebrospinal fluid may be inoculated into 5 ml of semisolid medium in 0.5 ml volumes.

Urine has to be taken at mid stream, preferably after administering a diuretic, and has to be alkaline. Therefore in carnivores urine should be made alkaline. In the acute phase the urine may not contain leptospires and leptospiruria is usually seen 14 to 28 days after the onset of the infection. As the sample could be heavily contaminated a selective media containing sulphathiazole (50 µg/ml, neomycin sulphate (5 µg/ml) and cycloheximide (0.5 µg/ml) is recommended. Due to the inhibitory effects of the selective agents subcultures are to be made within 48 hours of inoculation.

Tissues obtained at autopsy and from aborted or stillborn foetuses, foetal tissue, placenta and abortion products should be submitted for bacteriological examinations. Tissue material in one gram quantities are macerated in a small volume of sterile culture medium and the supernatant in 0.1 to 1 ml volumes at a dilution of 1:100 are inoculated into the EMJH medium. Blind subcultures would improve chances of isolation.

Treatment

Dihydrostreptomycin at the rate of 25 µg/kg, given two weeks apart by the intramuscular route had been reported to be effective against excretion of the organisms in the urine. When animals are to be transported the regime has been recommended with the second dose given within 24 hours of transport. For clinically ill cattle and pigs the same dose could be given for 3 consecutive days. Alternatively 5 g could be given twice a day for 3 consecutive days for cattle. Chemotherapy is expensive and, is justifiable in breeding animals or very valuable genetically superior animals (Faine,1993).

Prophylaxis

In developed countries prophylaxis has been carried out with the objective of reducing the incidence of the condition among humans. Basic hygienic measures such as proper clothing and safety-ware had been found to be very important. It is generally regarded that once a herd is infected it remains infected, because it is nearly impossible to break the chain of events which leads to the recurrence of infection, either by the same serovar or another. Elimination of rodents who are carriers of leptospires, prevention of animals entering possible environmental sources such as stagnating water pools or swampy areas are also important in curtailing the incidence of leptospirosis.

In some countries vaccination has been recommended as a routine control measure. In countries such as Australia and New Zealand vaccination is carried out with the ultimate objective of protection of humans rather than the livestock. The value of vaccines, however, is in doubt as its' efficacy has been found to be only 67 % in Australia. Bi-annual vaccinations have to be carried out, and this could be very costly (Hancock *et al.*, 1984).

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Chapter 4

VIRAL DISEASES

4.1 Foot and Mouth Disease

S.N. Kodituwakku

Foot and Mouth Disease (FMD) is a highly contagious viral infection of cloven-hoofed animals caused by aphthovirus belonging to the Picornaviridae family. It is one of the most economically important diseases included in List A of the World Animal Health Organization, the Office International des Epizooties (OIE). The significant economic impact of FMD has been well accepted by Asian countries.

In Asia, twenty two countries have officially reported the presence of FMD infection. Out of the seven serotypes of the FMD virus, four serotypes have been reported to cause the disease in Asia. They are "O", "A", "C" and "Asia 1" types. Type "O" predominates though types "Asia 1" and "A" are also found. Type "C" occurs rarely despite the susceptibility of a high proportion of animals to this serotype. It is not included very often in the vaccines.

Table 4.1.1 The distribution of the serotypes and occurrence of FMD in both cattle and buffaloes in Asia are given in the table below.

Country	FMD virus types*	Disease occurrence**
Bhutan	A, O	++
Bangladesh	A, O, C, Asia 1	++
Cambodia	O, Asia 1	++
India	A, O, C, Asia 1	+
Iran	A, O	++
Israel	O	+
Jordan	O	++
Kuwait	O	++
Laos	O, Asia 1	+++
Malaysia	A, O, Asia 1	( )
Mynmar	O, Asia 1	+++
Pakistan	A, O, C, Asia 1	++
Philippines	A, O, C	(+)
Sri Lanka	O	++
Thailand	O, Asia 1	++
Vietnam	O	++

*Source: Diagnosis and Epidemiology of Foot-and-Mouth disease in Southeast Asia. Proceedings of an International Workshop held at Lampang, Thailand. Sept. 6-9, 1993.

** Sources:
Report of the third meeting of the OIE sub-commission for Foot and Mouth disease in South-East Asia with the participation of FAO/IAEA, Manila, Philippines, 24-28 Feb. 1997, Monthly OIE Bulletin, Jan. to June 1997.

+	low sporadic occurrence	+ + +	high occurrence
+ +	enzootic	(+)	exceptional occurrence
( )	confined to certain regions		

Sri Lanka has reported the presence of only type "O" since 1985. FMD due to virus type "C" which was reported in Sri Lanka in 1970 has not been reported since 1984. It is believed to be introduced through cattle imported from India.

FMD virus frequently isolated from water buffaloes in Asia are type "O", "A", "Asia 1" . In India between 1973 and 1981 there had been 615 outbreaks of FMD and only 70 outbreaks involved buffaloes affecting 14 % of the buffalo population. The predominant serotype was "Asia 1" although serotypes "O" and "C" were also reported (Dutta *et al.* 1983).

The World Reference Laboratory (WRL) has reported simultaneous isolation of more than one serotype from a single sample of cattle epithelial tissues submitted from field cases (Donaldson 1993). It is also reported that samples from countries where the disease is endemic such as Turkey (Asia minor), Saudi Arabia, Kenya and Nepal the presence of either two or three serotypes have been revealed.

Transmission of FMD

FMD is capable of rapid horizontal transmission between infected and susceptible cloven-hoofed animals. The acute phase of disease usually lasts three to four days. During this phase all excretions and tissues contain virus and these animals become spreaders of the disease. It is well known that animals excrete the virus even before the manifestation of clinical signs. For example, excretion of virus in semen and milk has been detected four days before the clinical signs develop and in sheep excretion of virus in breath was reported 24 hours before the clinical signs were noticed (Sellers and Parker 1969; Burrows, 1968).

The behaviour of FMD virus in Asian buffalo (*Bubalus bubalis*) is less extensively documented when compared with the wild African buffalo (*Syncerus caffer*). Although these two types of buffaloes have a similar physical appearance, it is not possible to extrapolate the features of FMD virus interaction of African buffalo to Asian buffalo because they belong to different genera (Thomson, 1996). The number and pattern of their chromosomes differ. However it has been shown from studies in India that FMD spreads among water buffalo and also to in-contact goats and simultaneously infection in cattle, pigs and goats (Dutta *et al.* 1983). Under controlled laboratory conditions using FMD virus of bovine origin, the transmission of the virus from infected buffalo to susceptible in-contact cattle was similar to transmission between cattle (Gomes *et al.* 1997). This clearly shows that the FMD virus need not be adopted to cause disease in buffalo (Gomes *et al.* 1997). Transmission of virus from infected cattle to susceptible buffalo occurs in the same way as from buffalo to buffalo. During the recent outbreak of FMD in Sri Lanka, both cattle and buffaloes sharing common grazing fields were seen to be affected (author's experience).

The most important mechanism in the transmission of FMD is the movement of animals. Movement of contaminated animal products such as milk, meat, offal, untreated hides and skins fall into the second important mechanism of spread. Veterinarians and cattle keepers in- contact with incubating or infected animals, artificial inseminators do play a part in the transmission of FMD. In the early stages of outbreaks where the disease is not clearly manifested and recognised, the vehicles, milk collecting utensils and milk tankers play an important role in transmission. Lastly, the airborne spread and carrier state could also play a part in transmission (Donaldson, 1993).

Reports indicate that around 80% of FMD infected ruminants can become carriers after recovery and could initiate fresh outbreaks in fully susceptible animals when in contact (Donaldson, 1993). FMD vaccinated animals can also become carriers when exposed to infection. The site of carriage is the pharyngeal region. The duration of carrier state varies with the host species and the strain of virus. It is also accepted that, other unidentified factors too would determine the duration of carrier state. The role of African buffalo (*Syncerus caffer*) in maintaining FMD in Africa has been studied extensively and it is documented that African buffalo could carry the virus for up to 5 years. Experimental studies undertaken in Egypt has shown that water buffalo (*Bubalus bubalis*) can carry virus up to two months (Moussa, *et al.,* 1979). In Brazil, it has been reported that young and adult water buffaloes become carriers without showing lesions, while cattle sharing the same grazing land showed typical generalised lesions (Samara and Pinto, 1983).

Pathogenesis

Most commonly the FMD virus infects via the respiratory route especially in ruminants. A very small dose can initiate an infection (Sellers, 1971). The primary region of viral replication is in, and around the pharynx. The virus gain entry to pharynx through inhaled droplets or aerosol particles which enter directly or moved by mucociliary activity. Infection can also occur through a break in the skin or mucosa of an animal. Only a very small quantity of virus could bring about infection in such instances. Therefore, injection of improperly inactivated FMD vaccine, foot rot, feeding coarse fodder, harsh use of milking machines, surgical procedures and damage to animal's nostrils during restraint can all serve as entry points for FMD virus.

Multiplication of the virus takes place in the primary sites of the pharyngeal area and associated lymph glands. Later the virus enter the blood stream where the organisms are carried to secondary sites in glandular organs, lymphatic glands and epithelial tissues, in an around the mouth and feet where vesicles develop. Vesicles may also appear on the mammary glands of female animals. When the FMD virus invasion occurs through the lesions in the integument, replication commence in the epithelium and local lymph glands before entering the blood stream. Virus can also enter the blood stream directly with deeper introduction such as in surgical procedures (Donaldson, 1993).

The incubation period depends on the virus strain, dose of exposure and route of entry. Usually with high exposure dose through natural routes, the incubation period is short, around two to three days, but would extend up to ten to fourteen days with very low doses (Donaldson, 1987). The exposure dose in index cases is low and therefore the incubation period is long and the clinical signs may pass unnoticed. Amplification of virus takes place during the first cycle and thereafter the infection spread rapidly and the incubation period gets reduced.

The susceptibility of indigenous cattle and buffalo to FMD and its pathogenicity had been observed in Sri Lanka to vary from outbreak to outbreak (Fernando, 1969). Recent outbreaks of FMD in Sri Lanka after a lapse of two years (1995/96) revealed that the most common lesions in buffaloes were in the feet. Mouth lesions were mild. The pattern of infection of FMD virus in inoculated and in-contact buffaloes was similar to that observed in cattle (Gomes *et al.*, 1997). The virus replicated in the pharyngeal area and all the experimental animals had temperatures above 40^0 C on the day before or on the day the

characteristic vesicular lesions developed. However appearance of tongue lesions in the buffalo were rare and observed to be less severe than foot lesions.

Data from a questionnaire circulated by OIE to countries in South-East Asia indicated that water buffaloes are highly susceptible to FMD, and lesions produced are similar to those of cattle. Lameness appears to be a characteristic sign. The number of days for recovery varies from 12 to 21 days. It also appear that the disease in water buffalo occurs at certain times of the year (OIE Subcommission report, 1997). Gomes *et al.*, (1997) has observed that there is no difference between the persistence of the virus in the pharyngeal region of infected and in-contact cattle or buffalo for up to 35 days.

Clinical Diagnosis

In typical field cases, there may be an incubation period of 1 to 7 days. The onset of disease is manifested by a drop in milk yield, high fever (40 – 41^0 C), severe dejection, anorexia resulting from an acute painful stomatitis. There is salivation, characteristic smacking of the lips and chewing carefully. Vesicles appear in the buccal mucosa, on the dental pads, tongue, in the clefts and on the coronet of feet. These rupture within 24 hours, leaving a raw painful surface, causing severe discomfort to the animal. Hence, lameness and inappetance are the main signs that are usually observed by the farmer. The vesicles heal in about a week. The coronet could get swollen and the animal often become recumbant. Secondary bacterial infection of the lesions interfere with healing and may lead to the involvement of deep structures of the feet (Radostits, *et al.*, 1995). Young calves are highly susceptible and heavy mortality due to acute myocarditis is common even without typical vesicular lesions in the mouth and feet.

Differential Diagnosis

In buffaloes, foot lesions are most severe and common while the oral lesions are usually mild.

Laboratory Diagnosis

Diagnosis of FMD is carried out by direct detection of the virus in field samples by immunological methods such as micro-CFT and ELISA. Isolation of FMD virus in suckling mice or in cell culture is also practised when the field sample is not adequate to perform the conventional tests. Strain differentiation studies of FMD virus are carried out in India based on ELISA and two dimensional micro-Serum Neutralization Test (SNT). Modern technologies such as Polymerase Chain Reaction (PCR) for characterization of FMD virus is also available in India (Natarajan *et al.*, 1993).

Control

The strategies available for the control of FMD include the following:

• Stamping out	• Quarantine
• Tracing the origin of disease	• Movement control
• Combined strategies	• Vaccination
• Legislation	• Import/Export regulations
	• Zoosanitary measures

In Asia, vaccination is undertaken to control the disease. Routine vaccination of buffalo population against FMD is been practiced by some countries in Asia. The total cattle population in Asia is estimated to be more than 430 million heads and only 09 countries produce the vaccine locally. Thus it is evident that the vaccine produced in Asia is grossly inadequate to meet the requirements of the region.

Bhutan has introduced a control programme since July, 1996 where selective vaccination is done only in the most vulnerable herds and valuable stock owned by the Government and farmers' to minimize the expenses. On the other hand, India has introduced an extensive FMD control programme in selected areas so as to develop free zones through systematic vaccination of all susceptible animals. It is clearly evident that such a system would work only if movement of animals is strictly controlled. In Iran, during 1996, vaccination of susceptible animals has been carried out using locally produced inactivated bivalent vaccine. It is reported that 4.6 million heads of cattle and 15.7 million sheep and goats have been vaccinated the year. Since the local production of FMD vaccine does not meet the demand production capacity has been improved and expanded to produce 25 to 30 million doses of vaccine. In Jordan, cattle are vaccinated three times annually in high risk areas and twice in other parts of the country. Vaccinations have been extended to small ruminants in 1997. The control strategy adopted by Pakistan include vaccination of susceptible animals twice a year in February and December. In Sri Lanka routine vaccinations are confined to cattle and buffaloes. However, the vaccination coverage has always been low. In case of an outbreak, ring vaccination is carried out in surrounding disease-free areas.

The performance of FMD vaccine in the field depends on several factors, namely:

- Relationship between the vaccine virus and field virus strains
- Vaccine storage and maintenance of the cold chain
- Influence of passive maternal antibodies
- Interference of immuno suppressive infections or infestations
- The level of management and efficiency of vaccination programmes

It is unfortunate that for the control of FMD, vaccination and protection are not the same (Lombard and Scherumbrucker, 1993). This is clearly evident throughout the world where in some areas the prevalence of FMD continues despite comprehensive vaccination programmes. Major constraints in controlling FMD are similar in most of the countries in Asia. These include:

- Poor vaccination coverage
- Difficulty in maintaining the cold chain during transport of vaccine
- Existence of both exotic and indigenous animals with different levels of susceptibility
- Multiplicity of types and strains of FMD virus
- Wide host range
- Existence of carriers
- Unrestricted movement of animals
- Difficulty in enforcing legislative measures.

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NA-218-99

4.2 Rinderpest

R.Hettiarachchi

Rinderpest is an ancient *Asiatic plague* of cattle and buffalo and has been known and feared for as long as written records have been kept (Scott, 1981). It probably has caused more losses in cattle and water buffalo than any other single disease in the history of livestock production and have been witnessed since the initiation of veterinary education (De Tray, 1980).

Although natural infections occur in the even-toed *ungulates* belonging to the order *Artiodactyla*, the disease is most commonly observed in domestic *ungulates*, particularly buffaloes and cattle. Goats and sheep often contract mortal rinderpest in India, but elsewhere the disease has been recognized in these species only sporadically.

The causative agent is a member of the *Morbillivirus* genus in the family of *Paramyxo viridae*. The virus shares antigens with the other members in the genus, *viz* canine distemper, human measles and peste des petits ruminants viruses.

Although rinderpest had been introduced into all the continents in the world, subsequently it was confined to only eastern Africa and Asia. In Asia, it is found in India, Sri Lanka, Pakistan, Afghanistan, Saudi Arabia, United Arab Emirates, Oman, Iran, Iraq, Yemen and Turkey (Animal Health Year Book - 1995). In India, the disease was confined mainly to southern-most part of the country such as Tamil Nadu, Andra Pradhesh and Karnataka. Even in these areas, no clinical case has been observed since late 1995. Clinical evidence of rinderpest has not been detected after mid 1994 in Sri Lanka and Iran.

Transmission

Rinderpest virus is excreted in the expired air, nasal and oral secretions, and in the faeces of affected *febrile* animals. The primary modes of dissemination are the nasal secretions and faecal excretions (Liess and Plowright, 1964). The infected droplets are inhaled and the virus penetrates through the mucosa of the upper respiratory tract. Since infected droplets are relatively large they settle quickly and there is no risk of wind -borne transmission of the virus (Hyslop, 1972). Transmission requires close contact between sick and susceptible animals; the distance between sick and healthy animals has to be less than two meters and the contact has to last several hours (Idnani, 1944).

The rinderpest virus is unstable outside the animal's body. It is readily destroyed when the relative humidity lies between 50 and 60 percent. It is sensitive to heat, light and ultrasonic waves (Scott, 1959). High and low hydrogen-ion concentrations (pH) denature the virus consequently, rinderpest infected carcasses are rendered safe relatively quickly by the hydrogen-ion changes that follow autolysis and putrefaction, together with inactivating effect of high ambient temperature. As such, the virus is perpetuated by repeated short-cycle transmission from infected host to susceptible host (Scott, 1981). Recovered animals acquire an active resistance to the overt effects of re-infection which is generally assumed to be lifelong. As such, a frank clinical attack of rinderpest confers a lifelong immunity in these animals. Re-exposure results in an amnestic response in antibody levels (Plowright, 1962). Passive protection is conferred on the newborn calves of immune dams by the ingestion of

colostrum antibodies to rinderpest, and the protection once conferred persists for four to eight months (Brown, 1958). The ingested antibodies are not, however, secreted in the nasal mucus.

Pathogenesis

In cattle and buffaloes the virus invades the tissues of susceptible host through the mucus membranes lining the upper respiratory tract. Primary multiplication of the virus does not occur in the respiratory mucosae; the virus localizes instead in the pharyngeal and mandibular lymph nodes and in the palatal tonsil (Plowright, 1964). The virus multiplies in the above locations, and is disseminated in the blood attached to mononuclear cells (Scott *et al.*, 1986). Viraemia is evident one or two days before the onset of fever (Scott, 1955) and results in virus proliferation in the superficial and visceral lymph nodes, in the spleen and bone marrow, in the mucosae of the upper respiratory tract, in the lung and in the mucosae of the gastro-intestinal tract (Taylor *et al.*, 1965; Plowright 1964). The significant microscopic changes in rinderpest infections involve the lymphoid tissues (Maurer *et al.*, 1955). In particular, the lymphocytes in the germinal centres are destroyed. Multinucleated giant cells are present in stratified squamous epithelia and in lymphoid tissues being most readily detected in the tonsils (Plowright, 1965). Rinderpest virus induces a marked suppression of the humoral antibody response (Penhale and Pow, 1970) and cell - mediated immunity (Yamanouchi *et al.*, 1974).

Clinical Signs

Clinical signs in buffalo and cattle are similar and may be peracute, acute, subacute or even inapparent.

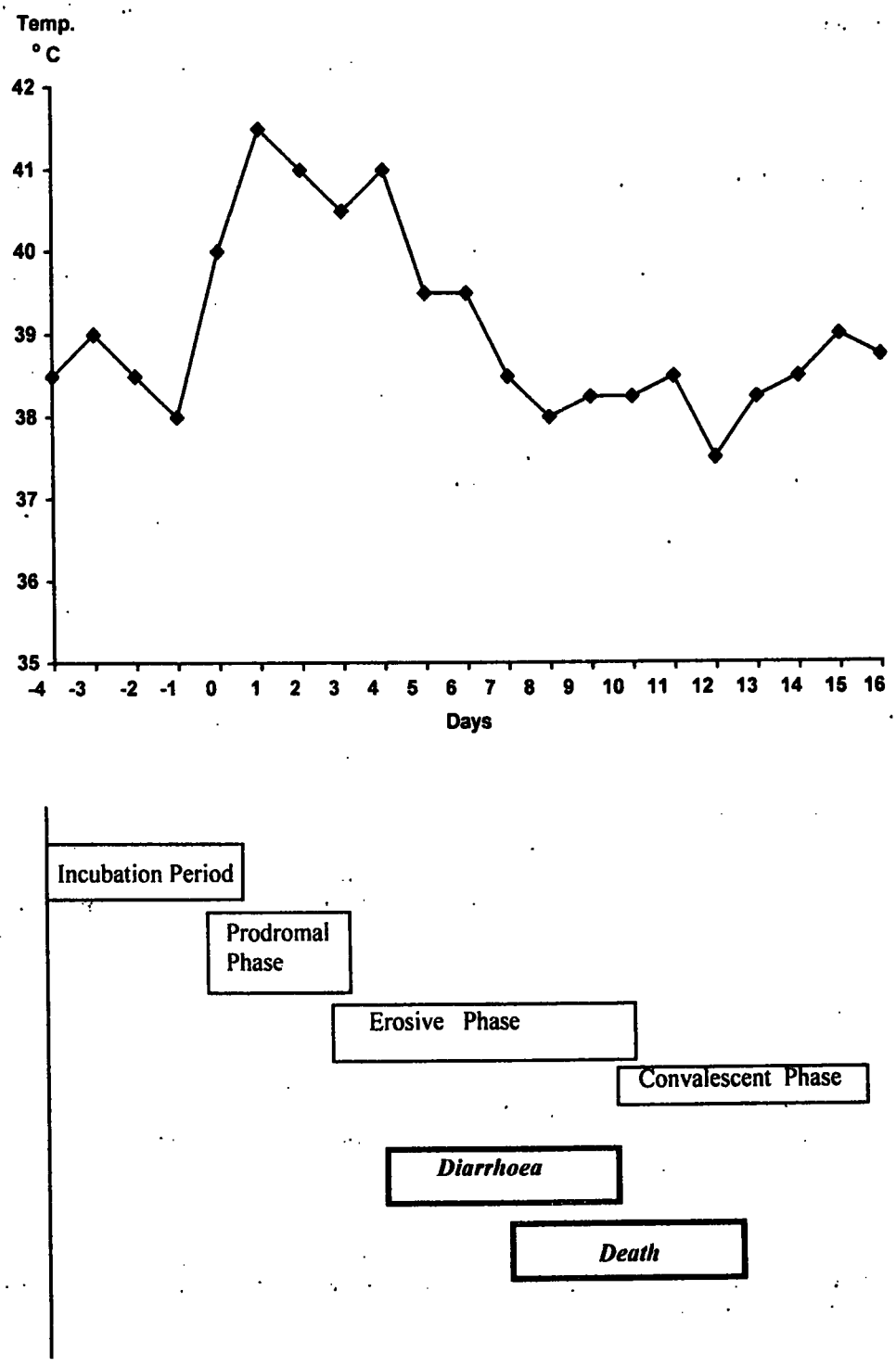
Peracute form

The onset of a peracute reaction is sudden and unexpected. It is manifested by inappetance, high fever, depression, severe congestion of visible mucosae, severe panting and racing pulse. Death supervenes within two or three days, even before mucosal erosions develop. Peracute reactions are not common, occurring most frequently in young calves and exotic animals.

Acute form

Acute reactions commonly occur in virgin epidemics involving animals with low innate resistance. The classic syndrome is divisible into three phases; the prodromal phase, the erosive phase and the convalescent phase in surviving animals (Figure 4.2.1).

Fig. 4.2.1 The clinical phases of classical rinderpest.



Incubation period ranges from 3 to 15 days being longest in animals having a high innate resistance. A sudden onset of fever marks the end of the incubation period and the start of the *prodromal phase* in which other clinical signs are minimal except in lactating cows whose milk yield falls. Overt illness is clearly evident 24 to 48 hours later; the animal becomes restless, depressed and then starts to manifest other clinical signs such as dry muzzle, staring coat, shallow rapid respirations, serous nasal and ocular discharges, congested mucous membranes, partial anorexia and constipation. Haematological examination reveals the onset of leucopaenia which particularly involves the lymphocytes.

The mucosal *erosive phase* begins two to five days after the onset of the prodromal fever with the appearance of raised necrotic pinheads of epithelium on the mucosae of the mouth, nostrils and uro-genital tract. The necrotic foci are readily abraded exposing shallow erosions with red raw floors. As the disease progresses the erosions enlarge and coalesce. Salivation is profuse. Thick yellow patches of necrotic cells begin to coat the nasal and lachrymal passages and mix with the secretions, producing mucopurulent nasal and lachrymal discharges. The breath is fetid. Respirations are laboured and painfull, characterized by an audible grunt when exhaling. At this stage, the affected animal appears obviously ill and very restless. It drinks copiously, stops eating and passes soft faeces at frequent intervals.

Diarrhoea starts as the fever falls, two to three days after the first appearance of the mucosal erosions. The fluid faeces are dark and often contain excess mucus, shreds of epithelium, necrotic debris and streaks of blood. The smell is fetid and offensive. Affected animals arch their backs and strain frequently, exposing congested and eroded rectal mucosae. Faeces are sometimes voided with considerable force resulting projectile diarrhoea. In fatal cases the diarrhoea worsens progressively, causing rapid dehydration and emaciation. Affected animals stand with lowered heads, sunken eyes and arched backs. The erosive phase does not usually last more than a week and animals may die at any time after the onset of diarrhoea. Deaths are attributed to the drain in electrolytes and body water (Heuschele and Barber, 1966). Animals surviving the erosive phase enter a convalescent phase during which there can be a remarkably quick re-growth of the oral epithelium and cessation of diarrhoea. Recovery of appetite and condition usually occur more slowly. Pregnant animals generally abort in the convalescent period.

Subacute Form

Subacute forms of rinderpest are encountered in immature and young adult stock indigenous to a country where the disease is enzootic. The incubation period tends to be longer than that of the acute form. The clinical signs are muted and often one or more of the cardinal features of the classical disease such as fever, mucosal erosions, mucopurulent nasal and ocular discharges or diarrhoea may be absent. Most of the affected animals will survive and the mortality rate is very low.

Rinderpest virus selectively destroys T and B lymphocytes but not memory cells (Penhale and Pow, 1970). Thus, latent infections, particularly protozoal infections, are exacerbated and frequently complicate the clinical picture.

Necropsy findings

Most buffaloes and cattle die 6-12 days after the onset of illness and, typically, the carcass is dehydrated, emaciated, fetid and soiled (Maurer *et al.*, 1956). The eyes are sunken, with the tear tracts scalded by a profuse muco-purulent discharge. The conjunctivas are congested and oedematous. Corneal ulceration occurs occasionally and bilateral corneal opacity rarely. The external nares and muzzle are encrusted with muco-purulent discharge. The hindquarters and flanks are soiled with the fetid fluid faeces.

In contrast, the carcasses of buffaloes and cattle that die early in the course of the disease, before the onset of profuse diarrhoea, are often in good condition, unsoiled and free of mucopurulent crusts and discharges.

The mucosa of the mouth and throat are eroded. Readily visible lesions in the forestomachs are minimal, whereas the pyloric region of the abomasum is always affected with necrotic patches of epithelium that slough to form bleeding ulcers, some of which contain black clots of blood. Payer's patches in the small intestine are severely affected; swollen, black from haemorrhage and friable from necrosis. Greatly distended capillaries packed with erythrocytes in the lamina propria form the so-called zebra stripes extending from the blind sac of the caecum to the anus; the lesions are prominent in the ileocaecal valve, the caecal tonsil and the crests of the folds of the caecal, colonic and rectal mucosae. In fresh carcasses of animals that die early in the course of the disease the stripes are bright red; but in the carcasses of animals that die later and in decomposed carcasses the stripes are greenish black. The severely eroded mucosa oozes blood into the lumen of the gut, which fills with dark, partially coagulated fluid.

The lungs are often normal except for a prominent emphysema in lingering cases when the animal dies after suffering severe respiratory distress. All lymphoid organs are affected, with the severest damage occurring in the mesenteric lymph nodes and the gut-associated lymphoid tissues. The lymph nodes are enlarged, soft and oedematous except in animals that die late in convalescence. Then, the lymph nodes are shrunken, greyish and show radial streaks in the cortex. The urinary bladder is congested and its mucosal surface is sometimes eroded and appears mottled with different shades of red.

Diagnosis

Provisional diagnosis is based on the history, clinical signs and postmortem lesions. Confirmation is made by (a) demonstrating the presence of virus antigen or genetic material, (b) by isolation of the live virus in cell-culture or susceptible cattle or buffalo, or (c) by the detection of specific antibody to the virus.

Samples for laboratory tests should be collected from live animals in the early stages of the disease or from fresh carcasses of animals that have died early in the course of the disease. Material for laboratory confirmation of rinderpest include biopsy samples of prescapular lymph nodes, samples of gum debris, tears, blood in anticoagulant and clotted blood. They have to be collected from live animals and transported to the laboratory in wet ice within 48 hours of collection. Prescapular and mesenteric lymph nodes, spleen and tonsil are selected from a fresh carcass for live virus isolation or antigen detection tests. Slices of these tissues

together with portions of affected mucosae placed in 10 percent formal-saline are suitable for histo pathological examination.

Agar gel immuno diffusion test (AGID), Counterimmunoelectrophoresis (CIEP) or Antigen-capture ELISA is commonly used to detect the antigen. The virus is isolated from buffy coat cells prepared from whole blood preserved with anticoagulants such as heparin or EDTA at concentrations of 10iu/ml or 0.5mg/ml respectively. Antibodies are demonstrated in serum either using an ELISA or virus neutralization test. Rinderpest antibodies are detected at seven days, post-infection. A rising titre in paired samples is necessary to confirm the disease.

Differential Diagnosis.

- Bovine virus diarrhoea and mucosal disease
- Malignant catarrhal fever.
- Foot-and-mouth disease (FMD).
- Peste des petits ruminants (PPR).
- Papular stomatitis.
- Infectious bovine rhino-tracheitis.
- Johne's disease.
- Pink-eye.
- Mycotic stomatitis.
- East coast fever.
- Gastrointestinal parasitosis.
- Poisoning.

Control and eradication.

Rinderpest free countries ensure their freedom by prohibiting imports of live ruminants and pigs from countries where the disease is present.

Control of virgin epizootics and eradication of disease is effective by slaughter policy with payment of compensation to the owners. This is the quickest, safest and least expensive method in a limited outbreak. Under the circumstances where the slaughter policy is unacceptable, isolation of sick cases and strict control over the movement of livestock will effectively control the spread of infection. These measures together with effective vaccination stamped out rinderpest from the Philippines and Ceylon when a slaughter policy could not be implemented (Capulong, 1965; Mahamooth, 1953).

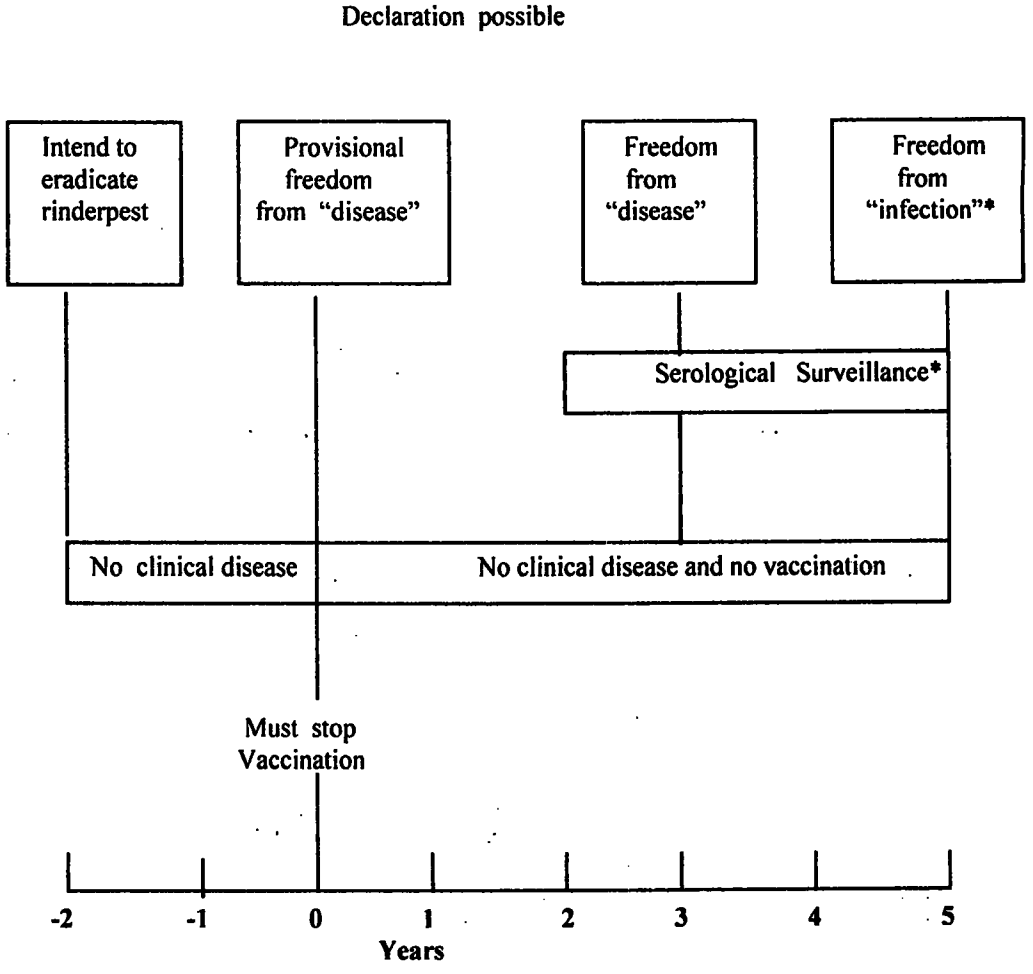
There is only one immunological type of rinderpest virus. Thus, the vaccine protects against all known strains of the virus. There is no post-vaccinal reaction and the immunity induced lasts for life. In practice, however, animals are usually vaccinated twice, once as calves and again one year later. Outbreaks in areas of endemic infection are usually tackled by immediate vaccination of all animals in the affected and neighbouring herds.

Global Rinderpest Eradication Programme (GREP) has been established in 1992 to achieve global eradication of rinderpest in 2010. The GREP co-ordinates the activities of three regional rinderpest eradication campaigns namely; PARC or Pan African Rinderpest Campaign, WAREC or West Asian Rinderpest Eradication Campaign and SAREC or South Asian Rinderpest Eradication Campaign. Office International des Epizooties (OIE) has defined three levels of technical accomplishment in achieving the goal of GREP. These are the absence of clinically observed disease for twenty four consecutive months, and

because of successful attainment of this first objective (provisional freedom from disease) a series of actions leading to a second (substantive freedom from disease) and third objective (freedom from infection), collectively termed *"The OIE Pathway"* (Figure 4.2.2).

In South Asia, except for Pakistan all other countries have achieved the level of absence of clinically observed disease for twenty four consecutive months which demands the ban on rinderpest vaccination and declaration of provisional freedom from disease.

Fig. 4.2.2 The OIE Pathway.



*If a country wants to be declared "free from infection" at the end of year 4, serological surveillance of unvaccinated animals must be in operation at the end of year 2, in order to prove that there has been no seropositive case in the country for at least two years.

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Chapter 5

METABOLIC DISEASES

R. Sivakanesan

The human-animal interaction, especially in the case of production animals, is aimed at maximising the yield in terms of milk, meat and offsprings. The advances made in the field of genetics and breeding are put to test in animal production systems and such experiments have proved valuable in extracting the maximum produce from the animals for human consumption. During such human approaches in animal breeding, the fundamental laws of physiology are completely ignored and the animals are considered to possess unlimited production potentials. To what extent the upgrading and cross breeding exercises will elevate the physiological capacity of an animal is difficult to quantify even though the production capacity of an animal could be measured in terms of litres of milk, kilograms of meat and number of offsprings. There is always the danger of crossing the barriers of physiological limits in an animal in most upgrading activities.

Criteria determining diseases of metabolic origin

Five criteria have been used to decide whether a condition can be regarded as metabolic or not.

- The condition should be non-infective in nature. However, one should be cautious of the fact that in most infections there is some disturbances of metabolic homeostasis, partly due to the direct effect of toxins on organs, and partly because of inappetance that accompanies the infection. Nevertheless, pathogenic organisms should not be involved in the aetiology of the condition.
- The condition should not arise as a consequence of a specific genetic disorder. Incidence of metabolic diseases are too frequent to be explained on the basis of genetic defects, because in the latter disorders the frequency is extremely low. In general, there is no specific localised gene effect, resulting in change at a particular site in a biochemical pathway.
- The condition should not be simply due to a dietary deficiency. The pattern of diet, however, can play an important part in the aetiology in some conditions. For example, in many cases of pregnancy toxaemia of sheep, inadequate intake of dietary energy has played some part. On the other hand, the disease cannot be produced simply by withdrawing food and it may even develop in ewes that have always been offered adequate food.
- There should be changes observable in the concentration of one or more blood metabolites. It should be emphasised that in subclinical states alterations in blood metabolites are seen even in the absence of clinical features. This is a feature to be recognised as a useful one to identify susceptible animals by performing metabolic profiles at crucial periods in the production cycle of an animal.
- The condition should usually develop at a time when the metabolic demand of an animal is highest as would be seen at its peak production or at critical points in the production cycle eg. parturition, terminal stages of pregnancy, peak lactation etc.

Factors causing metabolic diseases

Production cycle, metabolic demands and hormonal influence on production

In the life cycle of an animal the period around parturition could be regarded as the most critical period. It is around this period that the animal gives birth to the young, initiates the phase of lactation and is called upon to care and feed the newborn. The hormonal influence is strongest during this period with several reproductive hormones like oestrogen, progesterone, prolactin and oxytocin and others like insulin, growth hormone, thyroid hormones regulating the metabolic activities in an animal. The hormonal influence is so strong that it doesn't limit production. It is this physiological weakness in an animal that predisposes it to succumb to many conditions which are purely metabolic in nature. The hormonal grip on the metabolic events ensures a continued production governed by the hormonal metabolic drives. The physiological capacity of an animal in terms of quantity of nutrients ingested and processed cannot be expanded due to its fixed anatomical capacity. As such, if physiological capacity is unable to meet the nutrient need for hormonal driven metabolic events, nutrients will be drawn from the body reserves to maintain production. This creates a state of metabolic instability in an animal resulting in metabolic diseases.

Rumen microflora

The rumen microflora offer many advantages to the animal. Nutrient fermentation in the rumen converts indigestible components to utilizable products and the microbial synthesis results in the production of polysaccharides and good quality proteins. Even though this process is less efficient due to metabolic wastes in the form of methane and ammonia, crop residues and foliage which are available in plenty are effectively used in ruminant feeding. The quality of feed offered influences the product ultimately formed by microbial action. The predominant rumen microbial flora will decide on the direction of the fermentation pathway and the products yielded. The dominance of the microbes in the flora depends on the type of feed offered to the animal. Hence an interaction and an interdependence between the feed and the microbial flora can be seen in the rumen environment.

The requirements for metabolism and production in ruminants therefore largely depend on the activity of the rumen microbes. Production of less desirable products at times of increased metabolic demand may create an imbalance in nutrient flux to the sites of production leading to procurement of nutrients from body reserves for eg. ruminant ketosis develops as a result of a decrease in the supply of propionic acid, the glucogenic precursor due to alterations in the bacterial flora or type of feed. This emphasises the fact that alterations in the feeding system, feeding regime and management practices should be avoided at times of peak productivity in an animal.

Factors disturbing normal rumen function:

- Poor housing and overcrowding will result in inadequate rest and inefficient cudding with subsequent reduction in saliva, thereby resulting in the lowering of rumen pH.
- Sudden feed changes will lead to destruction or inadequate development of suitable flora.
- Excess carbohydrate feeding, especially the rapidly fermentable type results in rumenal acidosis with a consequent fall in pH.

- Excess protein feeding on the other hand, will result in alkalosis due to increased ammonia production.
- Improper silage production whereby there is butyric acid build up in the finished product resulting in the destruction of rumenal flora.
- Lack of coarse fibre results in lack of substrate for bacteria.

Milk fever (Parturient paresis)

Introduction

Milk fever is a metabolic disease commonly seen in high producing dairy buffaloes (Mohinder Singh, *et al* 1994; Sarne, *et al* 1957; Desai, 1953; Panda, 1952), similar to that seen in dairy cattle, and is characterised by general muscle weakness, incoordination and depressed consciousness within a few days before or after calving.

Incidence

A survey of 9432 parturated buffaloes at private and organized farms in the state of Punjab revealed a parturient paresis incidence of 3.1%, with 80% of cases occurring between July and December (Mohinder Singh *et al.*, 1994). A high incidence of milk fever is recorded in adult buffaloes in their third to sixth lactation and is more common in dry areas where straws and stovers are the staple roughage. The incidence of milk fever observed in the 2nd to 7th lactations respectively was 5.1%, 18%, 33.3%, 25.6%, 10.3% and 7.7%. (Mohinder Singh *et al.*, 1994).

Milk fever occurs during the last few days of pregnancy or within 2-3 days of parturition (Sharma, 1982). Rarely it may occur even up to 8 weeks after calving. Mohinder Singh *et al* (1994) observed that clinical milk fever develops 36 to 78 h post calving except in one buffalo, in which it developed after 58 days. Singh *et al.*, (1974) reported the onset was within 21 to 48 h of calving in buffaloes between the 3rd and 5th lactations.

Aetiology

Depression in the serum calcium level usually occurs in buffaloes at the time of calving and is greater in animals prone to milk fever. Usually three factors are responsible for the maintenance of the calcium level in the body fluids and disturbance in the function of one or more factors may initiate the development of milk fever. Serum calcium depression may occur due to either one or combination of many of the following factors.

Excessive calcium drainage in the colostrum: The amount of calcium in the colostrum is dependant on the volume of colostrum secreted. When the loss of calcium in colostrum is much higher than the normal capacity for its absorption from the intestine and mobilisation from the long bones, a decline in serum calcium could be observed.

Impaired absorption of dietary calcium from the intestine: Loss of appetite is seen in some animals at the time of parturition and these animals are more likely to be affected because of the reduced calcium absorption from the intestine.

Mobilisation: Slow and insufficient mobilisation of calcium from the skeletal reserves during the terminal stages of gestation.

Clinical pathology

Mohinder Singh *et al.*, (1994) observed that the buffaloes affected by milk fever have a lower mean plasma calcium level (4.91 mg/dl) than healthy animals (8.42 mg/dl). Singh *et al.*,

(1974) reported a mean serum calcium concentration of 4.68 and inorganic phosphorus 2.46 mg/100 ml (healthy animals 5.3-6.1 mg/dl; Ranjhan *and* Pathak, 1992).

Clinical findings

The incidence of milk fever in buffaloes is lower than in cows but the clinical manifestations are comparable (Mohinder Singh *et al.*, 1994; Singh *et al.*, 1974). Buffaloes with milk fever have lower ($P<0.01$) rectal temperature (99.26 *vs.* 100.5^{0}F), pulse rate (37.36 *vs.* 48.38), respiration rate (13.4 *vs.* 21.8/min) and ruminal movement (1.6 *vs.* 7.6/5 min) than healthy animals. The affected animals stop eating and is disinclined to move. The muzzle is dry and the body and extremities are cold to touch. The pupils are dilated and the animal appears to be depressed. The intensity of the heart sound is reduced and the pulse is weak. The animals are either unconscious or semiconscious. As the disease progresses, the buffaloes lie either in sternal or lateral recumbency. Mohinder Singh *et al.*, (1994) diagnosed 25.6, 43.6 and 30.8%, respectively of buffaloes with milk fever when in the standing posture, sternal recumbency and lateral recumbency.

When treatment is delayed the disease progresses rapidly and the animal becomes deeply unconscious. The condition deteriorates rapidly during a period of 12-24 hours and ultimately death takes place due to respiratory failure.

Treatment

Parenteral treatment with calcium borogluconate has been found to be very effective; relapses occur after 24 hours but could be successfully treated (Singh *et al.*, 1974). Therefore, under-dosing should be avoided. The dose depends on the body weight of the animal.

A satisfactory response has been reported following the administration of a 25% solution of calcium borogluconate partly intravenously (300-350 ml) and partly subcutaneously (200-250 ml). The drug should be infused slowly over 10-15 minutes. In relapsed cases, administration of a mixture of calcium gluconate 20.8%, boric acid 4.4%, magnesium hypophosphite 5% and dextrose 20% (all weight by volume) by the intravenous (350ml) and subcutaneous (200ml) route after 24 hrs has been found to be satisfactory (Singh *et al*, 1974).

In animals with continued excessive drainage of calcium in milk, calcium borogluconate may be administered 2 or 3 times at intervals of 12 hours. In milder cases calcium borogluconate may be fed at the rate of 60-100 g daily for 3-4 days following the initial parenteral administration.

Ketosis

Introduction

Ruminants in general are in a precarious situation with respect to their energy metabolism. The presence of ruminal microflora in the anterior part of the gastrointestinal system offers many advantages. The indigestible dietary polysaccharides, cellulose and hemicellulose, are effectively turned into volatile fatty acids by the microbial effect. This process, nevertheless, converts the digestible polysaccharides too into volatile fatty acids. The volatile fatty acid mixture produced in the rumen is predominantly ketogenic and this results in a decrease in efficiency in handling the digestible dietary polysaccharides. At times of peak production the demand for energy yielding nutrient glucose far exceed the rumen ability to provide glucogenic precursors. This leads to the development of ketosis.

Dairy buffaloes in early and at peak lactation are highly susceptible to ketosis. It is characterised by nervous signs, loss of body weight, reduced milk yield and ketones in the urine. The biochemical changes include hypoglycaemia, ketonaemia and ketonuria and the condition is associated with a low level of liver glycogen.

Aetiology

The increased demand for glucose during peak lactation or during the initial stages of milk production in high yielding animals restricts the oxaloacetate availability for metabolic reactions. This results in diminished activity of the tricarboxylic acid cycle and hence energy production and reduced rate of acetyl CoA catabolism. Restricted energy production results in increased breakdown of depot fats causing more production of acetyl CoA. The excess acetyl CoA is directed towards ketone body synthesis. Because of the diminished capability of the extrahepatic tissues to catabolise acetyl CoA, the blood ketone concentration is elevated causing ketonaemia and the appearance of ketone bodies in urine causing ketonuria. Ketonaemia and ketonuria together is referred to as ketosis. The composition and quantity of feed stuffs used for animals may be responsible for the development of ketosis.

Clinical findings

The disease is usually diagnosed after a period of subclinical illness. There is a gradual decrease in feed intake associated with a fall in milk production as observed in dairy cattle. Usually the concentrate part of the ration is refused by the sick animals but they continue to eat the dry roughage. After sometime signs of pica may be observed. There is considerable loss in body weight. The animals becomes stiff, dull and unwilling to move. The coat is dull, rough and dry (Ranjhan and Pathak, 1992).

The nervous form of the ketosis may occur in dairy buffaloes. The syndrome is sometimes confused with rabies. An affected animal moves aimlessly and may press its head against a wall and move in circles. A staggering gait and moderate tetany may be observed (Ranjhan and Pathak, 1992).

Treatment

The aim of the treatment should be to immediately restore the blood glucose concentration and to maintain it until such time the animal recovers, by parenteral or oral administration of glucose or glucose precursors. This will ensure the glucose supply to organs where metabolic pathways are dependant on glucose availability.

A 50% glucose solution, 500 ml, is administered intravenously and repeated for 3 to 5 days. To promote gluconeogenesis, intramuscular injection of 0.5 to 1.5 g glucocorticoids (cortisone acetate or hydrocortisone or hydrocortisone acetate) is given and repeated on the following day, if necessary. This is supplemented with oral administration of 250g propylene glycol mixed with an equal amount of water twice daily for 5 days. Alternatively, sodium propionate, 120 g or 250g of a lactate mixture (equal quantities of calcium lactate and sodium lactate) can be administered (Ranjhan and Pathak, 1992).

Hypomagnesaemic tetanies

The depression of serum magnesium in buffaloes, especially in calves and lactating animals produce tetany which is collectively referred to as hypomagnesaemic tetanies.

Hypomagnesaemic tetany of calves

Introduction

The absence of a homeostatic mechanism, the low amounts of magnesium in milk, decreased intestinal absorption of magnesium with advancing age and the increased demand of magnesium for growth places the juvenile buffalo calves in a vulnerable position. This problem however, is mostly seen in calves fed and raised solely on milk.

Aetiology

Calves reared indoors on whole milk without additives develop hypomagnesaemic tetany. Pramod Kumar, *et al.*, (1993) induced hypomagnesaemia in 6 buffalo calves by intra-ruminal administration of potassium chloride (1.3 g/kg) and citric acid (1.1 g/kg) for 5 consecutive days. Buffalo calves fed potassium iodide alone (100 mg/kg body weight) develop signs of hypomagnesaemic tetany after 5-8 days (El Sherif and Mottelib 1983).

Clinical pathology

A severe fall in serum magnesium (0.54-0.55 mg/dl) occurs in most clinical cases. In experimental hypomagnesaemia in calves the magnesium content decrease from day 2 to 20. Increases in total leukocyte count and decreases in haemoglobin content are evident. Differential leukocyte count reveal lymphocytosis, neutropenia and low monocyte counts on day 5 and lymphopenia, neutrophilia and monocytosis in the later stage of the disease. Eosinopenia is observed on days 2, 4, 5 and 10 (Pramod Kumar *et al.*, 1993). Analysis of blood chemistry reveal alkalosis, reduced calcium, magnesium, inorganic phosphorus, sodium and chloride, and increased concentrations of potassium, serum aspartate amino transaninase, alkaline phosphatase, lactate dehydrogenase, glucose, urea nitrogen, haematocrit and haemoglobin. Cholinesterase, serum alanine amino transaminanse, total serum protein, albumin and total globulin concentrations are unchanged (El Sherif and Mottelib 1983).

Clinical findings

Hypomagnesaemic calves show erection and backward carriage of ears, agitation, excitement, hypersensitivity, anorexia, sunken eye balls and staggering gait. Heart and respiration rates are increased, whereas rumen movements and body temperature are reduced (Pramod Kumar *et al.*, 1993). The animals are hypersensitive to external stimuli and show opisthotonus, ears folded backwards, retraction of eyelids, muscle twitching, tremor, tonic and clonic spasms, clonic convulsions and tetanic contractions (El Sherif and Mottelib, 1983). The tetanic symptoms include retraction of the head, kicking of the belly, clamping of the jaws and tonic and clonic convulsions (Awad *et al.*, 1979).

At postmortem examination, pale mucous membranes are found. There may be ascites. Various degrees of impaction with straw balls in the stomach may be observed. Pericardial and thoracic effusions and degeneration of adipose tissue are more common. There may be pulmonary lesions, enteritis, emaciation and dehydration (Awad *et al.*, 1979).

Treatment

Magnesium sulphate (10g) dissolved in sterile distilled water should be administered subcutaneously and followed by daily oral administration of 10-15g magnesium oxide. For the control of muscular tremors tranquilizers like acetylpromazine may be used. Xylazine (Rompun), Calcium and Magnesium salts (Calphon) and Phosphoric acid and cyanocobalanin (Catasol) and an electrolyte solution have been used for the treatment of experimentally induced hypomagnesaemia in buffalo calves (Sherif and Mottelib, 1983). Clinical signs abate and blood chemistry return to normal when calves are given the following on three consecutive days; xylazine (0.5 ml, intramuscular), 150 ml of Calphon intravenous and subcutaneously (containing calcium and magnesium salts), and Catosal 5 ml intramuscularly (containing phosphoric acid and cyanocobalamin). In addition 1 litre of an electrolyte solution containing 8.5 g sodium chloride, 0.4 g calcium chloride, 0.2 g caffeine sodium benzoate could also be administered.

Hypomagnesaemic tetany of dairy buffaloes

Introduction

Hypomagnesaemic tetany of dairy buffaloes occurs in lactating as well as non-lactating pregnant buffaloes fed on a large quantity of young tender herbage.

Aetiology

In pregnant and lactating buffaloes the developing foetus and drainage in milk demands a higher requirement of the mineral magnesium. A low level of magnesium in the feedstuffs and lack of mineral supplementation in the diets of these animals make them vulnerable to the condition (Ranjhan and Pathak, 1992).

Clinical findings

In acute cases clinical signs appear suddenly. Affected animals suddenly stop eating, show uneasiness and stand cautiously attentive. These signs are associated with the twitching of muscles. The animal may appear frightened or "mad". The gait is staggering; later the sick animal falls and moves its limbs in spasms. Death may occur in less than 4h after the appearance of the clinical signs (De and Goswami, 1960). There may be a sudden rise in body temperature after the tetanic attack. The pulse and respiration rates are increased. In sub-acute cases, a gradual loss in appetite results in an unthrifty condition. A loss of 22-55 kg in body weight of pregnant animals and about a 27% drop in milk yield of lactating animals has been recorded (Dahbash, 1976).

Treatment

Magnesium sulphate or magnesium borogluconate (25g) dissolved in about 400-500 ml of sterile distilled water should be injected subcutaneously at a very slow rate. The treatment should be followed by daily oral feeding of 50-60 g magnesium oxide for a period of 7-10 days. If the response is poor, calcium borogluconate is administered (Ranjhan and Pathak, 1992).

Post parturient haemoglobinuria

Introduction

Post parturient haemoglobinuria is a haemolytic syndrome associated with severe hypophosphataemia (Samad *et al.*, 1979). It has been observed that in post parturient

haemoglobinuria, the antioxidant potential of red blood cells becomes very low and the haemolysis is possibly due to oxidative damage of red blood cells (Mata *et al.*, 1994). Experiments with ascorbic acid proved effective in curing this disease (Chugh and Mata, 1997; Mata *et al.*, 1994).

Aetiology

Phosphorus deficiency haemoglobinuria is observed only in adult buffaloes, in advanced pregnancy and during the puerperal period; it is more frequent after the third pregnancy (Nagpal *et al.*, 1968). Feeding of low phosphorus feed for long periods have been incriminated as a cause (Nagpal *et al.*, 1968). The heavy feeding of sugar cane tops, sugar beet, kale, mustard, cabbage and lucerne which are known to contain inhibitory factors in feed such as metallic ions interfere with the absorption and assimilation of phosphorous (Freudenberg, 1955).

Most of the buffaloes affected with the disease have a history of calving within the last one month period indicating that the post parturient stage is most vulnerable to this disease. High producing animals with peak milk yield in the previous lactation ranging from 8-22 litres/ day are mostly affected. Buffaloes are more susceptible than cattle when sugar cane tops are continuously fed to them (Ranjhan and Pathak, 1992). Parturition appears to be a sparing factor whereas pregnancy or gestation and lactation stage are found to be the putative factors in the development of the condition (Samad, 1997).

Clinical findings

The first conspicuous signs of disease is the coffee-coloured urine. In most cases the appetite is normal but milk yield is significantly reduced. Constipation is common and faeces are hard and black tinged. The body temperature is either normal or slightly subnormal. Heart rate is slightly increased. The mucous membranes of the conjunctiva and vulva are discoloured or pale in appearance. Laboured breathing and jugular pulsation can be observed during the terminal stages of the disease. Clinical signs mostly appear after 4 months of pregnancy (Nagpal *et al.*, 1968). The disease is most commonly observed during summer when animals are fed straw from wheat or paddy, or stoves of maize, sorghum and pearl millet, which are all very poor sources of phosphorous.

Treatment

The intravenous injection of phosphorus in the form of monobasic sodium phosphate (60g) in 300 ml of sterile distilled water followed by oral administration of the same dose, twice daily for 3 days, has often been effective. In severe cases blood transfusions may be an useful supportive therapy (Ranjhan and Pathak, 1992). Ascorbic acid either at 5g (Mata *et al*, 1994) or 7.5g (Chugh and Mata, 1997) daily doses, administered intravenously in normal saline, is found to be 68.5 and 82% effective, respectively.

Lactic acidosis

Introduction

Lactic acidosis is caused by increased consumption of rapidly fermentable carbohydrates.

Aetiology

Under experimental conditions feeding of molasses (Randhawa *et al.*, 1993; Randhawa, *et al.*, 1989a; Randhawa *et al.*, 1989b; Choudhuri, *et al.*, 1980; Nauriyal and Baxi, 1978) ragi slurry (Hanumanthaiah *et al.*, 1990), cane sugar (Vihan, 1978), beet molasses (Angelov *et al.*, 1995), and wheat grains (Randhaw *et al.*, 1980a; Randhawa *et al.*, 1980b) have been observed to cause lactic acidosis.

Clinical pathology

Rumen: During acute ruminal acidosis induced in buffalo calves by a single oral feeding of molasses at the rate of 20 g/kg body weight (Randhawa *et al.*, 1989b), the rumen pH declines and this is associated with significant increase in the concentration of lactic acid in the rumen liquor, blood, faeces, cerebro spinal fluid and urine. In the rumen fluid, the decrease in pH is accompanied by a decrease in sodium and potassium and an increase in calcium, magnesium and inorganic phosphorus levels (Choudhuri *et al.*,1980; Randhaw *et al.*,1980a).

The condition is associated with marked changes in the colour, consistency and odour of the rumen liquor, with a significant increase in sedimentation activity test and cellulose digestion test values and a significant decrease in glucose fermentation test values. A complete absence of sedimentation activity and lack of glucose fermentation and the disappearance of the rumen protozoa could be observed in the acute form of the condition (Randhawa et al., 1989a). Total bacterial counts showed a significant decrease in both groups, and aerobic cultural isolation revealed a predominance of a Gram-positive flora, particularly *Streptococcus bovis* and Lactobacillus spp., which was directly related to the severity of the lactic acidosis. In the rumen liquor, the volatile fatty acids and ammonia-nitrogen significantly decreases, whereas the lactic acid concentration significantly increases.

Serum: In arterial blood, the pH, partial pressure of carbon dioxide, actual bicarbonate, carbonic acid and actual base excess decrease with a significant increase in the oxygen and haemoglobin contents (Randhawa *et al.*,1993). However, in venous blood significant increases in partial pressure of carbon dioxide and oxygen extraction ratio with reductions in partial pressure of oxygen and oxygen saturation are seen. Acute lactic acidosis results in metabolic acidosis, partially compensated by respiratory alkalosis reflecting inability of the cardiovascular and renal systems to compensate fully for the developing systemic acidosis.

Serum values of inorganic P and Na increase, while Ca, Mg and K decrease. The pH of urine decreases, and is usually associated with increased urinary excretion of inorganic phosphorus (Choudhuri *et al.*,1980). A significant increase in the activities of alanine aminotransferase, aspartate aminotransferase, glutamic dehydrogenase and arginase enzymes in the blood are observed (Randhawa *et al.*, 1989b). A positive correlation between blood serum alkaline phosphatase activity and rumen pH could be observed whereas lactic dehydrogenase activity and ascorbic acid concentration are not significantly affected (Vihan, 1978).

Histopathology: The histopathological changes in experimental lactic acidosis include mycotic rumenitis with microabscessation in rumen mucosa and liver. Lipid deposition and glycogen depletion with increase in activity of alkaline phosphatase, lactic dehydrogenase and decrease in the activity of succinic dehydrogenase, glucose-6-phosphatase and esterase in rumen and liver are evident on histochemical analysis. However, a decrease in the activity of alkaline

phosphatase is seen in bile canaliculi. In the kidneys, increased activity of alkaline phosphatase associated with decrease in the activity of succinic dehydrogenase with no change in the activities of lactic dehydrogenases, glucose-6-phosphatase and acid phosphatase in the tubular epithelial cells could be observed (Randhawa *et al.*,1989 b).

The brain shows marked congestion, perivascular lymphocytic cuffing, chromatolysis of neurons, satellitosis and neuronophagia. Nervous symptoms observed during acidosis were attributed to the changes in the CSF and brain (Randhaw, *et al.*, 1980a).

Cerebrospinal fluid: Cerebrospinal fluid (CSF) collected from 5 two year old buffalo calves before and after induction of acute ruminal acidosis by feeding crushed wheat grain had a yellowish tinge with turbidity and clots. Increases in glucose, total protein and total leukocyte count in the CSF are observed (Randhawa *et al.*, 1980a).

Clinical signs

Clinical signs in experimentally induced acidosis include general depression, anorexia, diarrhoea, laboured breathing, suspended rumination, and an increase in temperature, heart and respiration rate (Hanumanthaiah *et al.*,1990).

Treatment

Administration of fluids and alkalizers are effective in correcting rumen and metabolic acidosis (Randhawa *et al*, 1980b).

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Chapter 6

DISEASES OF YOUNG CALVES

6.1 Naval ill

N.U. Horadagoda

Aetiology

Naval ill or joint ill is a common condition in the calf. The condition is frequently seen in animals born in an unhygienic environment with no disinfection of the naval and have received little colostrum (Radostitis, *et al.,* 1994). The diseases can be caused by a single organism or a mixture. The bacteria involved include Streptococcus spp, *E.coli*, *Actinomyces pyogenes* and *Fusobacterium necrophorum.*

Pathogenesis

At birth, there is a change in the circulation from foetal to that of the new-born. The blood vessels of the umbilical cord rapidly lose most of the blood in it but still remain patent, thus allowing entry of infection. Once the infection enters it could cause infection at the site of entry, between muscle layers or in the peritoneum. Conversely, the bacteria may pass via the umbilical vein to the liver and eventually into circulation resulting in a septicaemia or chronic illness due to localisation in the joints as well as other organs such as heart, brain and eyes.

Clinical signs

The clinical signs of naval ill are highly variable. It may be restricted to local inflammation of the naval or abdominal wall muscles. In such cases the naval is swollen, soft and usually painful. On the other hand, where there is septicaemia the calf rapidly becomes ill. There is severe depression, pyrexia (40°C), congestion of the mucus membranes and accelerated respiratory and heart rates. There may also be a varying degree of dehydration followed by acidosis, recumbency and death. In contrast, clinical signs may be absent for several weeks or even months in cases where there is bacteraemia and consequent localisation of the organism. In these animals there is inappetance, dullness and an intermittently slightly raised temperature (38-39 °C). Other signs depend on the organ affected. For example, if localisation of infection is on the heart valves it causes endocarditis and results in cardiac murmurs, while the involvement of the eye will lead to panophthalmitis with hypopyon. The most common form of the disease is joint ill with the involvement of one or more joints. In most cases there is bilateral swelling mainly of the carpal joints. Affected animals tend to become lame and have an altered stance. Aspiration of affected joints usually reveals thick pus.

Necropsy findings

At postmortem examination there is the presence of infection in the umbilicus which may be accompanied by a local peritonitis. In the septicaemic form of the disease, petechial and

ecchymotic haemorrhages may be evident on the serosa and submucosa of various organs. In the chronic form of naval ill, inflammation and abscessation may occur in various organs.

Diagnosis

Clinical signs such as the swelling of the umbilicus of a young calf may aid diagnosis. The presence of the organism in blood culture and a neutrophilia on a differential white blood cell count may be helpful. However, naval ill need to be differentiated from other forms of septicaemia and locomotor problems such as muscular dystrophy.

Treatment and control

Treatment of septicaemia involves the use of antimicrobials administered intravenously. Amoxycillin, ampicillin, and oxytetracycline have been found to be effective (Hungerford, 1989). In less severe cases, penicillin and streptomycin may be given parenterally. The duration of treatment should be at least five days. Parental or oral administration of electrolyte solution may be needed to overcome dehydration. There are difficulties in the treatment of localised chronic infections such as joint ill. In cattle, the use of potentiated sulphonamides or lincomycin by injection have been found to give good results in some cases. Naval ill could be effectively controlled by disinfecting the naval with an appropriate disinfectant soon after birth. Tincture of iodine is very useful for this purpose, as it allows sterilisation and helps to cause desiccation.

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6.2 Bacterial diarrhoea

T.G. Wijewardana

Aetiology

Diarrhoea in neonatal calves is a condition distributed throughout the world causing enormous economic losses. The aetiology is multi-factorial with infectious agents and management, nutritional, physiological and environmental factors contributing to diarrhoea (Snodgrass *et a.l*, 1986). The aetiological agents causing diarrhoea in calves are well documented, with rotavirus, corona virus, enterotoxogenic *E.coli*, Salmonella species and cryptosporodium being regarded as the most important pathogens (Bulgin *et al.*, 1982, Moerman *et al.*, 1982, Tzipori, 1981, Morin *et al.*, 1978, Acres *et al.*, 1977, Moon *et al.*, 1976), however in the case of buffalo calves, available information is scanty. Enterotoxogenic *E.coli* and Salmonella species have been found to be the important bacterial pathogens in buffalo calves in Pakistan (Hafiz *et al.*, 1994). In Sri Lanka verocytotoxigenic *E.coli* had been associated with diarrhoeic buffalo calves (Mohammad *et al*, 1986).

Pathogenesis and Clinical Signs

E.coli is a normal inhabitant of the intestines of animals and man. Within hours of birth these organisms have been reported to colonise the intestines of calves. They possess fimbriae with which they adhere to the mucosa and produce an enterotoxin. This stimulates excessive secretion of fluid from the intestinal mucosa, which manifests as diarrhoea. Severe diarrhoea due to *E.coli* has been reported during the first two weeks of life (Barrandeguy *et al.*, 1988), with the highest occurrence observed among calves aged 1 to 3 days (Snodgrass *et al.*,1986). Neonates of ruminants are born without gammaglobulins. The colostrum offers the only source of vital immunoglobulins, which are absorbed into the circulation through micro-pinocytosis. Maximum absorption occurs within the first 6-8 hours after birth. During this period microorganisms compete with immunoglobulins for occupation of immunoglobulin receptor sites and at times succeed, thus resulting in agammaglobulinemia in neonatal calves (Khan *et al.*, 1991 b). Such calves are susceptible to a variety of infections including diarrhoea. It has been reported that calves with low levels of circulating immunoglobulins are twice as much more prone to sickness and four times more likely to die than calves with adequate levels of circulating immunoglobulins (White and Andrew, 1986).

Salmonella species cause calf scours with nausea, vomiting and cramps (Greene *et al.*, 1986). *Salmonella dublin* and *S.typhimurium* have been recorded to be associated with diarrhoea in cattle. In Sri Lanka, during a course of a disease investigation in a cattle farms, *S.dublin* was found to be the predominant pathogen associated with calf scours (Bandaranayake and Thambiaiyah, 1961). An abbatoir study carried out in Sri Lanka had revealed *Salmonella* carrier percentages of 10.1 and 5.2 for cattle and goats respectively (Nagaratnam and Ratnatunga, 1971), while subsequent study in cattle revealed a carrier rate of 0.5% for *Salmonella dublin* (Subasinghe *et al.*, 1975). Khan *et al.*, (1994) have reported that intensity of diarrhoea due to Salmonella is less than what is seen with *E.coli* and suggested that this could be due to the non production of an enterotoxin as done by *E.coli*. The same study had shown that deaths due to diarrhoea caused by Salmonella species was less than in diarrhoea associated with *E.coli*. Prevalence of diarrhoea due to Salmonella species is high up to the age of 3 months. In the older animals it may lead to septicaemia

and abortions. The organisms which lie dormant in the mesenteric lymph nodes play an important role in the epidemiology. They become active shedders when subjected to stress either physical or environmental.

Control

E.coli is endemic and its presence in the intestines do not necessarily lead to clinical illness. Unhygienic environment conditions, adverse weather and nutritional factors facilitate the manifestation of diarrhoea. Hence, proper management practices with adequate feeding of colostrum, particularly during the first 6 to 8 hours after birth could reduce the occurrence of diarrhoea due to *E.coli*. As infection with Salmonella species result in a carrier status, the culling of infected animals is recommended in place treatment.

Aetiology of diarrhoea is multifactorial. Therefore, very often, treatment is empirical. Proper nursing, keeping the diarrhoeic animal rehydrated and segregation of affected animals would minimise the deaths due to diarrhoea. In developed countries vaccination of pregnant animals against *E.coli* and Rota virus has been reported to be effective (Snodgrass *et al.*, 1986).

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NA-218-125

6.3 Respiratory diseases

N.U. Horadagoda

Introduction

Respiratory diseases constitutes one of the most important causes of morbidity and mortality of buffalo calves especially among those managed under an intensive system. Studies on calf mortality in the major state farms in Sri Lanka have revealed that respiratory tract infections are one of the most frequent cause of death in calves under 6 months of age (Subasinghe 1986; Bandaranayake, 1962). Similarly, studies in the government farms of the Haryana state in India have revealed that 41.3% of the deaths of buffalo calves was due to pneumonia (Verma and Kalra, 1974). Although calf pneumonia is and indeed, has for many years been an enormous problem, very few incidents of respiratory tract infections in buffalo calves have been investigated comprehensively.

Aetiology

Respiratory tract infections are indeed a major problem in cattle calves and laboratory investigations have lead to the isolations of different micro-organisms from lungs of affected animals. At present, the general view is that the mycoplasmas and viruses such as respiratory syncytial virus, parainfluenza III, bovine virus diarrhoea and infectious bovine rhinotracheitis virus are primary pathogens whereas commonly isolated bacteria like, *Pasteurella haemolytica*, *Pasteurella multocida*, *Actinomyces pyogenes*, *Staphylococcus aureus*, *Streptococcus pneumonia* only exert a significant clinical effect following bronchopulmonary damage caused by other agents. The three main species of mycoplasma associated with respiratory tract infection, particularly with chronic pneumonia are *M. dispar*, *M. bovis* and *ureaplasma* spp. Under certain circumstances it is likely that more than one agent is isolated from pneumonic lungs and that they may collectively play a role in initiating the infection and influencing the severity of the disease. Although a large number of different infectious agents have been isolated from pneumonic lungs of calves there inability to produce the clinical disease has led to the concept of a "multi-factor aetiology" for calf pneumonia. In this concept the effect of potential respiratory pathogens is modified by "stress" of other diseases or physical factors such as overcrowding, wide fluctuations of environmental temperatures and mixing of calves of various ages and sources.

Although pneumonia is recognised as a major problem in buffalo calves, very little information is available on the aetiological agents responsible for the disease. De Alwis *et al.*, (1975) reported the occurrence of an acute fibrinous bronchopneumonia in buffalo calves associated with *Pasteurella multocida* serotype B:2, the organism responsible for haemorrhagic septicaemia. The role of viruses and mycoplasmas in buffalo calf pneumonia is not fully investigated, but it would not be unjustifiable to assume that many of the organism responsible for pneumonia in cattle calves are potential pathogens in buffalo calves as well. As far as the pathogenesis is concerned, there seems to be a close parallel between cattle and buffalo calves in that many of the potential pathogens inhabit the upper respiratory tract as part of the normal flora and produce disease when the animals are "stressed". This is demonstrated in the studies reported by De Alwis (1977) in which a higher incidence of *Actinomyces pyogenes* isolations was made from the nasopharynx of weaned buffalo calves (45%) compared to pre-weaned animals (2%) in livestock farms where this organism was a common pathogen isolated from pneumonic lungs. The latter

findings indicate the early colonisation of the organism in the nasopharynx and rapid proliferation when calves are stressed at weaning.

Clinical signs

The clinical signs would manifest itself as a chronic or acute disease depending on the organism. In the chronic form the onset of the infection is gradual and the animals remain bright and alert but slight mucoid or mucopurulent discharge may be present. The temperature may be slightly increased while the respiratory rate may remain normal or be elevated to around to 100 per minute accompanied with a dry, explosive cough, which is usually produced singly. In the acute form of the disease there is usually a reduction in the feed intake, pyrexia and widespread coughing is apparent. The affected animals become dull, and the head tends to be carried lower than normal. Other signs include a mucoid or mucopurulent ocular-nasal discharge and tachypnoea (respiration rate generally over 40 per minute).

Necropsy

The lesions are usually confined to the anterior ventral parts of the lung lobes and involve the apical, cardiac and cranial parts of the caudal lobes. The involvement may vary from 10 to 70 per cent of the lung tissue depending on whether the pneumonia is acute or chronic. In the latter form a lesser area of the lung is involved and it may take several months for the lesions to resolve even in uncomplicated cases. In the acute form of the disease, a greater part of the lung is affected and is often complicated with suppuration or necrosis depending on the organism. In addition, there is gross enlargement and congestion of the mediastinal and bronchial lymph nodes. In some cases there may be sero-sanguinous fluid in the thorax and pericardial sac, fibrinous pleurisy, enlargement of the heart with the presence of subendocardial and subepicardial haemorrhages.

Treatment

Most of the information in the treatment of pneumonia is based on studies performed in cattle. Broad spectrum antimicrobials with bactericidal properties are recommended in the treatment of pneumonia in calves. The choice will most probably have to be based on the previous successful use of the preparation. It is best not to use a long-acting preparation because if there no response to treatment it will make the initiation of subsequent therapy difficult. If an animal dies, cultures and subsequent antibiotic sensitivity test performed on isolates, preferably obtained from untreated animals will be very useful in deciding on the antimicrobial to be employed. Treatment is usually continued for three to five days depending of the drug used and the response to treatment. Commonly used drugs include Danofloxacin or Trimethoprim and sulphadiazine combination.

The use of corticosteriods concurrently with antimicrobials has been found helpful in the symptomatic relief, although it does not have a direct effect on the cause of the disease. The use of non-steroidal anti-inflammatory drugs such as flunixin meglumine have also been found to be useful to reduce the inflammatory response in calf pneumonia. During the clinical course of the disease many animals become partially or completely anorexic and it has been suggested that supportive therapy in the form of multi-vitamin injections,

particularly of the vitamin B group may be of use in overcoming any temporary deficiency that may result from low vitamin storage. Proper nursing of affected animals by providing plenty of dry bedding, allowing easy access to water and good quality concentrate, avoiding draughts are some of the important aspects which must be considered to allow the rapid recovery of the sick animals.

Prevention and control

As the diseases of the respiratory system are multi-factorial, preventive measures include attention to management as well as prophylactic therapies. Ensuring that calves receive the maximum amount of colostrum (up to 6 pints) within 6 hours of birth, thorough cleaning and regular disinfection of calf pens, rearing calves of the same age in small groups are some of the management practices that would minimise respiratory tract infections. Although vaccines are available against many of the micro-organisms responsible for calf pneumonia there efficacy in preventing lung infections in buffalo calves has not been examined.

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Chapter 7

HEALTH CARE AND DISEASE PREVENTION

D.H.A. Subasinghe

Factors influencing health of buffaloes

Disease could be defined as any impairment that interferes with the performance of the normal functions of an animal. It may include responses to environmental factors such as climate, nutrition, infectious agents, toxicants, metabolic disorders or a combination of the above (Webster 1982). Under natural conditions the environment is complex and variable. It could either be favourable or hostile. Variations in temperature, weather, rainfall and sunshine in combination with seasonal shortages of feed and water could influence the status of health and disease. Buffaloes like other domestic animals are exposed to parasitic infestations, microbial infections, toxic agents and even dietary deficiencies. They usually possess an innate ability to adapt or cope with such hazards in most situations. However, if the natural resistance is inadequate to withstaid the impending "insult", they will suffer and die. On the other hand, if the body defences are adequate to overcome the invading disease agents, the animal will develop resistance to the disease and survive (Harting 1994). Buffaloes living in the wild as well as domesticated buffaloes that are reared in large herds generally under extensive management are constantly under environmental stress, which is a threat to their survival. Such situations could cause considerable economic losses through poor performance and mortality.

When an animal is exposed to a causative agent of disease, whether it would succumb to the disease or not is dependant on several factors such as the virulence of the causative agent, presence of a favourable environment to establish an infection and the capacity of the host animal to resist the infection. Occurrence of disease would be evident only when the defence mechanisms of the body are compromised. Factors associated with multi-factoral disease are complex and only some of the more relevant aspects are discussed here, with possible healthcare measures that could be adapted to prevent the occurrence of disease or minimise the severity of the condition.

Some of the more important external factors that can influence the health and well being of domestic animals including the buffalo may be categorised as follows:

Environment:
Physical factors: Variations in ambient temperature, relative humidity, wind velocity and air circulation, excess rain or drought.
Chemical factors: Toxic gasses and chemicals mixed with the air or water in the environment.
Biological factors: Pathogenic microorganisms (bacteria, viruses, fungi) parasites and reptile venom.

Housing
Type of housing, floor construction, ventilation, tethering system, bedding, removal of manure and cleaning, feed and water supply.

Management

Stocking density, management system adopted, body temperature regulation, method of milking, animal care, treatment and medication, vaccination, disinfection of premises and hygienic procedure.

Nutrition

Food and water supply - quality and quantity of food and water available to the animal, prevention of contaminants in feed and water.

A diagrammatic illustration of the influence of external factors on the health and welfare of the animal is given in Fig.7.1. Any of these factors interacting with the animal singly or in combination can cause disease, if the body defenses are unable to withstand the challenge.

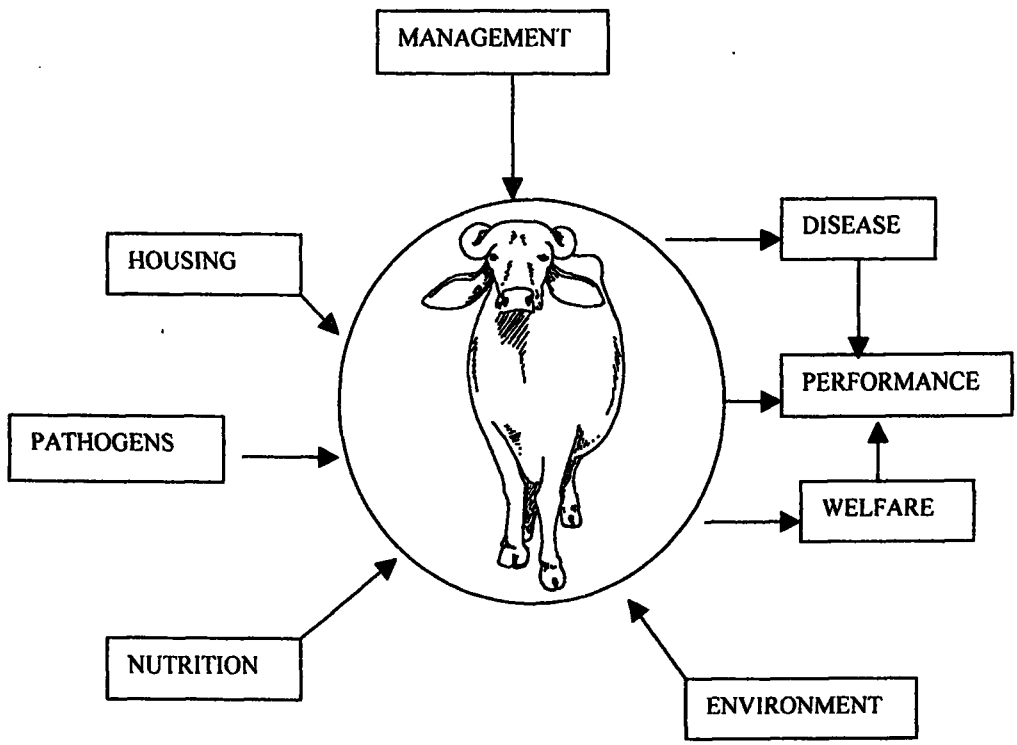

Fig. 7.1 External factors and pathogens acting on the performance, health and welfare of the animal

Environment

Collection of all the environmental factors that would adversely affect the health and well being of the animal may be defined as stress. For example excess of heat or cold or strong wind drafts cannot be tolerated by most animals, generally if it has not had such experiences

before. It may be described as "response to challenge" or " the ability to cope with the environmental demands". The ability of young calves to cope with this demand is much less in relation to adults. This fact should therefore be taken into consideration when dairy owners provide facilities to reduce environmental stress (Harting, 1994). The buffalo cannot tolerate direct sunshine for prolonged periods and are therefore distressed by it. They cannot tolerate extreme cold as well. Sudden drop in ambient temperature or exposure to cold draft may cause severe chilling or lead to pneumonia and probably death in young calves. Hence the need to provide a comfortable environment so as to maintain them in good health. Under natural conditions buffaloes are used to wallowing in water specially during mid day in the hot tropics, to dissipate the excess of body heat. However, under intensive management such facilities are not always available. Hence, alternative provisions have to be made to relieve them of heat stress. This could be achieved by pouring or sprinkling water on them three to four times a day during the critical period in which the effect of heat is great. Buffalo calves suffer very much from heat stress. Some of the visible signs may be an increase in body temperature, respiration and pulse rate. Sometimes rumination also could stop and the animal may show signs of discomfort (Cockrill 1974). Immediate removal of the animal to the shade and splashing water on its body will accelerate the cooling down process by removal of excess body heat and hastens recovery.

Animal housing

Buffaloes managed under extensive and semi-intensive management systems in the tropics are generally not housed or kept indoors. However, intensively managed dairy buffalo cows and their young ones need proper housing with good ventilation and air circulation. Fresh air is important for animals that are housed indoors since the respiratory system is in direct contact with the surrounding air which may be polluted with numerous contaminants that could precipitate respiratory disease. Toxic gases, dust and pathogenic microbes carried through the air in the environment enhances the risk of infection. Direct drafts blowing through animal housing has also been identified as an important factor in climatic stress and the influence of this is greater in young calves. Poorly constructed animal housing with inadequate ventilation, bad flooring and wet bedding increases the risk of infections, causing diseases such as pneumonia, gastro-enteritis and parasitic infestations, specially in young calves and mastitis in cows. Pathogenic micro-organisms and parasitic larvae are generally found in abundance in such unhygienic environment. Therefore, well constructed hygienic housing is an essential requirement for good health and welfare of the stock. Provision of such facilities would improve the micro climate of the environment and keep the animals in prime health and productivity.

Animal management:

Animals kept as a closed herd are normally less exposed to the risk of new infections, if the management and handling procedures are executed well. Animals within the farm usually develop a herd immunity to the microbial population in the environment. Animal handling, milking and calf management should always be done by farmers and stockmen with proper skill and care, to maintain the health and productivity of the herd at optimal level. New animals introduced to the herd should be quarantined for a specific period before they are allowed to mix up with the rest of the herd. This would prevent the introduction of diseases to the herd through newly acquired animals. Domestic animals also need constant care and attention. They will respond with maximal efficiency only under favourable environmental

conditions, good handling and management. Hence, stock owners should ensure to provide such conditions and derive the ensuing benefits. .

Nutrition

It is quite obvious that nutrition plays a very vital role in the health and productivity of an animal. It is pointed out elsewhere in this chapter the importance of colostrum to the vitality of the neonatal calf and its survival. As the physiological requirements of the animal changes with growth, production and reproductive functions of the body, the nutritional demands also increase in proportion to age and functions of the animal. For example, as the animal matures from calfhood to an adult, nutritional requirements will increase for optimal performance of the various physiological functions. This feature is illustrated in Table 7.1. The essential basic components of the diet in the buffalo comprise of water, energy (carbohydrates), proteins and minerals. They have to be supplied to the animal in adequate and balanced quantities, for optimal performance of the physiological functions and productivity. The nutritional requirements of animals vary with individuals and their physiological states (eg. for maintenance, growth, pregnancy, lactation etc.) Hence, their dietary requirements have to be determined individually or as similar groups on a scientific basis, depending on factors such as the age, body weight, physiological state (eg. pregnancy, lactation and milk yield). Having determined the individual dietary requirement, it is also necessary to consider the biological value of each of the feeds available to the stock owner for feeding his stock economically. A balanced ration could then be formulated, based on the nutritional needs of his stock.

It is therefore the task of the stockmen or the dairy owner to prepare a balanced diet for each of his animals depending on each situation, so that his animals will perform at optimal productivity. A well nourished animal will be a healthy animal and will always be able to withstand disease, whereas an ill nourished one is liable to contract disease easily and also succumb to its adverse effects. The importance of good nutrition in health care management of buffaloes has therefore been emphasized (Subasinghe and Horadagoda, 1998, Ibrahim 1998).

Table 7.1 Nutritional needs of animals at different physiological stages in life.

	Maintenance	Growth	Pregnancy	Lactation
Calf	+	+	-	-
Heifer (non pregnant)	+	+	-	-
Heifer (pregnant)	+	+	+	-
Lactating cow	+	-	-	+
Lactating cow (pregnant)	+	-	+	+
Dry cow (pregnant)	+	-	+	-

Prevention and control of common diseases in the buffalo calf

In the buffalo most of the disease problems occur during calfhood. Mortality studies conducted in buffalo farms of Sri Lanka revealed an overall calf mortality figure of 22.2 % and deaths were higher at the age of 0 – 1 month and at 4 – 6 month (Subasinghe, 1981). Subsequent studies conducted in major buffalo farms of Sri Lanka also showed a similar pattern, where the overall calf mortality was 24.6%, out of a total number of 6500 pregnancies examined. Pre-natal and post-natal deaths were 6.0% and 19.8% respectively (Subasinghe, 1986). Earlier studies among indigenous buffaloes owned by smallholder farmers also showed a similar pattern (Kumaratilleke, 1979, de Silva *et al* 1985). Most common causes of death among buffalo calves were, diarrhoea, respiratory disease and parasitic infections.

Mortality in calves has always been a significant economic wastage. The magnitude of these losses could however be minimised by taking precautionary measures to reduce the various factors that contribute to the disease condition. eg. *Management factors:* Improvement in animals handling, management and stockmanship would be one of them. Training of dairy men in skills and attitudes would greatly increase the efficiency of management (Varley, 1992). *Immunological factors:* Rapid acquisition of immunity to the pathogens in the immediate environment of the neonatal calf is very essential for the survival and subsequent vigor of the growing calf. It is absolutely necessary for the neonatal calf to acquire the maximum amount of immunity possible, through the colostral antibodies from its dam. The colostrum feed has to be suckled quickly and effectively by the calf, preferably within the first 12 hours after birth when maximum absorption from the intestines is possible. There are three main types of immunoglobulines (IgG, IgM and IgA) in the colostrum. The IgG class is heterogeneous and can be further subdivided into IgG_1 and IgG_2. Of these IgG_1 accounts for 81% of the antibodies in colostrum and constitute the major class of immunoglobin in the adult sera as well. IgG_1 is actually concentrated in the serum during the final month of pregnancy in the dam. Patency of the small intestines of the neonatal animal varies between animals, immunoglobulin class and the method of measurement. Patency for IgG lasts for about 24 – 27 hours, while the patency period for IgM is the shortest: 16 hours (White, 1991).

Immunological studies on neonatal buffalo calves in Sri Lanka demonstrated that immunity to FMD in the newborn calf is transferred solely through the colostrum ingested from the dam and the level of maternally derived antibodies (MDA) in the calf is proportional to the serum antibody level of the dam (Subasinghe and Fernando, 1988) This signifies the importance of maintaining a high level of immunity to common infectious diseases of the buffalo (like FMD and HS) in the pregnant dams immediately prior to parturition.

Measures recommended to improve the status of passive immunity in the neonatal calf are:

- Dry the cow 6-8 week prior to calving and improve her plane of nutrition.
- Immunize the cow against the common infectious diseases (HS, FMD, BQ) early in this period, to increase the circulating antibody levels in the serum and also colostrum, at parturition.

- Maintain the pregnant cow on a high plane of nutrition and a body condition score of 3.5 to 4 at late pregnancy.
- Provide a calving area with a nonslip floor or a clean dry paddock in a quiet environment.
- Ensure that the new born calf receives sufficient colostrum. (3 – 4 l) within the first 6 hours and a second feed within the next 6 hours.
- Check the udder of the parturient cows every 4 hours after birth of the calf. If sufficient quantity of colostrum has not been ingested, supplement colostrum intake from an alternative source.
- If the dam has had dystocia, milk fever or mastitis, feed the calf from a bottle.
- If the dam dies at parturition, frozen colostrum (after thawing) or colostrum from another dam in the herd may be fed to the calf (use only frozen colostrum from the first milk of a non mastitic, vaccinated, healthy cow in the 2nd or 3rd lactation).

Scientific evidence show that survival prospects of the new born calf is strongly linked to the intake of immunoglobulins from the dam (Varley, 1992). The high concentration of energy and protein in the colostrum is in itself a survival package for the neonatal calf. Neonates are invariably born with low energy reserves and in the absence of appropriate nutrition hypoglycemia, coma and death can occur rapidly. Since diseases are multi-factoral in origin, it should be possible to improve the situation by an increased understanding of these factors to avoid high risk situation and also utilize the available resources appropriately to overcome problems encountered in calf management.

The ensuing sections describe some of the commonly encountered calfhood diseases, their prevention and control. Since they are described in detail under chapter 6, only the latter aspect is emphasized here.

Neo-natal infections

In neonatal calves of both cattle and buffaloes naval ill is a common ailment with a high incidence, specially in calves that did not receive adequate feed of colostrum during the first day of its life and are also reared under an unhygienic environment. Bacteria such as *E.coli*, *Actinomyces pyogenes* and Streptococcal species become opportunistic invaders in the above situation. The reader is referred to chapter 6.1 for more details.

Treatment:

- Intravenous administration of antibiotics like ampicillin, amoxycillin and oxytetracycline in acutely ill septicaemic cases.
- Less severe cases may be treated parenterally with penicillin and streptomycin for 4 - 5 days.
- Chronic cases of joint ill can sometimes be refractory to treatment with antibiotics. In such situations potentiated sulphonamides can be used parenterally for treatment, with reasonable success.
- In cases of dehydration, electrolytes should be administered to restore the electrolyte balance.

Prevention and Control:

- Strict hygienic measures should be adapted at calving.
- Application of an appropriate disinfectant like tincture iodine on the naval of the new-born calf immediately after calving. Such procedures could prevent external infections to a great extent and the occurrence of naval ill.

Calf scours

Diarrhoea is a common ailment of calves causing considerable economic loss in dairy production. It has a multifactoral aetiology such as infectious agents (bacteria and viruses), parasites, toxicants, nutritional, physiological, management and environmental factors contributing singly or in combination to cause diarrhoea. However, bacterial, viral and parasitic diarrhoeas are the most common forms of diarrhoea in buffalo calves. We have therefore limited our discussion to these.

Bacterial diarrhoea: Organisms commonly associated with calf diarrhoea in cattle and buffaloes in Sri Lanka and other countries of the Asian region are *E.coli* and Salmonella species. In the neonatal calf *E.coli* diarrhoea generally takes a severe form, sometimes causing death due to dehydration. Incidence of diarrhoea due to Salmonella is generally high in calves up to 3 months of age. Serotypes such as *S.dublin and S.typhimurium* have often been associated with Salmonella diarrhoea. In addition to the diarrhoea it causes nausea and vomiting. Please see Chapter 6.2 for more details.

Treatment:

- The affected calves may be treated by parenteral or oral administration of appropriate antibiotic therapy.
- Salmonella infections may also be treated similarly using appropriate drugs.
- Rehydration therapy is important in the treatment of diarrhoea cases.

Control:

- Since *E.coli* is normally present in the environment and in the intestines of animals, its role as a pathogen becomes important only under adverse weather, unhygienic environment, poor nutrition and management. Therefore, this situation could be prevented to a great extent by providing good nutrition and management, good housing and hygienic environment.
- Allow the neonatal calf to suckle adequate amount of colostrum from the dam within the first few hours of its life (up to 12 h), to ensure that it has ingested sufficient gamma globulin with the colostrum, that could afford protection to the above infections.

Viral diarrhoea: Current evidence indicate that certain viruses like rotaviruses and coronaviruses can be the primary cause of diarrhoea in young calves of dairy cows (Radostits *et al.*, 1994). Multiple mixed viral infections have also been recognised recently from the same diarrhoea calf, in the presence or absence of *E.coli* infections.

Health care and disease prevention

Reported cases of viral diarrhoea in buffalo calves are scanty. However diarrhoea associated with rotavirus in buffalo calves in the age group of 1-150 days has been reported in Sri Lanka. It was found that there is a strong association between the presence of viral antigens in the faeces and diarrhoea. Serological studies conducted on buffalo calf sera (using the ELISA technique) have also shown the presence of viral antibodies in 68.5% of the samples examined (Sunil Chandra and Mahalingam, 1994). Enteric corona viruses are also associated with diarrhoea in dairy calves below one month of age (Radostits *et al* 1994), but very little information is available on this infection in buffaloes. Faecal samples of diarrhoeic buffalo calves and non diarrhoeic buffalo calves below 5 months of age examined by ELISA technique were all negative for corona virus. (Ariyaratne and Mahalingam, 1995).

In rotavirus infection the most clinically significant strain in animals as well as the human population belongs to Group A. Mature animals can be the source of infection for the neonatal calf. Factors that can influence the infection are the age of the calf, its immune status, absorption of colostrum antibodies, ambient temperature, degree of viral exposure (dose of virus) and the presence of other enteropathogens. Mortality is heavy in calves that did not receive adequate colostrum after birth. (Rodostits *et al.*, 1994). Colostrum provide immunity for the first few days after birth at which time rotavirus specific antibodies are prevalent in the colostrum. Viral diarrhoeas are normally explosive outbreaks characterised by profuse liquid diarrhoea with pale yellow faeces and sometimes with flecks of blood. They recover in a few days with fluid therapy. If *E coli* infection is concurrently present, severity of the condition can increase.

Prevention and Control:

- Avoid over crowding in calf pens.
- Facilitate new born calves to obtain the maximum amount of colostrum from the dam during the first twelve hours after birth
- Maintain the calf pens clean and dry.
- Provide clean drinking water and good nutrition.
- Isolate sick cases immediately on detection and give prompt treatment.
- No specific antiviral drugs are available for virus diarrhoea. However, supportive treatment such as rehydration fluid therapy of the calves is very important.

Parasitic diarrhoea: Common causes of parasitic diarrhoea which significantly affect the health of buffalo calves is due to nematode infection by helminth parasite *Toxocara vitulorum* or due to an intestinal protozan infection known as coccidiosis.

Helminth diarrhoea: Gastro intestinal infections of buffalo calves are of great concern to farmers. The most pathogenic of the helminth species to buffaloes is *Toxocara vitulorum*.The young calf acquires the infection from its dam primarily through the milk ingested within the first 8 days of its life (Roberts 1990a). The infective larvae moult in the small intestines of the calf to develop into adult parasites. In an infected calf the number of mature parasites may increase and parasitic eggs appear in the faeces from 20 – 30 days. The peak egg production is around 5 – 7 weeks of age. At this stage the calf carries a heavy pathogenic worm burden and clinical signs of the infection appear usually at this time.

Common clinical signs are diarrhoea, dehydration, abdominal pain and butiric odour in the breath. Constipation or intestinal obstruction may be observed sometimes.

Treatment and control:

- A single treatment of calves between 10–16 days after birth with an appropriate anthelmintic has been very effective, in eliminating inmature parasites (Roberts and Fernando, 1988/89, Roberts, 1990b).
- Pyrantel (8 mg/kg body weight) or Levamisol. (1.5 % levamisole HCl) at 7.5 mg/kg body weight are effective against immature and mature parasites.
- Management of calves under a good hygienic environment, routine deworming at the critical time periods and proper disposal of the faeces can prevent such parasitic infections.

Coccidial diarrhoea: Buffalo calf diarrhoea due to coccidiosis is a problem of concern in Sri Lanka. Nine species of Eimeria have been identified as causative organisms of this condition in buffalo calves (Bahirathan *et al* 1995). The calves are very vulnerable to the infection when they are young. The disease is severe in calves up to about two months of age. Prevalence of coccidiosis in buffalo calves is at its peak at 3-5 weeks of age (Weilgama, presonal communication). They show severe diarrhoea associated with the shedding of massive numbers of coccidial oocysts to the environment. With the development of schizonts and liberation of the merozoites, distortion and disruption of the intestinal villi occur. In severe infections the crypts of the intestine are damaged and blood leaks into the intestinal lumen resulting in severe haemorrhagic diarrhoea. Diagnosis is based on clinical signs and demonstration of oocysts in the faeces. The reader is referred to chapter 1.1 for more details.

Treatment and control:

- Sulphadimidine at a dosage rate of 140 mg/kg body weight can be given orally for 3 days, together with parental fluid therapy.
- Amprolium 10 mg/kg body weight daily for 5 days given per os has also been used in dairy calves.
- Overcrowding of calf pens has to be avoided at all times.
- The pens should also be hygienically clean and dry.
- Feed troughs should be elevated from the ground to prent contamination of the feed with infective material in the faces.
- Disposal of the faeces has to be regular and properly done, to prevent reinfection.
- Sick animals should be isolated and treated.

Pneumonia in buffalo calves

Calf pneumonia is one of the most important causes of buffalo calf mortality in Sri Lanka (Bandaranayake, 1962; Subasinghe, 1986). In respiratory infections of calves a variety of viruses (eg. parainflunza–3, adenovirus, rhinovirus, reovirus, bovoid hespesvirus (IBR) are considered to play the role of primary pathogens that initiate the infection, while the bacterial infection sets in as secondary invaders, under the lowered resistance of the animal host. A variety of bacterial species (*pasteurella, streptococcus, staphylococcus, actinomyces)* have often been associated with pneumonia in calves. Nevertheless, inability of such organisms to

reproduce pneumonia on their own has lead to the belief that several factors such as variation in ambient temperature, poor management and environmental stress could be pre-disposing causes that aggravate the condition. A more informative description of this condition is available in chapter 6.3.

Treatment and control.

- Identify and isolate affected cases and maintain surveillance on the rest of the herd for early detection of sick animals.
- Administer specific/broad spectrum antibiotics for a period of 4-5 days duration depending on each case. (Danoflaxin, trimethoprim and sulphadiazine have been used with success).
- Animals with severe pneumonia should be treated daily for several days.
- Common causes of failure to respond rapidly may be due to: a) advanced disease, b) development of pleurisy, c) presence of pulmonary abscesses, d) drug-resistant bacteria, e) inadequate dosage and f) other diseases which do not respond to antibiotics
- For viral pneumonia, there is actually no specific treatment. However, non-steroid anti-inflammatory drugs (NSAIDS) are sometimes used as adjunctive therapy. Anti-inflammatory drugs, multi- vitamins or vitamin B have been found useful as supportive therapy.
- Sick calves should be housed in warm, well ventilated housing with adequate accommodation and free from drafts.
- They should also be provided with ample fresh water and nourishing food.
- Avoid exposure to inclement weather. If the animal is off feed, parenteral feeding may have to be resorted, to maintain the animal alive.
- Expectorants may be of value during convalescence.

Prevention:

- Ensure that the neonatal calf receives adequate amounts of colostrum within the first 6 h after birth and a second feed within the next 6 hours to ensure that it has received 10% of its body weight of colostrum, while the intestines are still patent to gamma globulin.
- Regular cleaning and disinfection of the calf pens, provision of clean dry bedding for the calves, housing calves of similar age in small groups and improvement in calf management using appropriate technology and skilled stockman will prevent the incidence of disease considerably.
- In intensively managed state dairy buffalo farms where calf pneumonia was a constant problem in Sri Lanka in the mid nineteen seventies, formalised vaccines prepared at the Veterinary Research Institutes, Peradeniya from bacteria such as Actinomyces and staphylococcus *spp.* isolated from nasal swabs taken from sick calves and from infected lung tissue at necropsy, has had reasonable success in reducing the severity of the disease and incidence of pneumonia cases during the said period (author's experience).

Diseases associated with fever, haemorrhage and sudden death

In the buffalo there are several disease syndromes exhibiting signs of high fever, haemorrhage and sudden death. Of these, the most economically important diseases

concerning the Asian region are Haemorrhagic septicaemia (HS), Anthrax and Black Quarter (BQ). In the sections that follow, attention has therefore been focussed on these three diseases, placing special emphasis on diagnosis, prevention and control as the other aspects are described in detail under chapter 2 of this book.

Haemorrhagic septicaemia (HS): Haemorrhagic septicaemia is the most serious disease that affects the water buffalo in the Asian region and is also the most susceptible animal to this disease. It causes a heavy death toll and considerable economic loss (De Alwis and Vipulasiri 1980) The disease is fatal to cattle and buffaloes and is caused by *Pasteurella multocida* (Carter's type B). Younger stock are more susceptible than the adults (De Alwis *et al* 1986). Disease outbreaks usually occur with the onset of monsoon rains which normally precede long dry periods. Diagnosis of the disease is based on the history, clinical signs, necropsy lesions and laboratory confirmation.

Commonly observed signs are; inappetance, elevated temperature, respiratory distress, recumbancy and death. Sub-mandibular oedema extending sometimes to the brisket region may also be observed at times. Most field cases are peracute, associated with sudden death. Protracted pneumonic cases have also been reported in Sri Lanka (De Alwis *et al.*, 1995). Further details on pathological lesions are described in chapter 2.1.

Treatment:

In large herds treatment is not practical. However in small dairies and individual animals parenteral treatment with antibiotics like penicillin, cephalothin, enrofloxin and oxytetracyclines may be successful, if it is undertaken early (Abeynayake *et al.*,1993).

Prevention and control:

- Isolation of sick and incontact animals.
- Restriction in the movement of livestock within the infected area and out of the infected area.
- Implementation of State legislation to facilitate effective control of the disease.
- Vaccination: In Sri Lanka two types of vaccines are locally manufactured and used in the field. i) Alum precipitated vaccine (APV) providing quick immunity of 3 - 4 months duration. This is used on the face of a disease outbreak. ii) Oil adjuvant vaccine (OAV) providing a slow but longer immunity (up to one year) is used in routine immunisation.
- Immunisation programme recommended for cattle and buffaloes in Sri Lanka: Immunise calves at 4 months of age with OAV and follow up with a booster vaccination in 3 months (using the same vaccine). Vaccination should thereafter be repeated annually (De Alwis *et al*, 1978).

Black Quarter (BQ)

Black Quarter is also an acute contagious disease of cattle and buffaloes which show signs of high fever, and sudden death in peracute cases. Clinical signs of oedema, discolouration and gas formation under the skin can be observed often in the upper regions of the fore or the hind limbs. The disease is characterised by high mortality, severe toxaemia, inflammation of the muscles particularly in the limbs. Gas formation under the skin of the affected muscle is a

notable feature observed by the application of slight pressure on the region with the fingers. This action creates a slight sound termed "crepetus" and can be used as a diagnostic feature. Buffaloes and cattle are equally susceptible and animals between the ages of six months to two years are the most susceptible. Laboratory confirmation of the disease is made by the isolation and identification of the causative organism *Clostridium Chauvoe* from the affected parts of the animal. The reader referred to chapter 2.2 for further details.

Prevention and control:

- Parenteral treatment with antibiotics in large doses can be successful in the early stages, but not practical in large herds.
- Recommended method of control is preventive vaccination. All animals between the age of 4 months to 2 years should be vaccinated.
- Immunisation should commence at 4-6 months of age and followed up with a second vaccination at 13-15 months. A booster vaccination should there after be given at 22-24 months of age.
- In Sri Lanka BQ vaccine is manufactured locally to meet the requirement of the country.

Anthrax

Anthrax is a highly contagious disease affecting cattle and buffaloes characterised by sudden death accompanied by oozing of unclotted blood from the natural orifices and incomplete rigor mortis. No pathognomonic lesions are observed. Differential diagnosis from other syndromes such as lightning stroke, Black Quarter (peracute form), clostridial infections, leptospirosis(acute form), hypomagnesemia, lead poisoning and snake bite is important. Anthrax is a zoonotic disease with worldwide distribution and is caused by *Bacillus anthracis.* In the developing countries of the world incidence of the disease is high due to certain factors such as, poor diagnostic and reporting systems, indiscriminate slaughter of sick animals for meat and hides and improper disposal of infected carcasses. In enzootic areas, specially in marshy lands where buffaloes wallow, the soil remains infected with the organism (spores) for many years (Cockril 1974). This disease was present in Sri Lanka in the past, but it has not been reported in this country since 1969. However, the risk of re-introduction through new imports cannot be ruled out.

Prevention and control

- Although early treatment with antibiotics (e.g. penicillin, streptomycin, oxytetracyclines) can be used, it is not recommended, because of the risk of human infection and the highly infectious nature of the disease.
- Strict hygienic measures should be adapted in handling sick cases and carcases, in order to prevent contamination of the environment. Blood samples for laboratory diagnosis are taken from the ear vein or caudal vein.
- The carcase should be burried in a deep pit (2m) with the contaminated bedding and soil sprinkled with quick lime.
- Infected premisses should be decontaminated with a strong and effective disinfectant.
- All incontact animals should be segregated and kept in quarantine, to prevent the spread of infection.

- All susceptible cattle and buffaloes should be vaccinated annually as a prophylactic measure. An avirulent strain of *Bacillus anthrasis* (34F2 Sterne) is used worldwide in the manufacture of this vaccine. Vaccination against anthrax is not undertaken presently in Sri Lanka, as the disease has not been reported after 1969.

Occular disease in buffaloes

Factors causing occular disease in the buffalo are many. A commonly occuring occular malady is an affection of the conjunctival sac which is caused by the invasion of foreign bodies such as dust, grass seeds, straw and such material that enter the eye during grazing. Parasites such as *Thelezia sp.* have also been incriminated as a causative agent of conjunctival sac infections in the buffalo. This condition has been recorded in India, Sri Lanka and Egypt (Cockril 1974). Invasion of the conjunctival sac by foreign bodies or microbes induce a catarrhal conjunctivitis which becomes mucopurulent with secondary bacterial infection and could sometimes lead to keratitis. Among the various occular affections only Infectious Bovine Keratitis and its treatment has been described, because of the common occurrence and its significance in the buffalo.

Infectious Bovine Keratitis (IBK)

Among the occular infections in the buffalo, IBK which is caused by a bacterial organism *Morexella bovis* is considered the most important. It is of common occurrence in cattle and buffaloes with a high incidence during the dry seasons. Spread of the infection is facilitated by the increase in fly population during this period. Carrier animals are also considered epedemiologically significant in the transmission of the disease (Wijewardana *et al.*, 1984). Incidence of the disease is higher in buffalo calves than adults. Close herding of animals for long periods in dusty environment can facilitate the spread of disease.

The disease is characterised by reddning of one or both eyes, blepherospasm (spasmodic winking), photophobia, occular discomfort and epiphoria (tearing) followed by conjunctivitis, hyperaemia and oedema with copious serous discharge from the eyes. Cornea becomes oedematous and may slough after rapid enlargement forming an ulcer. The ulcer may heal in 4 -6 weeks. However, if the condition becomes serious, the animal may become blind.

Treatment and control:

- Infected cases should be isolated from the rest and treated promptly with appropriate antibiotics.
- Opthalmic ointments containing suitable antibiotics and cortisone can be used. Many such preparations are available in the market.
- Application of neomycin and hydrocortisone in the form of an ointment twice a day has been successful.
- Parenteral treatment with long acting oxytetracyclines early in the infection has also been effective.

Diseases associated with oral lesions

This section deals with two highly contagious viral diseases of buffaloes namely, Foot and Mouth Disease (FMD) and Rinderpest (RP) which are of great economic significance to dairy/buffalo farmers, particularly in the Asian region as they have been endemic in the region for many decades. Both these diseases are characterised by similar clinical signs such as high fever and severe oral lesions, but the dissimilarity is that RP is severely fatal, whereas FMD is rarely fatal in adult animals. Oral lesions of FMD are vesicular in nature, while those that appear in RP are never vesicular, but occur as superficial erosions and proceed to develop as ulcers . FMD should also be differentiated from vesicular stomatitis (VS).

Foot and Mouth Disease (FMD)

Foot and Mouth Disease is a highly contagious acute viral disease of cloven footed animals including the buffalo and is of great economic importance. The virus has 7 sero types of which 4 are present in Asia (O,A,C and Asia 1). Type "O" is the predominant one out of the above. Although virus type "C" was reported to be present in Sri Lanka in 1970, only type "O" is present now. Severity of the disease in buffaloes vary between outbreaks, but is much less evident compared to cattle in Sri Lanka. It is speculated, that the wild buffalo may serve as a reservoir (Fernando 1980). As the transmission and pathogenesis of the disease has been described in detail under chapter 4.1, this chapter only deals with the prevention and control of the disease.

Prevention and Control:

Strategies adopted to control the disease vary with each country. In countries like Australia and U.K. which are free from FMD control measures are very stringent. However, in Asian countries where the disease is endemic, various measures indicated below are implemented for its control.

- Implementation of import control regulations with regard to livestock and livestock products to prevent the entry of virus into the country particularly new strains.
- Movement control of animals from infected areas to disease free regions of the country, through the implementation of legislative regulations and procedures.
- Implementation of animal quarantine measures.
- Implementation of zoosanitary measures.
- Implementation of regular vaccination programmes

Some of the countries in Asia use their own vaccines in the immunisation programmes(eg. India, Pakistan and in Sri Lanka until recently), while others use vaccines imported from reputed vaccine manufacturers. Vaccines used in the immunisation programmes of the different countries in the region vary according to the needs of each area. Most countries, where several serotypes are present, they use multivalent vaccines. However, Sri Lanka uses only monovalent vaccine containing virus type "O", as it is the only serotype present in the country now. Routine vaccinations in Sri Lanka are undertaken in cattle and buffaloes. At present ring vaccination is carried out in the disease free areas around the focus of infection, using imported vaccine. However, in the state farms and well managed private sector dairies routine vaccination programme are carried out at regular intervals. The FMD vaccination schedule recommended for Sri Lanka is given below.

- Primary vaccination in calves at 4 months of age.
- Second vaccination in calves at 5 months of age.
- Third vaccination in calves at 7 months of age.
- Booster vaccination in calves at 12 months of age and twice/year thereafter. Vaccinal immunity to FMD is known to last only for a period of six months. Hence the need to vaccinate twice a year.
- The success of an immunisation programme would depend on several factors listed below:
  - The efficacy of the vaccine used should provide protection against the strain of field virus causing the outbreak.
  - Maintenance of the cold chain of the vaccine in the field.
  - In calfhood, vaccinations there should be minimal interfearance from maternally derived antibodies (MDA). It is for this reason that the first calf hood vaccination is done at 4 months of age.
  - At the time of vaccination one has to be cautious of concurrent disease in the animal.

Rinderpest

It is a highly fatal virus disease of cloven footed animals like cattle, buffalo, goats and sheep characterised by high fever, necrotic stomatitis, diarrhoea and high mortality. Transmission of the disease is by close contact between sick animals and susceptible ones. The virus is excreted by infected animals in the urine, faeces, nasal discharges and sweat. Transmission occurs trough contaminated feed or aerosol. The virus is unstable outside the animal body and is quickly destroyed at ambient temperature, sunlight and variation in the pH. Rinderpest has been present in a number of countries of the Asian region such as India, Pakistan, Sri Lanka and Afganistan and in the middle east countries. However, with regular and sustained vaccination prevalence rate has been significantly reduced in certain countries in recent years eg. India. Diagnosis is based on the history, clinical signs and necropsy lesions, while confirmation is through isolation of the virus in cell culture and the detection of virus specific antibodies in the serum of infected animals.

Prevention and Control:

- Prohibition of the import of live ruminants and pigs from countries where the disease is present to countries free of disease.
- In the case of new outbreaks eradication could be attempted by adapting a "slaughter policy". ie. the slaughter of affected animals with the payment of compensation to stock owners.
- When the slaughter policy cannot be applied, spread of the disease could be prevented by immediate isolation of the sick cases in the infected area and control of the movement of animals.
- Vaccination of the susceptible livestock with rinderpest vaccine, which protects
- The animals against all strains of rinderpest virus.
- Immunity induced is generally life long.
- Susceptible animals should be vaccinated twice, first at a young age of 4-6 months and again after one year.

- On the face of an outbreak of rinderpest immediate vaccination is done on all susceptible animals in the entire neighbourhood. .
- A Global Rinderpest Eradication Programme (GREP) has been launched in 1992, to achieve the eradication of rinderpest in the year 2010.

Disease affecting the mammary glands

Mastitis

The most important disease of the mammary glands in dairy cows and buffaloes is mastitis and is of great economic importance to the farmer. Buffalo cows are relatively resistant to udder infections compared to dairy cows (Wanasinghe 1985). However, it does not mean that the problem could be taken lightly, because in large dairy herds and even in small dairies where the management levels are poor and unhygienic incidence of mastitis has been significantly higher. Probability of mastitis incidence also increase with age and lactation number. (Mitra *et al.*1995). In most countries annual economic loss from mastitis could be significantly high. Therefore it is important for any sized dairy buffalo operation to undertake an effective programme of mastitis control and prevention. The first step that has to be taken in such a control programme is to detect the infected cases (clinical and sub-clinical) in the entire herd. Preliminary screening could be done by using the California Mastitis Test (CMT). This test has recently been adapted for use in buffaloes in Sri Lanka (Silva *et al.* 1994). The organisms known to cause mastitis in buffalo cows are many and varied. Some of the more important ones among them are: Staphylococcus, Streptococcus, Escherichia and Corynebacterium species. Mycoplasma and fungi may also be involved occasionally. A detailed description of the disease is given under chapter 2.3 of this book. Our emphasis here is only on the practical aspects of treatment, prevention and control.

Treatment of clinical mastitis:

- Clinical cases should be treated promptly, because any delay could cause considerable damage to the udder and mammary tissues resulting in the loss of milk production .
- Milk from clinical cases should be withheld from home consumption and from the market, for the duration recommended by the manufacturer of the drug used for treatment.
- Milk samples from clinical cases should be sent to the laboratory for bacteriological culture prior to treatment, for identification of the causative organisms.
- In the laboratory, antibiotic sensitivity tests should also be performed, so as to determine the most appropriate drug suitable for treatment and to change the drug in case the initial treatment was ineffective.
- Clinical symptoms are generally caused by toxic microbial products. Therefore, frequent removal of such products from the udder would provide relief to the animal. This is achieved normally by milking each infected quarter prior to intramammary treatment.
- Intramammary antibiotic preparations should be judiciously chosen and administered daily to each infected quarter at 12 –24 hour intervals for 3- 4 days. Manufacturer's recommendations should be followed in this regard.
- In the treatment of clinical cases following points should be taken into consideration.
    - The invading organism should be sensitive to the antibiotic used.
    - The antibiotic preparation should be non-irritant

- The antibiotic concentration should be adequate to overcome the damaging effects of the invading organism.
- Efficacy of the antibiotic in the udder in the presence milk should be of reasonable duration.
- The vehicle in which the drug is presented should be a slow releasing base having prolonged activity in the udder
- Absorption and distribution of the antibiotic in the mammary tissues should be efficient.

Treatment of subclinical mastitis:

- All subclinical cases detected through the preliminary screening test using the CMT and isolation procedure should be treated, at the time of drying off.
- This is popularly called "dry cow therapy". It may be done 6 – 8 weeks prior to parturition, because the rate of cure in this situation is greater than when the animal is in lactation.
- The other advantages of this method are:
  - Antibiotic requirements for treatment are less,
  - Loss of milk due to therapy is minimal,
  - Antibiotic efficacy is greater in the absence of milk in the udder,
  - Regeneration of damaged mammary tissue is better,
  - There is no antibiotic residue in the milk at calving.
- It is generally recommended that all clinical cases be treated at the end of the lactation as dry cow therapy.
- All cows showing a 1+ or greater reactivity by the CMT should also be subjected to dry cow therapy.

Prevention and control of mastitis:

Since mastitis is an infection which affects mostly high producing dairy and buffalo cows an effective programme of prevention and control would be the most economic proposition for a dairy operation. Therefore, the following preventive programme could be recommended:

- Preventing the introduction of new infections by following hygienic pre and postmilking procedures.
- Testing new cows prior to their introduction into the herd.
- Maintaining the milking machines if available in good working order.
- The infection present in the herd should be eliminated by treating the clinical and subclinical cases early and culling the chronic cases.
- Regular monitoring of the control programme which could be achieved by
  - Examining bulk milk samples routinely and keeping records.
  - Early detection of clinical cases and identifying subclinical cases.
  - Keeping records of treatment, including the antibiotic sensitivity and response to treatment.

Table 7.2 Antibiotics commonly used in mastitis treatment.

Causative organism	Antibiotic recommended for use in order of sensitivity
Staphylococcus aureus and other staphylococeus species.	chloramphenicol[1], gentamycin[2], oxytetracyclin[3], neomycin[4], erythromycin[5], kenemycin[6], cloxacillin[7], ampicillin[8], cefaloridine[9], sulphonamides[10].
E.coli	1,2,3,4,5,9,10, and strepto-penicillin[11].
Streptoccal species.	1,3,5,6,7,8,10,11.
Mycoplasma	1,4,5, thiamphenicol, spiramycin, vibramycin, lincomycin, ledermycin, tetracycline.

Refer chapter 2.3 for details

Diseases associated with abortion

This section describes two important reproductive diseases of bacteriological aetiology causing abortion in buffaloes. Both diseases are significant economically and from zoonotic aspect. They could cause human infections of a serious nature if appropriate preventive measures are not taken.

Brucellosis

Brucellosis is a diseases primarily affecting ruminants such as cattle, buffaloes and sheep and non ruminants like pigs and is communicable to humans. The disease in bovines primarily affects the reproductive system causing major economic loss through abortions, infertility and lowered milk production. In countries where the disease is endemic. Humans generally contract the disease by consumption of raw milk. The causative agent of brucellosis in cattle and buffaloes is usually *Brucella abortus.* It is endemec in most developing countries in Asia. Organisms are present in massive numbers in the aborted foetus, placental tissues, vaginal discharges and in the milk. After calving the environment gets heavily contaminated with brucella organisms, that could survive for a long period in the pasture and soil. The organisms are however destroyed under direct sunlight or heat. Infection from animal to animal occurs normally through direct contact or through the nasopharynx, conjunctiva and skin aberations. Calves born to infected dams can get infected either congenitally or through the ingestion of milk from the infected dam. The disease becomes evident in the female only at parturition or after abortion. Infected animals can become carriers for life. Abortion usually occurs after 5 months of pregnancy. Retention of placenta and metritis are common complications in such cases. In the case of infected bulls orchitis and epididimitis may be observed. Direct sexual transmission of the disease is rare. The chances of infection may be greater if the bull semen is used for artificial insemination.

Prevention, Control and Eradication:

- Suspected herds should be examined regularly for the detection of infected cases to facilitate early preventive measures to arrest the spread of infection.

- Brucellosis should be regarded as a heard problem and basic hygienic measures taken to contain the infection within the infected premises and to prevent a human infection.
- Infected pregnant cows should be kept in isolation from 4 days prior to parturition up to atleast one month after calving.
- At parturition the placenta and all of the after birth material should be collected carefully and burried or preferably incinerated.
- In the event of an abortion too similar procedure should be followed.
- After a calving or an abortion, the dairy premises should be thoroughly disinfected, using a good disinfectant.
- Vaccination will reduce the incidence of abortion in the herd. About 2/3 the animals in the herd would develop resistance to exposure.
- When the prevalence of infection in the herd falls below 0.2%, vaccination of the herd may be terminated.
- If the economic and social status of the country permits, a total eradication programme could also be followed.

Leptospirosis

Leptospirosis affects most mammalian species including cattle, buffaloes and man and has a worldwide distribution. It is also of zoonotic importance because it is transmitted from animals to man. The organism responsible for the disease is a bacterium of the genus Leptospira. This is a disease of cattle and buffaloes that affects predominantly the reproductive system causing economic loss through abortion, still births, infertility and decreased milk production. The organism is excreted in the urine and milk. Infection may enter the body through the aberated skin, inhalation or through ingestion. Leptospirosis could manifest in several forms such as per-acute, acute and sub-acute farms. Clinical signs in the buffalo and cattle are not pathognomonic for leptospira. In sub acute cases the animal is weak, depressed, anorexic, anaemic with high fever and diarrhoea. Lactating cows may show discoloration of the milk with clots in it and a reduction in yield. Jaundice and signs of encephalitis may appear in severe cases. In pregnant animals, abortion and stillbirths may be observed. For a confirmative diagnosis serological tests and bacterial cultures have to be done. Urine, blood, peritoneal fluids and pleural exudate from infected cases are used as diagnostic material

Treatment and control:

- Dihydrostreptomycin at a dosage rate of 25 ug/kg administered intramuscularly for a period of 14 days has been found effective.
- Prophylactic treatment is undertaken in developed countries, mainly to prevent human infection, rather than of the livestock.
- Vaccination is costly, yet the efficacy is not satisfactory.
- As a preventive measure, attempts have been made to control field rodents such as rats and mice that could transmit the disease to ruminants and man.
- Preventing buffaloes from wallowing in waterpools contaminated with leptospira organisms has been another control measure

Diseases associated with production

When the physiological capability of the animal body fails to meet the nutritional demands for its metabolic activities, nutrients will be drawn from the body reserves to maintain production functions. This situation creates a metabolic imbalance resulting in disease related to production. Such diseases are described in detail under chapter 5. In here we have briefly outlined only some of the more important metabolic diseases commonly occurring in buffaloes and the line of treatment.

Milk Fever

This is a disease condition that is seen commonly in high producing dairy buffaloes and cows. The disease is of sudden onset and is caused by the lowering of serum calcium in the cow. It may be due to (a) excessive drainage of calcium through the colostrum or milk, (b) impaired absorption of dietary calcium, or (c) slow mobilisation of calcium from the skeletal reserves at the terminal stage of gestation. The condition is generally characterised by depression, muscle weakness and incoordination few days prior to or after calving. The pulse rate, respiratory rate and body temperature are lower than in a healthy cow. Muzzle becomes dry and body extremities are cold. The cow generally stops eating and movement become slow. She may become recumbent in the lateral or sternal recumbency. If immediate treatment is not given, she may loose consciousness and die in 12 to 24 hrs.

Treatment

- Parenteral administration of a 25% solution of calcium borogluconate. 300 - 350 ml given intravenously (i/v), followed by another 200 - 350ml subcutaneously(s/c).
- In case of a relapse following treatment can be given: A mixture of calcium gluconate 20.8% + boric acid 4.4% + magnesium hypophosphite 5% and dextrose 20% (w/v) administered at a dosage rate of 350 ml i/v and 200ml s/c.

Ketosis.

Dairy buffaloes in peak lactation are susceptible to ketosis. It is a multifactoral disorder of energy metabolism. The disease is characterised by a drop in feed intake, nervous signs, reduction in milk yield, ketones in the urine and loss of body weight. At peak production the increase in demand for glucose often exceeds the ability of the rumen to supply glucogenic precursors which result in ketosis. The blood ketone concentration is increased causing ketoneamia and ketonuria. The two conditions taken together is termed ketosis.

Treatment.

- Immediate restoration of blood glucose level and maintain it until recovery. This can be achieved through parenteral or oral administration of glucose or glucose precursors. Administer 500ml of 50% glucose (dextrose) solution i/v daily for 4 - 5 days.
- Glucocorticoids (cortisone acetate or hydrocortisone acetate) 0.5 to 1.5g may be given i/v to promote gluconeogenesis.
- The above therapy could be supported by the oral administration of 250g propylene glycol mixed with equal amounts of water twice per day for 5 days.
- Propylene glycol or glycerine (225g twice/d for 2 days followed by 110g/d for 2 days) given by mouth as a drench has been effective in dairy cattle.

Hypomagnesaemia in dairy buffaloes

Disease in cows: Hypomagnesaemia may occur in lactating as well as in nonlactating pregnant buffalo cows. Due to the high milk yield and the development of the foetus in such animals, the dietary magnesium requirement increases proportionately. Deficiencies in the dietary magnesium cause this condition. The onset of clinical disease is sudden and the animal becomes uneasy, excitable and off feed. Twitching of the muscles and staggering gait is generally seen. The animal may even fall down (De and Goswanne 1960). After a tetanic attack there is a sudden rise in body temperature, pulse and respiratory rates.

Treatment:

- Magnesium borogluconate or magnesium sulphate (25g) dissolved in 400 to 500ml sterile distilled water can be administered by s/c route and followed up with 50g magnesium oxide per day for 7 days given by mouth.
- Subcutaneous injection of about 200 ml of 50% solution of magnesium sulphate in sterile distilled water has been recommended for cattle for optimal results (Radostitis *et al.*, 1994).

Disease in calves: Hypomagnesaemia. occurs in buffalo calves that do not receive adequate amounts of magnesium through intestinal absorption. Clinically, there is a severe fall in serum magnesium level. Hypomagnesaemic calves show erection and backward carriage of ears, excitement, hypersensitivity, anorexia, sunken eye balls, staggering gait, increased respiration and heart rate, reduction in body temperature and ruminant movement.

Treatment:

- Parenteral administration of magnesium sulphate (10g dissolved in sterile distilled water) subcutaneously and followed by oral administration of 10 - 15g magnesium oxide daily.
- Tranquilizers like acetylpromazine may be used for the control of muscular tremors.

Insidious bacterial diseases of the buffalo

Tuberculosis

Tuberculosis is a chronic disease manifested by progressive development of tubercles in any part of the animal body. The organism pathogenic to buffaloes and cattle is *Mycobacterium tuberculosis*. This organism survives for a long period in warm and moist covered environment. Although the incidence of tuberculosis in buffaloes has been reported to be higher than in cattle, it is seldom seen in Sri Lanka. Most significant factor concerning tuberculosis in buffaloes and cattle is its zoonotic property. Humans could contract the disease by drinking raw milk of tuberculous cows or through the aerosol route. Details on the epidemiology of the disease are described in chapter 2.4.3.

Prevention and control:

- Adequate boiling, pasteurization or sterilisation prior to the consumption of milk and milk products could prevent human infection through milk.
- Thorough antimortem inspection as well as precautions taken at meat inspection to eliminate the affected organs and parts could further prevent the risk of human infection.
- Treatment of the disease in the buffalo is of very little practical value. Control of the disease could be achieved by the slaughter of all tuberculin positive animals if the economic situation permits.
- In the developing countries of the Asian region like Sri Lanka, the above slaughter policy may not be economically feasible. In such a situation one could resort to the slaughter of clinical cases detected and testing the rest of the herd twice a year.
- The herd should then be divided into reactors and non-reactors and the reactors should be kept under close observation for clinical signs and eliminated, eventually.
- At all times, the dairy premises should be kept clean, to prevent the spread of disease.

Johne's disease (Paratuberculosis)

This is an infectious enteritis in the buffalo and in cattle caused by *Mycobacterium paratuberculosis* and is almost a fatal condition. It is manifested by chronic diarrhoea and wasting. The organism is relatively resistant to sunlight and disinfectants and could survive for long periods in water, soil and faeces. Transmission is by ingestion of contaminated food and water or through direct contact from infected animals. The disease is wide spread in buffaloes in India with a high level of susceptibility of the young (Mohan 1968). However, the condition is not so common in Sri Lanka.

Prevention and control:

- Treatment is not effective.
- Infected animals and their faeces should be disposed of properly and the premises disinfected thoroughly.
- Culling the infected cases would be the most prudent step to eliminate the infection from the herd.

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