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A REVIEW OF USE OF PROBIOTIC AS GROWTH PROMOTER IN ANIMAL FEEDING

N. Priyankarage¹ B.V.Sc, Ph.D, S.S.P. Silva¹ B.V.Sc, Ph.D, and S.P. Gunaratne² B.V.Sc, Ph.D

¹Animal Nutrition Division, Veterinary Research Institute, Gannoruwa, Peradeniya
²Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya

INTRODUCTION

With the introduction of penicillin in late 1940s, large number of antibiotics have been discovered and used to treat infections. These antibiotics are mainly used as therapeutic agents but also been used for prevention of bacterial diseases as well as growth or production enhancer (Jin et al., 1997 a). Due to the intensive production pressure, the farm animals especially poultry are subjected to various kinds of stresses in the present farming systems, which adversely affects the performances. The stress factors such as overcrowding, transportation and vaccination can cause imbalances in the gut microflora and lowering of the body defense mechanism (Jin et al., 1997 a). Under such circumstances, sub therapeutic levels of antibiotics have been used as feed additives to suppress the harmful organisms and improve the growth and feed conversion efficiency (Francois and Michel, 1968; Armstrong, 1984; Jin et al., 1997 a). However, continued use of sub therapeutic levels of antibiotics in animal feeding cause several public health hazards like development of antibiotic resistant microbes and harmful residues in animal products (Blackmen, 2000). As a consequence, the use of antibiotics as feed additive has been highly regulated and even totally banned in some parts of the world. However, it is well accepted that antibiotic growth promoters have had significant role in modern day animal production. Therefore suitable alternatives are essential to replace the use of antibiotics in feed and research has been focused for looking at appropriate substitutes during the last few years.

The use of nonantibiotic growth promoters such as probiotics, prebiotics, organic acids as well as exogenous enzymes have been claimed to be more consumer friendly alternatives to antibiotics. These growth promoters mainly act on the intestinal microflora of host animal and beneficially alter the microflora in order to get the positive effects on performance and prevention of certain bacterial infections in animals, while exogenous enzymes improve the digestion and nutrient utilization of animals (Blackmen, 2000).

Prebiotics are mainly oligosaccharides of galactose, fructose or mannose (Mingan, 2001) and it beneficially affect the host by selectively stimulating the growth or activity of one or limited number of beneficial bacterial species already resident in the digestive tract and thus improves host health (Wenk, 2000). Organic acids such as propionic, acetic, formic, citric and tartaric acids can be added to the animal feed to enhance the digestion and influence the intestinal flora of host animal in a positive way (Health Council Report, 1998). Exogenous enzymes are added to enhance the digestion of particular components mainly Non Starch Polysaccharides (NSP) in grains and meals that are used in animal feed production. Studies have been conducted with the inclusion of exogenous enzymes to the diets based on rice by products (Silva et al., 2003), rye (Silva and Smithard, 2002) and barley (Jensen et al., 1957) and positive effects on animals performance have been well demonstrated.

HISTORY OF PROBIOTICS

At the beginning of the 20th century, the beneficial effects of consuming large quantities of yought containing Lactobacillus were first recognized by Metchnikoff(1907) due to the favourable influence on the intestinal microflora by replacing toxin producing bacteria which results in better health and increased lifespan. Rettger and Chaplin (1921) again confirmed these findings and assumed that this beneficial effect was due to the colonization of the gut by Lactobacillus acidophilus. Use of these live microbes in poultry preparations was pioneered by Tortureo (1973) who observed that the increased weight gain and better feed conversion were comparable to that of antibiotic growth promoters. During the same period Nurmi and Rantala (1973) observed that feeding of faeces of adult domestic fowl to the newly hatched chicks result the restriction of colonization of Salmonella infantis in cecum. Since then the use of live microbes in livestock and poultry feeding has been receiving attention and studies have been conducted to determine the effects and mechanism involved.

DEFINITION OF PROBIOTIC

The term "Probiotic" is derived from Greek word “for life” (Jin et al., 1997 a; Gibson and Fuller, 2000) and the innovators of this term to live microbial feed additives were Lilly and Stillwell (1965) who used to describe the growth promoting effects of the live microbes. Later Crawford (1979) described the term "Probiotic" as a...
culture of specific living microorganisms which implants in the animal to which it is fed and ensures the effective establishment of the intestinal population of beneficial organisms. He also mentioned that the culture must consist of minimum number of specific bacteria maintained in a dry and stable form for storage purposes, temperature dependent and produce a maximum response with a specific dose range.

The term "Probiotics" was suggested to be replaced by the term "Direct Fed Microbes (DFM)" by Miles et al (1991) who defined DFM as "a source of live naturally occurring microorganisms which included bacteria, fungi and yeast. However, most of the products contained bacteria and therefore the term "Probiotic" remains unchanged (Jin et al., 1997a).

PROBIOTIC ORGANISMS AND THEIR ROUTE OF ADMINISTRATION

Some bacterial species are widely used as probiotic organisms. Lactobacillus species are the commonly used bacteria and other bacterial species like Streptococcus, Enterococcus, Bifidobacterium and some strains of Escherichia coli are also used in probiotic preparations. In addition to bacteria and fungi, live yeast have also been used as probiotics in animal feeding. These organisms have been used alone or as a mixture to obtain the growth promoting effect. The importance of multiple strain preparations is recognized as they are active against a wider range of adverse conditions and in a wider range of animal species. In addition, probiotics have been used in combination with other growth promoters (Samanta and Biswas, 1995).

Probiotics can be introduced to animals through drinking water as well as mixed with feed and these preparations are available in different forms such as capsules, paste, powder or granules. In most instances, after termination of probiotic feeding, the effects produced were there only for a limited period (Fuller, 1989; Shome et al., 2000). Therefore, the best method of administration of probiotics is continuous feeding to the animal. However, it has been shown that low doses even for shorter periods is effective (Watkins and Kratzer, 1983).

CHARACTERISTICS OF PROBIOTICS

Microorganisms should have some specific characters such as growth promotion and resistance to disease to the host animal to be classified as probiotic organisms. It should be non pathogenic and not have any adverse effects and contain viable cells, preferably in large numbers, although the minimum effective dose is not known, to deliver large number of microbes into the lower intestine. It must be capable of surviving in the intestine, be resistant to low pH, organic acids and other antibacterial substances present in the intestine. It should also be stable and capable of retaining its viability for long periods during long storage under field conditions (Fuller, 1989; Simmering and Blaut, 2001).

Probiotics with all these features has considerable advantages. They do not induce resistance to antibiotics and they are not toxic and not producing any undesirable side effects, when being fed and in the case of food animals, will not produce toxic residues in the carcass (Fuller, 1989). They stimulate immunity and immune status (Gibson and Fuller, 2000; Hershberg and Mayer, 2000) and remains unaffected by antibiotics (Isolauri et al., 1998; Perdigon et al., 1990).

However, presence of low viable cell counts in the commercial probiotic preparations (Clements et al., 1983) and presence of totally different bacterial species in the preparations (Fowler, 1969; Gilliland, 1981) can affect the quality and the outcome of the probiotic products.

MAJOR EFFECTS OF PROBIOTICS

Maintaining beneficial microflora in the alimentary tract

The foetus in utero is sterile, but soon after birth all warm blooded animals acquires microorganisms and establish them in the digestive tract. In most cases, these established microflora are the forerunners of the final organisms, which will colonize and persist in the digestive tract throughout the adult life of the animal. Most of these are beneficial organisms, specially Lactobacilli, form a symbiotic relationship with the host and help to regulate the composition of its intestinal microflora. In this phenomenon host provide favourable temperature, constant supply of nutrients and essential fluids to microorganisms and host benefit by maintaining a microflora that does not cause any disease state. This relationship will help to maintain the microbial balance in order to obtain the well-functioning intestinal tract with efficient conversion of feed for maintenance, growth and production (Fuller, 1973, 1989).

However, in modern intensive animal rearing systems, due to the available hygienic conditions, acquiring of full complement of characteristic microbes is difficult. Further, due to certain stress factors and antibiotic therapy the established flora can be altered. Therefore, continuous feeding of probiotics to animals will enable the host animal to return to normal. It has been found that probiotics can maintain beneficial microflora in two ways; by competitive exclusion and antagonistic activity towards the pathogens (Jin et al., 1997a).

Competitive Exclusion

The competitive exclusion of undesirable microbes by the beneficial microbes has been suggested as the major mechanism of action of probiotics in animal feeds. This was first applied to the domestic fowl by Nurmi and Rantala (1973) when they attempted to control a severe outbreak of salmonella infection in broiler flocks. In this study, it was determined that very low doses of salmonella were sufficient to initiate salmonella infection in chicks and inoculating them with adult fowl intestinal contents orally, increase the resistance of young chicks to salmonella infection. Since then several studies were carried out to confirm the concept of competitive...
Table. Commonly used Probiotic organisms

<table>
<thead>
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<th>Probiotic organisms</th>
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<tbody>
<tr>
<td><strong>1. Bacterial species</strong></td>
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<tr>
<td>A. Lactobacillus species</td>
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<tr>
<td>L. Acidophilus</td>
<td>Ahmed et al., 1994; Priyankarage et al., 2004</td>
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<tr>
<td>L. sporogenes</td>
<td>Jin et al., 2000</td>
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<td>L. fermentum</td>
<td>Jin et al., 1998 a, b</td>
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<td>L. casei</td>
<td>Priyankarage et al., 2003, 2004</td>
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<td>L. helveticus</td>
<td>Fuller, 1989</td>
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<td>L. lactis</td>
<td>Fuller, 1989</td>
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<tr>
<td>L. salivarius</td>
<td>Fuller, 1989</td>
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<tr>
<td>L. Plantarum</td>
<td>Fuller, 1989</td>
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<tr>
<td>L. Reuteri</td>
<td>Dunham et al., 1993</td>
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<tr>
<td>L. gasseri</td>
<td>Usman, 2001</td>
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<td>L. Johnsonii</td>
<td>Denou et al., 2008</td>
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<tr>
<td>L. Paracasei</td>
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<tr>
<td>L. Rhamnosus</td>
<td>Lesniewska et al., 2006</td>
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<tr>
<td>L. bulgaricus</td>
<td>Bhatt et al., 1995; Priyankarage et al., 2003</td>
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<tr>
<td>B. Bifidobacterium species</td>
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<tr>
<td>B. bifidum</td>
<td>Panda et al., 2000; Priyankarae et al., 2004</td>
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<td>B. breve</td>
<td>Mullie et al., 2004</td>
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<td>B. lactis</td>
<td>Sanders, 2006; Lesniewska et al., 2006</td>
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<td>B. longum</td>
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<tr>
<td>C. Streptococcus species</td>
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<tr>
<td>S. thermophilus</td>
<td>Priyankarage et al., 2003; Palliyaguru et al., 2004</td>
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<td>S. faecium</td>
<td>Gohain et al., 1998; Priyankarage et al., 2004</td>
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<tr>
<td>D. Enterococcus species</td>
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<tr>
<td>E. Faecium</td>
<td>Scharek et al., 2007</td>
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<tr>
<td>E. faecalis</td>
<td>Fuller, 1989</td>
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<tr>
<td>E. Bacillus</td>
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<tr>
<td>B. cereus</td>
<td>Lodemann et al., 2008; Scharek et al., 2007</td>
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<tr>
<td>B. subtilis</td>
<td>Mutus et al., 2006</td>
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<tr>
<td>B. Licheniformis</td>
<td>Alexopoulos et al., 2004</td>
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<tr>
<td>E. Other Bacterial species</td>
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<tr>
<td>Escherichia coli</td>
<td>Kleta et al., 2006</td>
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<td><strong>2. Fungal species</strong></td>
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<tr>
<td>Aspergillus oryzae</td>
<td>Han et al., 1999; Priyankarage et al., 2004</td>
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<tr>
<td>Eremothecium ashbyi</td>
<td>Narahari et al., 1997</td>
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<tr>
<td>Saccharomyces cerevisiae (live yeast)</td>
<td>Sarkar et al., 1997; Besnard et al., 2000; Priyankarage et al., 2004</td>
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exclusion. These studies conducted with undefined mixed bacterial cultures from adult faecal materials, cecal contents (Starovic et al., 1991; Baba et al., 1991; Corrier et al., 1995) as well as with defined bacterial cultures (Watkins and Kratzer 1983; Watkins and Miller, 1983; Buenrostro and Kratzer, 1983; Priyankarage et al., 2003, 2004). The competitive exclusion has been brought about by several means in gastro intestinal tract, namely (1) Creation of micro ecology that is hostile to other bacterial species, (2) Elimination of other bacterial receptor sites, (3) Production and secretion of antimicrobials and (4) Selective & competitive depletion of essential nutrients (Rolfé, 1991).

**Competitive exclusion with undefined bacterial cultures**

Undefined treatments have been shown to be more effective than defined culture treatments (Starvic et al., 1991). The number of Salmonella organisms in the gut has been decreased in Salmonella typhimurium inoculated broiler chicks when treated with mixed cecal bacterial cultures obtained from adult broilers (Corrier et al., 1995). Further, they have also indicated that volatile fatty acid producing bacteria present in the mixed cecal bacterial culture rapidly establish in the ceca of the treated chicks and effectively reduce the S. typhimurium colonization. The caecal contents from adult birds are much more effective than caecal contents of newly hatched chicks in reducing the colonization of S. typhimurium in the gut (Baba et al., 1991).

**Competitive exclusion with defined bacterial cultures**

Lactic acid producing bacteria (mainly Lactobacillus) have been the main component of defined cultures and in defined mixture containing strains from a single genus were ineffective in comparison with mixtures containing strains from different genus (Starvic et al., 1991). The efficacy of defined mixtures gradually decreases as the time of storage increases explaining the poor efficacy (Starvic et al., 1991). Neither host specific strains nor non-host specific strains of Lactobacillus have been found to be effective in competitive exclusion of bacteria in the gut of chicks reared under clean environmental conditions (Watkins and Kratzer, 1983). However, with some stresses, host specific Lactobacilli strains may aid the colonization process than non-host specific strains. The number of E. coli organisms in crop and small intestine of chicks has been reduced by strains of the host specific Lactobacilli (strains 59 & 74/1) but not in the cecum (Fuller, 1977). Similar findings have been found by Watkins and Kratzer (1983) who indicated that chicks dosed with Lactobacilli strain 59 lowered the coliform numbers in crop contents and suggested that strain 59 is a more competitive Lactobacillus in the avian crop. Edens et al (1997) also found that in vivo administration of Lactobacillus reuteri has effectively increased the rate of gut colonization and reduced the E. coli and Salmonella colonization of chicks. Further increasing the dietary levels of L. acidophilus elevated the Lactobacillus colonization and reduced coliform number in all sections in the intestine except the caecum suggesting that the mechanism is not effective in caecum (Miles et al., 1981; Jin et al., 1996 a).

Competitive exclusion has been much stronger with Lactobacillus species than most other species of probiotic bacteria. When compared the ability of adherence, L. acidophilus has shown better gut colonisation than Streptococcus thermophilus (Conway, 1987; Baba et al., 1991) indicating that combination of Lactobacilli with non-pathogenic E. coli is far more effective in excluding S. typhimurium from the gut. However, there are instances where the probiotics are ineffective to competitively exclude Salmonella in challenged experiments (Stavric et al., 1992; Pivnick and Nurmi, 1982; Hinton and Mead, 1992; Priyankarage et al., 2004). Although, some strains of L. acidophilus and L. fermentum are able to reduce the attachment of specific salmonella species in chicken Ileal Epithelial Cells (IES), some strains are incapable of displacing S. typhimurium, S. pullorum and S. enteritidis once they are attached to chicken IES (Jin et al., 1996 b) and the most adherent strain was the Lactobacillus acidophilus 126.

Mead and Barrow (1990) discussed that the treatment with probiotics is prophylactic when used prior to Salmonella challenge and such protection has been demonstrated against at least 10 Salmonella sero types including both invasive and non-invasive strains. However, the treatment is partially effective against the host specific Salmonella gallinarum, which causes systemic infection and mortality in chicks since this sero type does not persistently colonize in the cecum.

**Antagonistic activity**

The ability of lactic acid bacteria to produce antimicrobial substances has been used for a long time to preserve food. This preservation is based on the fermentation of carbohydrates resulting in small molecular mass organic molecules, which exhibit the antimicrobial activity (Ouwehand, 1998). These bacteriocidal substances contribute to antagonistic activity and lactic acid bacteria are the major producer of these substances. Among those substances bacteriocins, organic acids and hydrogen peroxide has been the main products of Lactic acid bacteria (Jin et al., 1997 b). The bacteriocins are comprised of well-characterized bacteriocins (DeKlerk and Smith, 1967; Barefoot and Klaenhammer, 1983) and bacteriocins like substances such as lactocidins (Vincent et al., 1959). Bacteriocins are the compounds that contain biologically active protein moiety with bactericidal action (Tagg et al., 1976). These substances are not affected by low pH, insensitive to catalase and non-dialysable. These substances could perform an important role in controlling unfavourable microflora by inhibiting the activity of numerous bacterial genera including Proteus spp, Salmonella spp, E. coli species and Streptococcus species in the intestinal tract of animals (Vincent et al., 1959).

Weak acids such as lactic and acetic are the major organic acids produced by the bacteria. They have more
powerful antibacterial activity at low pH (Simon and Blackman, 1949) and undissociated forms of these acids are more toxic and reduce the growth of other bacteria. The dissociated forms have also been observed to inhibit the microbial growth (Ekluand, 1983). Undissociated form of these acids are lipid soluble and they diffuse across the cell membrane (Bearso et al., 1977) and after entering into the cell, the acid will be dissociated since the pH in the cytoplasm is around neutral (Padan et al., 1981). Some researchers have suggested that, after dissociation of the acids, accumulation of protons in the cytoplasm cause acidification and results in growth inhibition (Salmond et al., 1984), where as others think that the accumulation of anion in cytoplasm reduces the rate of macromolecular synthesis (reviewed in Cherrington et al., 1991) and this affects the cell membrane transport causing growth inhibition (Ekluand, 1980).

Dahiya and Speck (1968) found that the Lactobacillus lactis and Lactobacillus bulgaricus isolated from yogurt starter culture have ability to inhibit the growth of Staphylococcus aureus. The inhibitor was identified as hydrogen peroxide (H₂O₂) and the concentration of which increased on storage of Lactobacilli at 5°C, neutral pH and maximum at 5 days. However carbohydrate source was necessary for the formation of inhibitor. It was also noted that the formation of H₂O₂ depends on the temperature; higher the temperature lowest the concentration of H₂O₂. H₂O₂ has strong oxidizing effect on the bacterial cell, sulphhydryl groups of cell proteins and membrane lipids (Morris, 1976). The main antimicrobial effect of H₂O₂ is due to blocking of glycolysis by inhibiting glucose transport, hexokinase activity and glyceraldehyde 3-phosphate dehydrogenase activity due to the oxidation of sulphhydryl groups in these enzymes (reviewed in Ouwehand, 1998). The activity of H₂O₂ toward gram-positive including lactic acid bacteria is bacteriostatic and many gram-negative bacteria are rapidly killed (Condon, 1987).

In vitro studies have shown the inhibitory activity of lactic acid bacteria against the poultry pathogens (Chateau et al., 1993). About 103 Lactobacillus species from two probiotic products have been tested to observe their ability to inhibit some strains of poultry pathogens (4 strains of Listeria monocytogenes, Salmonella enteritidis, S. typhimurium, 6 strains of E. coli). Fifty three percent out of 103 Lactobacillus isolates were able to inhibit 2 Salmonella species, 56% were able to inhibit 6 strains of E. coli and 66% of Lactobacillus isolates were able to inhibit 4 strains of Listeria monocytogenes (Chateau et al., 1993) and the inhibition was attributed to the antagonistic activity.

OTHER EFFECTS OF PROBIOTICS

Effect on gut morphology

The microbial effects on structure of the digestive tract of host animal have been reviewed by March (1979). The thickness of the intestinal wall of germ free animal is lower than the conventional animal and the proportion of lamina propria is also reduced in germ free animals. The reason for the thinning of intestinal wall of germ free animal is the rate of turnover of mucosal cells. There are differences between germ free and conventional animal in the rate of turnover of the mucosal cells and the presence of bacterial flora is associated with more rapid cellular proliferation in the epithelium (March, 1979). The supplementation of antibiotics cause permanent shortening and blunt of the ileum villus of the intestinal epithelium (Coates et al., 1955) where as the supplementation of Lactobacillus increases the villus height and crypt depth of the intestine (Edens et al., 1997). Therefore, it has been suggested that alteration to gut morphology may have been one of the mechanisms of actions of probiotics.

Effect on gut physiology and microflora

In poultry, highest populations of bacteria are in caecum and lowest in the duodenum and majority were gram positive. Lactobacillus and Streptococcus were the predominant type of bacteria found in the duodenum and jejunum and Staphylococcus, E. coli, Fusobacterium and anaerobic cocci were found in lesser numbers. However, high anaerobic population and less number of other microbes such as Lactobacillus, Streptococcus, Staphylococcus and E. coli were found in the cecum (Jin et al., 1997b).

With the dietary supplementation of Streptococcus faecium M-74, more S. faecium colonies were found in cecum than the other portions of the intestine (Owings et al., 1990). It was also observed that the age of broilers influenced the number of S. faecium colonies in the duodenum, jejunum, ileum and cecum and number of colonies was highest in day 7 and decreased with the age.

With the supplementation of mixture of L. acidophilus (NCDC 4) and L. salivarius (NCDC 11 & 13) through the drinking water to native chickens, number of lactobacilli counts was significantly higher and pH of the crop was significantly lower after 24 hours (Shome et al., 2000). The lactobacilli counts were higher in crop than the cecum and mortality among treated birds was dramatically reduced suggesting that the higher mortality in control group was mainly due to alteration of enteropathogens (Shome et al., 2000). In addition to the bacteria, the supplementation of Aspergillus oryzae significantly increased the Lactobacillus species in the GI tract and significantly lowered the faecal E. coli counts suggesting synergistic interactions between Lactobacillus species and A. oryzae (Han et al., 1999).

Nutrient Utilization

The intestinal micro flora of animals has an important effect on digestion and absorption of the feed, metabolism of dietary nutrients such as carbohydrate, protein, fat and minerals and the synthesis of essential vitamins (Jin et al., 1997b). Many experiments have been carried out to determine the digestion and nutrient utilization of the feed with the supplementation of live microbes and the results have been inconsistent. March (1979) discussed in his review that there must be an interrelationship between the
intestinal microflora and their host with nitrogen utilization and the presence of non-pathogenic microflora in the digestive tract affects the fatty acid absorption by affecting the bile salt secretion and fat utilisation. Nahason et al. (1996) indicated that the Lactobacillus cultures were able to increase the daily feed consumption of pullets and enhance calcium, phosphorus and nitrogen retention in layers during laying phase, but no difference was found in fat retention among treatments groups in the experiment. Han et al. (1999) have found that the supplementation of A. oryzae as a probiotic significantly improved the gross energy and dry matter metabolizability, but not the metabolizabilities of crude protein and crude fat. Rasool et al. (2000) studied the influence of microbial cultures in drinking water on nutrient digestibility and found that the digestibility of dry matter and crude protein has been improved.

However, some studies indicated no significant treatment effects, but in most cases only a numerical improvement had been observed in organic matter, nitrogen, calcium and phosphorus utilization with the probiotic feeding (Mudalgi et al., 1993). Similar findings have been reported when broiler diets were supplemented with live yeast (Singh et al., 2000) where, there was no significant improvement in dry matter, organic matter, ether extract digestibility and calcium and phosphorus retention.

### Digestive and bacterial enzyme activity

Several studies have been carried out to evaluate the effects of live microbials on digestive enzyme activity and some scientists have attributed the improved performance of animals fed with probiotic containing diets to the enhanced activity of enzymes. March (1979) discussed in his review that the pancreatic and intestinal enzymes secreted into the intestine might be inactivated by bacterial action but the digestive capacity of lower intestine might be enhanced by bacterial enzymes.

Collington et al. (1990) studied the influence of antibiotic and probiotic on the development of digestive enzyme activity in pre and post weaned pigs mainly concentrating on carbohydrases (sucrase and lactase) and peptidases (dipeptidase and tripeptidase) activity in the mucosa. Both treatments, antibiotic and probiotic had effects on the development of digestive enzyme function within the small intestinal mucosa and these functions were more prominent in the pre weaning period than the post weaning period.

Jin et al. (2000) reported that adherent Lactobacillus cultures have increased the amylolytic enzyme activity but not the lipolytic and proteolytic enzyme activities in the small intestine. These findings are comparable with that of Sissons (1989) who indicated that supplementation of Lactobacillus increases the intestinal amylase activity and Salter et al. (1974) found that the presence of bacteria had no effect on digestion of dietary protein in the chicken.

Bacterial enzymes such as β-glucosidase and β-glucuronidase are the major microbial glycosidases in the intestinal tract and these are responsible for the production of toxic metabolites from non-toxic glycosides (Jin et al., 2000). β-Glucuronidase is believed to be responsible for the hydrolysis of glucuronides in the lumen of gut and this generates toxic and carcinogenic compounds. β-Glucosidase is also responsible for the production of toxic cyanide (Goldin and Gorbach, 1984). Although, these harmful compounds may not cause any harm to chickens, they may reduce the performance and feed utilization. Jin et al. (2000) found that, with the supplementation of L. acidophilus and mixture of 12 Lactobacillus strains in broiler significantly reduced the intestinal β-glucuronidase levels but fecal β-glucuronidase activity significantly reduced only by L. acidophilus. However, fecal β-glucosidase activity has been significantly reduced by both treatments, but without significant effect on intestinal β-glucosidase activity.

### Stimulation of immune system

Gut associated lymphoid tissue is the largest lymphoid tissue of the body and connected with other mucosal systems and systemic immunity. It is one of the first contacts for food components, variety of antigens from beneficial and pathogenic bacteria, which are exogenous to the body (Isolauri et al., 1998). Normal intestinal microflora in the epithelial cells act as a barrier and prevent the movement of pathogenic bacteria, antigens and other harmful substances from the gut lumen. Altering of the permeability of this barrier by the pathogenic bacteria, viruses, chemicals and other antigens, facilitate the invasion of pathogens, foreign antigens and other harmful substances. This may lead to diarrhea, mucosal inflammation and activation of harmful components in intestinal contents (Salminen et al., 1996). Altered intestinal microflora can be corrected by oral intake of lactic acid bacteria and other probiotic bacteria which have the ability to survive in gastric condition and colonize in the intestine by adhering to the intestinal epithelium (Isolauri et al., 1998). These beneficial bacteria especially Lactobacilli could be important in the development of immunity in young animals (Perdigon et al., 1990).

Many animal studies indicated that probiotic bacteria may interfere with systemic immunity. Selected strains of lactic acid bacteria and Bifidobacteria can stimulate non-specific immunity via stimulating interleukin factors 6 and 10 (Isolauri et al., 1998).

Raza et al. (1995) have shown the effectiveness of Lactobacillus treatment on rotavirus diarrhoea and watery diarrhoea in human. Isolauri et al. (1998) indicated that with the supplementation of Lactobacillus strains especially Lactobacillus GG on rotaviral infection in children, enhance the serum antibody level (IgA) and specific antibody secreting cells (sASC). Therefore, it has been suggested that certain strains of lactic acid bacteria especially Lactobacillus GG promote systemic and local immunity to rotavirus. Majamaa et al. (1995) compared the immunological effects of viable and heat inactivated lactobacillus and suggested that the viability of the strain is important to induce the immune stimulation.

Ability of the strain to survive in acid and bile, viability of the strain in the gastrointestinal tract, response on
bacterial surface, survival and contact with the intestinal barrier, adherence to the intestinal mucosa and uptake of lactic acid bacteria by mucosa are some of the properties of lactic acid bacteria to stimulate the immune response (Isolauri et al., 1998).

Ammonia Production

The harmfulness of ammonia to the health and performance of broilers have been well documented (Reece et al., 1980; Jin et al., 1997b). The production of ammonia by ureolysis in the intestinal mucosa could cause significant damage to the surface of the cell (Jin et al., 1997b). This also could adversely affect the animal health and performance and suppressing ammonia production and urease activity could be beneficial for animals. Yeo and Kim (1997) found that supplementation of probiotic (L. casei) in the diet, significantly suppressed the growth of urease producing bacteria in chicken gut during their young ages (first 3 weeks) but that was not evident when they were older (6 weeks). Chiang and Hsieh (1995) indicated that dietary supplementation of probiotic (mixture of L. acidophilus, Bacillus subtilis and Streptococcus faecium) significantly reduced the excreta and litter ammonia levels and it was suggested that this may be due to increase of dietary protein digestibility by probiotics and thereby reducing excreta nitrogen.

Anti carcinogenic activity

Some scientists suggest that the probiotic bacteria especially Lactobacillus species are able to produce antitumor activities. Fuller (1989) discussed in his review that the anti carcinogenic properties of lactobacilli can be attributed to three different mechanisms (a) inhibition of tumor cells (Reddy et al., 1973), (b) suppression of bacteria which produce enzymes such as β-glucuronidase and β-glucosidase which are responsible for the production of toxic metabolites from non-toxic glycosides (Jin et al., 2000), (c) suppression of nitroreductase which is also a bacterial enzyme and involved in the synthesis of carcinogens such as nitrosamines (Goldin and Gorbach, 1984).

Cholesterol metabolism

A number of studies have been carried out to see the effects of probiotics on plasma cholesterol levels in human and animals. In human studies, Lichtenstein and Goldin (1998) reported in their review that the consumption of both skimmed and full fat yoghurt significantly decreased the plasma cholesterol level. Gilliland and Walker (1990) found L. acidophilus has ability to assimilate cholesterol and thereby lowering plasma cholesterol in humans. However, there are studies with no significant effect of probiotics on plasma cholesterol level (Pulusani and Rao, 1983; Lin et al., 1989).

In animal experiments, Panda et al (2000) reported that probiotics (L. acidophilus, L. casei, B. bifidum, S. faecium, A. oryzae and Torulopsis species) significantly reduced the serum cholesterol levels of broilers and the effects have been attributed to reduced absorption or reduced synthesis of cholesterol in the gastro-intestinal tract. Serum cholesterol levels have been significantly lowered in broilers supplemented with Lactobacillus cultures (12 strains of Lactobacillus which belong to four species such as L. acidophilus, L. fermentum, L. crispatus and L. brevis) after day 20 of age (Jin et al., 1998a) and indicated that the assimilation or uptake of cholesterol by Lactobacillus could be the possible reasons for the cholesterol lowering effect. The cholesterol lowering effects of L. acidophilus have been observed in layers as well (Abdulrahim et al., 1996) where effect was found both in serum as well as in egg yolk. Gohain and Sapcota (1998) have also reported marginally lower serum cholesterol in probiotic fed birds.

EFFECTS OF PROBIOTICS ON DIFFERENT ANIMALS

Inconsistency in results has been reported on growth performance and production of animals with supplementation of probiotics. This has been attributed to several factors; degree of attachment between strains of the same species of microbes (Fuller, 1989), host specificity of microbes (Watkins and Kratzem, 1983) and the presence of stressful conditions (Jin et al., 1997a).

Broilers

The addition of probiotics to the broiler diets has yielded mixed results. Dietary supplementation of different strains of L. bulgaricus have significantly increased the live weight gain, feed conversion ratios and reduced the chick mortality during the starter phase but not in finisher phase (Yeo and Kim, 1997; Tortuero and Fernandez, 1973). However, same species of Lactobacillus [but different strain (L4 strain)] significantly improved the feed conversion ratio and live weight gain of broiler during the finisher stage (Bhatt et al., 1995). Similar finding was observed by Gohain and Sapcota (1998) where, the body weight gains of broilers were significantly improved by feeding probiotics and the effect was more conspicuous at 6th and 7th weeks of age. Meanwhile, Jin et al (1998b) found that broilers fed with mixed Lactobacillus cultures have significantly improved body weight gain and feed conversion efficiency in both starter (0 to 3 weeks) and finisher (4 to 6 weeks) stages. Jin et al (1996c) studied the effects of B. subtilis and Lactobacilli cultures as a probiotic in broiler feed and illustrated that there was a significant improvement in body weight gain and feed conversion efficiency. Similar beneficial effect was observed by Jin et al (2000) by the inclusion of mixture of 12 Lactobacillus strains (2 strains of L. acidophilus, 3 strains of L. Fermentum, 1 strain of L. crispatus and 6 strains of L. brevis) to the broiler diets. Growth rate, nutrient utilization and digestibility of feed have been improved by the supplementation of effective microbes (Em) to broilers via drinking water (Rasool et al., 2000) and recommended that 0.02% level was more effective than 0.01 or 0.04% levels. Zanzad et al (2000)
compared three probiotic organisms (Saccharomyces cerevisiae, Lactobacillus sporogenus and Saccharomyces boulardii) with control group in broilers and concluded that all three probiotic organisms improve the growth rate and feed conversion efficiency but L. sporogenus was rated best among the three organisms. Improved performance has been observed with the supplementation of L. acidophilus, B. subtilis and S. faecium as probiotic organisms to broilers (Chiang and Hsieh, 1995). Although performance was improved, there was no significant difference in the feed intake of different groups. However, Gohain and Sapcota (1998) reported that the feed intakes of broilers fed probiotic supplemented diet was lower than the control birds and found no significant differences in feed conversion ratios between probiotic fed and control groups. Similar findings were reported by Takalikar et al (1992). Having acknowledged the inconsistency of results of probiotics, findings of Banday and Risam, (2001) has been self-explanatory for at least some of the inconsistencies. Banday and Risam, (2001) found that feed conversion efficiency, body weight gain and feed intake of broilers can be improved significantly by the inclusion of higher levels of probiotic to the broiler feed. Significant improvements of growth rate and feed efficiency have been reported with the use of fungal broth (Eremothecium ashbyii) in broiler feeds (Narahari and Omprakash, 1997). The results of Owings et al (1990) showed that supplementation of S. faecium M-74 has improved the body weight and feed conversion efficiency up to 36 days of age with unsexed/straight run broilers.

Meanwhile there has been a suggestion that addition of probiotics does not necessarily guarantee a favourable response. With the inclusion of probiotic to broiler feed, Mudalgi et al (1993) found that there was no significant improvement of live weight gain, feed conversion efficiency and feed intake, apart from a small numerical improvements with the addition of L. bulgaricus. Similar effects were reported by Toker (2000) and Baidya et al (1994) with the supplementation of probiotics and antibiotic to broiler feed and concluded that there were no advantages of inclusion of either probiotics or antibiotics to the feed. However, Choudhury et al (1998) found that although the supplementation of probiotics to broilers did not have any growth promoting effect, growth promoting antibiotic significantly improved the growth rate and feed efficiency of broilers. No beneficial effects were found in growth of broilers fed with live yeast (Singh et al., 2000) but numerical improvements in body weight gain were noticed with the higher doses. Similar findings were reported by Sarkar et al (1997) with the supplementation of different varieties of yeast and suggested that numerical improvements may be due to synthesis of different nutrients in the intestine. Samanta and Biswas (1995) showed that there were no any beneficial effects of broiler performance with the addition of probiotic or weak acids such as lactic. Watkins and Kratzer (1983) found that the supplementation of higher doses (above 5.0 log<sub>10</sub> cfu/chick) of either host specific strains of lactobacilli or non host specific strains may even be detrimental where they found growth of chicks were depressed marginally.

Authors also suggested that the oral administration of high numbers of different Lactobacillus strains does not have a significant effect on early broiler chick growth.

When considering the carcass characters, no significant difference was found in dressing percentages (Gohain and Sapcota, 1998) and abdominal fat contents (Chiang and Hsieh, 1995) with the supplementation of probiotics. Nonetheless, Banday and Risam (2001) found significant improvement in dressing, eviscerated and edible meat yields, but no difference between the weights of organs (Liver, Kidney, Pancreas and Spleen) of treated groups with the addition of probiotic to the feed and control birds. There were no advantages in carcass characteristics, internal organ weights and edible giblet (Heart & Liver) weights by inclusion of either probiotics or antibiotics to the feed (Toker, 2000; Choudhury et al., 1998; Baidya et al., 1994; Sarkar et al., 1997). Owings et al (1990) indicated that dietary supplementation of S. faecium M-74 has no effect on carcass yield and percentage of moisture, fat and protein of breast and thigh meat and skin. Mahajan et al (2000) studied the effect of probiotics (contained live yeast culture, L. acidophilus and S. faecium) and season on the different quality characteristics of poultry meat and found that the proximate composition except fat percentage was significantly increased in probiotic fed broilers. They also indicated that the microbial counts including total viable count, coliforms, streptococci and staphylococci were lowered in the leg and breast of the probiotic fed birds.

**Layers**

It is apparent that the effects of probiotics on the performance of layers are much more consistent than that of broilers. Abdulrahim et al (1996) compared the effects of L. acidophilus, Zine bacitracin, alone and combination of these two on layers and reported that the addition of L. acidophilus at the concentration of 4 X 10<sup>7</sup> colony forming units per gram (cfu/g) significantly improved the egg production, feed conversion efficiency and marginal elevation of egg weight but the treatment effect of bacitracin was insignificant. They have also suggested that there was a relationship between number of lactobacilli and nutrient uptake; high numbers of lactobacilli might improve the nutrient uptake and or absorption. However there was no significant difference between treatments and control regarding the eggshell thickness and total lipids and triglycerides in both plasma and egg yolk. Supplementation of L. sporogenes through the drinking water significantly increased the egg production, egg size and numerically improved feed efficiency and maintained the peak egg production up to 42 weeks of age (Ahmed et al., 1994). They have also indicted that there was no beneficial effect of acidification of probiotic by mixing organic acid. Miles et al (1981) studied the effect of inclusion of L. acidophilus at three different levels (0.0125, 0.0375 and 0.0625 %) to the diets of layers and conducted their experiments in three geographical locations to ascertain the effects of climate on layer performance. Although they have used same diets and probiotics, it was noticeable that probiotic did
significantly improve the hen-day egg production and feed efficiency in one location, numerically improved in another and no difference was observed in the other location with the two levels (0.0125 & 0.375%) of probiotics. With the highest levels (0.0625%) no improvements were observed in production performances possibly due to (a) the presence of excessive numbers of organisms may increase the gut motility and alter the nutrient availability for absorption, (b) other beneficial bacterial populations may be altered and thereby disrupting the cohabitation of the established microflora. Nevertheless, this finding has shed the light that efficacy of probiotics may well be influenced by the environment. Therefore finding of the efficacy of given probiotic in one part of the world may not necessarily be the same in some other parts of the world. They also reported that egg weight and egg quality were not influenced by the inclusion of probiotics. Similarly, Hane et al (1999) indicated that there were no significant effects on egg qualities (egg weight, yolk colour and egg shell thickness) with the addition of A. oryzae to the layer feed. Supplementation of mixed culture of L. acidophilus and L. casei significantly improved the hen-day egg production, feed conversion ratio, egg weight and albumin quality (Tortuero and Fernandez, 1995).

However, it is evident that not all the probiotics are effective and Goodling et al (1987) studied the effects of liquid non-viable Lactobacillus product, dried non-viable Lactobacillus fermentation product and dried viable Lactobacillus fermentation product on layer performance and found that there was no significant effect on hen-day egg production, feed efficiency and egg size. On the other hand in experiment with birds kept under extra hygienic conditions, it has been speculated that hygienic conditions be the reason for the ineffectiveness of the Lactobacillus products. Again in contrary, Nahashon et al (1996) reported that there was significant improvement in daily feed consumption and body weight gains during the pullet stage (7-19 weeks of age) with the addition of Lactobacillus, though no difference in feed conversion efficiency was observed. However, during the laying phase the findings were similar to that of Goodling et al (1987) where there was no significant improvement in egg production and feed conversion. They too assumed that rearing layers in ideal condition may be attributed to the failure of Lactobacillus to show a significant effect. Egg weight and size were significantly improved with the Lactobacillus and suggested that improved retention of calcium and nitrogen may be the possible reason for the improvement (Nahashon et al., 1996). Balevi et al (2001) studied the effect of supplementation of different doses (250, 500 and 750 ppm) of probiotics (protextin) for 40 week old layers and found that there was no significant difference between the groups fed 250 and 750 ppm but significant improvement in feed consumption, feed conversion and lowering of the damaged eggs with 500 ppm supplementation.

**Pigs**

The use of probiotics in pigs too has been practiced for long period. The inclusion of probiotics to the pig diets has also produced variable results. Lopez et al (1994) found that the supplementation of probiotic has improved the daily weight gain of piglets at weaning and minimized the severity of diarrhoea. Average daily body weight gain and the efficiency of feed conversion significantly improved in growing pigs with the supplementation of live yeast culture, and lactic acid bacteria (Gombos et al., 1995). Similar results were obtained by Jeon et al (1996) in growing pigs with enzymes, probiotics and Yucca powder (Yucca schidigera). They also found that ammonia N concentration in faeces was also reduced by all treatment diets. Roberton et al (1994) illustrated that the inclusion of probiotics to gestation and lactation rations has improved the breeding career of the sow and feed efficiency. It has also improved the health of the sow and early performance of the piglets. Sows with 20-30 % MMA (Mastitis Metritis Agalactia) were fed with lactic acid producing S. faecium Cornelle 68 (Cernivet LBC 350) and found that the percentage of sows that required treatments for MMA was lower in probiotic treated group and oral probiotic treatment of the sow up to the point of farrowing was able to reduce the frequency of diarrhoea in the litter (Jensen et al., 1994). With the supplementation of digested bacterial cell powder from Bravibacterium lactofermentum to post weaning and suckling piglets, Toride et al (1998) found that low diarrhoeal morbidity (P<0.05) and high survival rate (P<0.01) in both post weaning and suckling piglets.Pollmann et al (1980) indicated that the probiotics can be used as promising alternative to antibiotic and lactobacillus containing products are more superior to the streptococcal products. They have also suggested that the lactic acid produced as a metabolite during fermentation of a lactic acid producing bacterial culture is the cause for performance improvement.

However, there are studies indicating the negative effects with the use probiotic in pigs. Kim et al (1998) studied the performance, carcass characters and nutrient retention in finishing pigs with the supplementation of cellulase enzymes and L. acidophilus and concluded that the addition of cellulolytic enzymes or bacterial feed additives have no effect on growth performance, carcass merit and nutrient utilization. Yang et al (1998) indicated that there was only a marginal improvement, but no significant improvements in daily weight gains, feed intake, feed conversion ratios and carcass characters in pigs fed with probiotics.

**Ruminants**

Although the use of probiotics in ruminants is not as common with monogastric animals, some preparations have been used in ruminants as well. Further the consistency of results with probiotics in ruminants appears to be far more greater than their monogastric counterparts. It is however noticeable that the commonest organism that has been used in ruminants is yeast where as Lactobacillus is the predominant probiotic organism in monogastric feeding.

Malik et al (1998b) studied the effects of supplementing probiotic (S. Cerevisiae and L. acidophilus in 1:1 ratio) to
young calves (1 week old) and found significantly higher live weight gain, and lower incidence of diarrhoea. Positive effects were prominent during the period of providing probiotic and disappeared when the supplementation ceased (post supplementation period). Malik et al (1998a) carried out an in-vitro study to evaluate the effect of different probiotic organisms (L. acidophilus-1, L. acidophilus-R, Streptococcus thermophilus-HST, S. thermophilus-CH, S.cerevisiae-522 and S. cerevisiae-B) in rumen by incubating with rumen fluid and found that the strains S. cerevisiae and L. acidophilus were able to increase the dry matter and organic matter digestibility and S. cerevisiae-B has increased the volatile fatty acid production than the other strains and concluded that the strains of S. cerevisiae can be used successfully as feed supplement in ruminants. However, Singh et al (1998) found that the inclusion of yeast (S. cerevisiae ITCCF 2094) to calf feed has no effect on feed intake, nutrient digestibility, feed to gain ratio and nitrogen retention but there have been a marginal improvement in live weight gain. Daily milk yield, protein and fat content of the milk have been improved marginally with the addition of yeast to feed ration in dairy cows (Skoko et al., 1993). Similar results were found by Popovic et al (1998) with live yeast in diet of dairy cows and it has also lowered the incidence of mastitis in cows. Alshaikh et al (2002) found that the Holstein cows fed with yeast cultures from different sources have significantly increased the daily milk yield and milk composition (percentage of fat, protein, lactose, total solids and solid non fat in milk). They have also found that the mean concentration of rumen ammonia nitrogen decreased significantly with probiotic supplementation. They suggested that the reduction may be due to the increased incorporation of ammonia into microbial protein, which may in turn, be the direct result of stimulated microbial activity. However some studies showed that the milk yield or milk composition was not significantly altered by yeast supplementation (Blauwinkel et al., 1995; Robinson, 1997). Alshaikh et al (2002) suggested that the discrepancies could be associated with differences in breeds, stage of lactation, type of forage given, the source of the yeast culture and feeding strategy of the studies.

In small ruminants, significantly improved live weight gain, average daily gain and lowered incidence of diarrhoea were observed with the supplementation of curds in crossbred kids (Anandan et al., 1999). In growing goats average daily weight gain and feed conversion were improved and the rumen ammonia nitrogen concentration lowered with the supplementation of yeast (Gado et al., 1998). It also enhanced the fibre digestibility though there was no improvement in roughage intake. Rumen pH, volatile fatty acids level and rumen microorganisms were not altered by addition of different concentration of yeast but the density of cellulolytic bacteria in rumen increased with the dose of 1 g/kg of yeast. Daily feed intake of hay in lactating goats has increased with the inclusion of yeast but the lower dose of yeast has increased the milk yield only. Milk fat and lactose was not influenced by the yeast supplementation (Badawi et al., 1998).

Other animals

Average weight gain of the native chicken has increased during first to forth week with the supplementation of mixture of L. acidophilus and L. salivarius (Shome et al., 2000) and suggested that the effect of early colonization of lactobacillus in the gut and regular supply of Lactobacillus via drinking water are the possible reasons for better performance. The effect was later minimized by the withdrawal of lactobacillus. This statement is comparable with that of Fuller (1989) who indicated that the best method of administration of probiotics is continuous feeding to obtain the better performance. Kumararaj et al (1997) studied about the supplementation of probiotics to quails and indicated that there was a significant improvement in body weight and numerical improvement in feed intake and efficiency. However, these parameters were not statistically significant. Neither the carcass characters nor the mortality was influenced by probiotic feeding in quails.

Intestinal viscosity is a principle indicator of digestibility and absorption of nutrients in the intestine especially in small intestine as it influences intestinal motility (Ermainer and Strukle, 1999). Ermainer and Strukle (1999) studied the effect of probiotics on the intestinal viscosity mainly in small intestine and cecum of rabbit and found that there was no significant effect with the use of probiotics (Bacillus toyoi, Bacillus CIP 5832 and S. cerevisiae) on intestinal viscosity.

CONCLUSION

The lack of consistency in the results with the use of probiotics in animal feeding has caused many people to be sceptical about the positive effects of probiotics. Differences in the bacterial strains, forms of bacteria used and the live bacterial concentrations of dietary supplementation may contribute to the inconsistent results. However, supplementing right strains of bacteria, optimal concentration of viable cells and stressful conditions could be attributed to the beneficial effect of the probiotics. Even though with all uncertainties of results of probiotics in animal feeding, scientists still believe that probiotic is one of the most potential substitutes for antibiotic growth promoters.

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Probiotic as growth promoter


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FOOT AND MOUTH DISEASE: EPIDEMIOLOGICAL STUDY OF AN ATYPICAL EPIDEMIC IN SRI LANKA

M. Kodituwakku, B.V.Sc. and S.M.K. Karunaratne

Department of Animal Production and Health, Peradeniya, Sri Lanka

Summary: Foot and mouth disease has been reported in Sri Lanka since mid nineties causing epidemics in every 4-6 years. An observational study was carried out to comprehend the epidemiological features of an atypical epidemic of 1997 in the country. Circumstantial evidences suggested that the epidemic originated from infective foci at national wild life sanctuaries and wild life reservoirs in the endemic zone which includes North Central Province (NCP), North Western Province (NWP) and North & Eastern Province (NEP). Commencement of the epidemic coincided with the seasonal movement of livestock. Disease lasted for 32 months among domestic livestock population and 34,472 cases were recorded of which 84% were found in the endemic zone. Seasonal peaks were evident during North- East monsoon. The highest prevalence rate of 4.03% was found in NCP in 1997. Infection spread to all the Provinces in the country advocated by the cattle movement. However, the origin of infection at Jaffna Peninsula remains unknown. The causative foot and mouth virus belonged to ME-SA topotype of serotype ‘O’. Observed mortality rate in this epidemic was 0.029%.

INTRODUCTION

The official reporting of Foot and Mouth Disease in Sri Lanka (Ceylon) began in 1900 (Sturgess, 1900) concurrently with the formation of Department of Veterinary Services in the country. However, a similar disease condition causing “sore mouth and sore hoof” in cattle had been recognized in 1869 or even much earlier (AGA, 1869). Though foot and mouth disease (FMD) is endemic in the island, epidemics affecting all the Provinces have been recorded regularly in every 4-6 years (Fernando, 1969).

FMD in the recent decades

A massive epidemic in 1987 swept through all the Provinces resulting in 85,641 cases and 604 deaths, the highest number recorded so far. Subsequent epidemic was observed four years later (1991-1992) infecting all the Provinces except Sabaragamuwa Province (Anonymous, 1991; Anonymous 1992). Yearly incidence of the disease since 1987 indicated a lull period that lasted for two years of 1995 and 1996 (Kodituwakku, 1999). This was followed by an atypical epidemic which lingered on for 32 months in the country.

METHODOLOGY

An observational study was carried out for a period of 34 months (January 1997-October 1999). Primary data were obtained by personal interrogation of herd owners at the time of disease outbreaks. Secondary data gathered from the preliminary disease outbreak investigation and weekly disease reports submitted by the Government Veterinary Surgeons of Department of Animal Production and Health in the field and the Veterinary Investigation Officers in the regions. Furthermore, the secondary data were edited or supplemented substantively by the information collected at field visits made by the authors. The computer software programmes namely 'Arc GIS 9.3' and 'TADinfo' were utilized to facilitate spatial and numerical data analysis.

Samples were collected from clinical cases in various locations and subjected to enzyme linked immunosorbent assay (ELISA) at the Veterinary Research Institute. Furthermore, virus isolation was performed at World Reference Laboratory for Foot and Mouth Disease (WRLFMD), Pirbright, U.K., on two occasions followed by characterization to determine the topotype of the circulating field virus.

RESULTS AND DISCUSSION

Findings on 1997 Epidemic

Primary Outbreaks
The 1997 epidemic was detected in January 1997; traced back to the index case on 29th December 1996 at Old Eluwankulama, a village in the southern border of Wilpattu National Sanctuary (WNS). Disease spread to 14 contiguous villages affecting cattle and buffaloes in large herds managed under free grazing system and thereafter move southwards infecting Karuwalagaswewa. A buffalo herd moved from here carried the disease further south to Swarnapaliyagama, a village in Anamaduwa Divisional Secretary Division (DSD) in early February. Later on, cases were detected at
This traveled far eastwards infecting six more Veterinary ranges. The attack lasted for ten weeks, recording 3,994 cases and 308 deaths (Table 1). While the infection originated in the border of WNS and lasted until February 1998, it was moving southwards in Puttalam District and gaining entry to Kurunegala District, a separate focus of infection. Cases were found in the southeast border of the North-Western Province (NWP) in four locations for a period of one month.

The third focus of infection was detected on 2 February 1997 at Nelumwewa, a village in Polonnaruwa District. This was confined to 41 animals in three neighbouring herds. However, two months later, a larger infected area was detected in 3 adjoining Veterinary ranges namely in the vicinity of Kantale Tank, began in January 1998 at Welikanda, Medirigiriya and Valaichchenai (Figure 2). It spread southwards and affected seven more Veterinary ranges (Figure 3a) and eventually, the disease became very mild and did not warrant much attention and appeared to be subsided by early August.

Nevertheless, the next wave of infection became evident in October affecting seven of the above areas and four villages in Anuradhapura District and lingered on in the National Sanctuary. The last vestige of this attack was noticed on 2 February 1999 at Battulu Oya, a village in Anuradhapura District. These herds were known to be grazing near the Kantale tank in the southwest border of Nawagaththegama Veterinary ranges infecting 42 villages in Mundel DSD. However, the terminal cases of Wilpattu focus in 1997 were noticed in June-July 1999 at Ihalamaragahawewa, a village in Nochchiyagama DSD located in the northeast border of the National Sanctuary.

Secondary and subsequent outbreaks were noticed in August 1998. Fifteen calves at Padaviya and six calves at Trincomalee succumbed. Early cases of the tertiary outbreak were noticed in August 1998 at Anamaduwa, Thambuttegama and Nochchiyagama at Kalae Siyambalawa, a village in the northern boundary of Anuradhapura District. It spread southwards and affected seven of the above areas and four villages in Anuradhapura District and lingered on in the National Sanctuary. The last vestige of this attack was noticed on 2 February 1999 at Battulu Oya, a village in Anuradhapura District. These herds were known to be grazing near the Kantale tank in the southwest border of Nawagaththegama Veterinary ranges infecting 42 villages in Mundel DSD. However, the terminal cases of Wilpattu focus in 1997 were noticed in June-July 1999 at Ihalamaragahawewa, a village in Nochchiyagama DSD located in the northeast border of the National Sanctuary.

Table 1. Data on FMD primary attack near WSN in Jan – March 1997.

<table>
<thead>
<tr>
<th>ID No</th>
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<th>Deaths</th>
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<td>Bingiriya</td>
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</tbody>
</table>

Figure 1. Spread of FMD primary attack near WNS

Figure 2. Primary foci of infection in 1997 epidemic
Serukelle, Galgamuwa and Bingiriya (Figure 1). This attack lasted for ten weeks recording 3,994 cases and 308 deaths (Table 1).

While the infection originated in the border of WNS was moving southwards in Puttalam District and gaining entry to Kurunegala District, a separate focus of infection was detected on 12 January 1997 at Dekethipoththa, a village in the southeast border of Horawapothana DSD in Anuradhapura District. These herds were known to be grazing near the Kantale tank in the southwest border of Trincomalee District. The disease spread extensively moving towards northwest up to Rambewa and southwest up to Kekirawa Veterinary ranges infecting 42 villages in five clusters (Figure 2); 12,162 animals experienced the infection clinically and 197 of them died over a period of four months.

The third focus of infection was detected on 2 February 1997 at Nelumwewa a village in Polonnaruwa District. This was confined to 41 animals in three neighbouring herds. However, two months later, a larger infected area was detected in 3 adjoining Veterinary ranges namely Welikanda, Medirigiriya and Valaichchenai (Figure 2).

Secondary and subsequent outbreaks
Spread from origin at Wilpattu

The secondary outbreak of the primary infection originated near WNS began in late May 1997 in Anamaduwa, Thambuttegama and Nochchiyagama Veterinary ranges. It spread southwards and affected seven more Veterinary ranges (Figure 3a) and eventually, the disease became very mild and did not warrant much attention and appeared to be subsided by early August.

Nevertheless, the next wave of infection became evident in October affecting seven of the above areas and traveled far eastwards infecting six more Veterinary ranges in Kurunegala District and also leaked into the western part of Sabaragamuwa Province via Rambukkana (Figure 3b) and lasted until February 1998.

After a lull period of 3 months in late May 1998, fresh cases were found in the southeast border of the North-Western Province (NWP) in four locations for a period of one month. The last attack of the 1997 Epidemic in the NWP began on 2nd of November 1998 in Nawagaththegama DSD and dispersed among eight Veterinary ranges. The last vestige of this attack was noticed on 2nd of February 1999 at Battulu Oya, a village in Mundel DSD. However, the terminal cases of Wilpattu focus in 1997 were noticed in June-July 1999 at Ihalamaragahawewa, a village in Nochchiyagama DSD located in the northeast border of the National Sanctuary (Figure 3c).

Spread from origin in the vicinity Kantale Tank

The secondary outbreak of the primary focus originated in the vicinity of Kantale Tank, began in January 1998 at Thakvanagar in Kuchchuveli DSD in Trincomalee District and moved in the northwest direction affecting Anuradhapura District. A total of 994 cases were recorded in this attack which lasted for two months. Fifteen calves at Padaviya and six calves at Trincomalee succumbed. Early cases of the tertiary outbreak were noticed in August 1998 at Kalae Siyambalawa a village in the northern boundary of Anuradhapura District and lingered on in the surrounding areas for eight months; fresh cases noticed in Kilinochchi and Mullaitivu Districts in the north, Trincomalee District in the east and up to Rambewa and Wilachchiya in Anuradhapura District in the south (Figure 4).

Figure 3. Secondary, Tertiary and Subsequent outbreak waves of WNS origin infection
Further, in late 1998, it was introduced to Jaffna Peninsula infecting 5 separate locations namely Velanai, Chankanai, Kopay, Jaffna and Kayts. Total recorded cases in this attack numbered 6,291 which may underestimate and not reflect the actual situation since there was limitation in accessibility to this infected area due to ethnic war.

**Spread from the origin near Somawathie Chaithiya Sanctuary**

Secondary outbreak was noticed at Polonnaruwa in November and December 1997 at two separate locations; one at Polonnaruwa and one at Bakamuna Veterinary range. Fresh cases continued till February 1998 recording 90 cases in these attacks.

**Introduction of Infection into other areas**

**FMD in Western Province**

The first case of the 1997 epidemic in the Western Province was observed in early February 1997 at Negombo among the slaughter cattle brought in from Puttalam. Since then, cases were detected in thirteen more Veterinary ranges namely Divulapitiya, Kadawathe Mirigama, Dompe, Kotadeniyawa, Gampaha, Kosgama, Udahamulla, Kolonnawa, Homagama, Agalawaththa, Mathugama and Kalutara until March 1998 (Figure 5a).

**FMD in Central Province**

Animals salvaged from slaughterhouse at Maharagama in the Western Province and distributed among the farmers introduced the disease into the Central Province in late 1997. The village named Panwila in Kandy District served as the entry point of the infection into this Province. Subsequently, cases were noticed at five more Veterinary ranges in Kandy District. A similar occurrence took place in January 1999 at Galagedera Veterinary range via slaughter cattle from Colombo. The disease was also detected at Naula and Galewela Veterinary ranges perhaps introduced from Polonnaruwa (Figure 5b).

**FMD in Southern Province**

The 1997 epidemic intruded the Southern Province in December 1998 via cattle and goats brought in from North Central Province (NCP) under a Project implemented by the Southern Development Authority. Index case was noticed at Walasgala, a village in Dickwela DSD in Matara District. Subsequently, it spread to the adjoining Veterinary ranges namely Akuressa, Tangalle, Matara, Tissamaharama, Kamburupitiya, Hambantota and Sooriyawewa too (Figure 5c).

**FMD in Uva Province**

Slaughter cattle from Lunugamwehera at Tissamaharama took the infection to Pahalagama, a village in Buttala Veterinary range in Moneragala District in May 1999. Subsequently cases were also detected in the vicinity of slaughter houses at Bibile and Badulla (Figure 5c).

WRLFMD, Pirbright, U.K. revealed that the field virus belonged to ME-SA topotype of foot and mouth serotype 'O' and the virus strains isolated in both occasions were closely related to each other.
Maharagama in the Western Province and distributed closely related to each other. FMD in Central Province
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Sanctuary
Spread from the origin near Somawathie Chaithiya
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Secondary outbreak was noticed at Polonnaruwa in
The first case of the 1997 epidemic in the Western Sooriyawewa too (Figure 5c).
Animals salvaged from slaughterhouse at 'O' and the virus strains isolated in both occasions were
as a part of livestock management practice in extensively (1969) mentioned that spontaneous outbreaks had been
seasonal movement of livestock, returning to the villages samples for further epidemiological studies. Fernando,
February (Kodituwakku, 1993). This coincides with the field investigation and failure to collect diagnostic
adjoining NEP. elsewhere in the country arouse the probability of its
created in the border of NWP and NCP, the Provinces inability to associate the possible origin to outbreaks since 1990. Appearance of FMD cases in late 1998 and the
remaining unnoticed in the area between NCP and NEP and eventually became stabilized in an endemic form. Disease
shifted towards the national sanctuaries and reservoirs where significant number of wild animal Jaffna Peninsula remained free of clinical cases of FMD population is maintained. Thereby, new foci have been since 1990. Appearance of FMD cases in late 1998 and the
have contributed to the 1997 FMD epidemic in Sri Lanka. Hoole (1901), Crawford (1936) and Fernando (1969)
Consequently, infective foci have shifted towards the national sanctuaries and reservoirs where significant number of wild animal population is maintained. Thereby, new foci have been since 1990. Appearance of FMD cases in late 1998 and the
in the villages, thus the return of livestock after paddy harvesting. Further, disease incidence has been high due to
occurrence of index cases of 1997 epidemic too remains these outbreaks could not be determined.

DISCUSSION

Three separate foci of FMD infection namely Wilpattu National Sanctuary, Kantale Tank vicinity and Somawathie Chaithiya Sanctuary could be identified to
have contributed to the 1997 FMD epidemic in Sri Lanka. Hoole (1901), Crawford (1936) and Fernando (1969)
Three separate foci of FMD infection namely Wilpattu National Sanctuary, Kantale Tank vicinity and Somawathie Chaithiya Sanctuary could be identified to
have contributed to the 1997 FMD epidemic in Sri Lanka. Hoole (1901), Crawford (1936) and Fernando (1969)

Morbidity and Mortality rates

Yearly prevalence of FMD in the country during the study period of 1997, 1998 & 1999 (up to Oct.1999) was
found to be 1.01%, 0.1% and 0.39% respectively. The highest prevalence was found in NCP, the prevalence
rates were 4.03% (1997), 0.28% (1998) and 1.74%
(1999). Disease prevalence in NWP was also notably high in 1997 (1.5%) and 1998 (0.13%). Mortality rates were
0.025% (1997), 0.001% (1998) and 0.003% (1999).

The epidemic curve of the outbreaks that primarily originated near Wilpattu National Sanctuary had major
peaks in December-February and minor peaks in June-July. The major peaks indicate the implication of seasonal
agricultural practice associated with livestock managed under extensive system. Disease prevalence shows a
direct relationship to the presence of susceptible animals in the villages, thus the return of livestock after paddy
harvesting. Further, disease incidence has been high due to the spread of disease to other herds in the villages.
Donaldson (1993) also implied that the seasonal incidence of outbreaks in villages was found to be increasing in Sri
Lanka when the animals return from forest grazing areas.
The minor peaks of the curve suggest that the vaccination coverage and other zoo-sanitary measures have been
inadequate in arresting the spread of infection.
The outbreaks in the southwest border of NCP (Nocchiyagama and Thambuttegama) originated at
Wilpattu National Sanctuary. However, major portion of the NCP were affected from the infection originated near
Kantale Tank. The disease moved northwards and remained unnoticed in the area between NCP and NEP and
eventually became stabilized in an endemic form. Disease surveillance and disease control measures were not
effective as the accessibility to this area was very much limited due to civil unrest in 1997-1998.
Jaffna Peninsula remained free of clinical cases of FMD since 1990. Appearance of FMD cases in late 1998 and the
inability to associate the possible origin to outbreaks elsewhere in the country arouse the probability of its
introduction from India with return of refugees. However, the fact remains neither proved nor refuted due to poor
field investigation and failure to collect diagnostic samples for further epidemiological studies. Fernando,
(1969) mentioned that spontaneous outbreaks had been experienced in Jaffna Peninsula in 1968 and the origin of
these outbreaks could not be determined.

Figure 5. Spatial distribution of cases in Provinces outside the endemic zone

![Figure 5a. Western Province](image)
![Figure 5b. Central Province](image)
![Figure 5c. Southern & Uva Provinces](image)
FMD spread within NWP, NCP and NEP mainly by the movement of livestock as part of their management practice, whereas transport of animals for various reasons introduced the disease into other Provinces. Demand for beef is higher in Western Province than in any other part of the country. It is usually met by traffic of cattle and buffaloes for slaughter from NWP, NCP and NEP. Flow of slaughter cattle into Western Province significantly increase whenever there is occurrence of infectious diseases in the above three Provinces. Generally, cattle brokers purchase animals in the infected villages for very low price and transport them for slaughter in urban areas. Consequently infection is introduced into Western Province. It was true in 1997 FMD epidemic too. Index case at Western Province was detected among slaughter cattle brought in from Puttalam in February 1997. Sri Lanka being a Buddhist country the credence of salvaging cattle in order to receive merit in life is well appreciated. Hence cattle salvage programmes have gained more recognition in mid nineties. Cattle brought for slaughter are released and in many occasions, these animals are supplied to cattle farmers in rural areas. FMD was introduced into Central Province via these animals in November 1997. However, prompt control measures brought the disease under control; no clinical cases were observed after two months time. Small herd size (3-10 animals) and the management system (stall-fed) may have contributed to the successful control of infection. A similar introduction at Galagedara in January 1999 too was controlled effectively.

Cattle and goats from NCP were transported to Southern Province under the Provincial Livestock Development Project with inadequate health precautions and resulted in FMD infection in Southern Province in December 1998. Disease spread within the Province mainly by direct contact of animals in large herds managed under free-range system. The immune belt developed by vaccination near the border of Southern Province could not protect the Uva Province. Slaughter cattle were transported across this area and thereby the disease was introduced initially at Buttala in December 1998 and later on at Bibile and Badulla. Though, the 1997 FMD epidemic moved in various directions invading into all the Provinces by December 1998, disease control measures contributed to arrest the spread wherever and whenever possible. Thus, the disease was brought under control to a greater extent in September 1999; the clinical cases were confined to the NEP where the civil war disturbed the field level implementation of disease control activities. The highest prevalence of FMD was found in NCP which has common borders with the NEP where the disease surveillance was relatively poor and disease control programmes were very much limited. Disease prevalence in NWP was also notably high in 1997 and 1998 perhaps due to the large number of animal population and its location being adjacent to NCP. Mortality rates varied according to the prevalence rates and it was found to be high in 1997.

CONCLUSION

The 1997 epidemic indicate that historical foci of FMD in the country have been shifted and new foci created in the borders of NWP and NCP. Disease is endemic in three Provinces namely, NEP, NCP and NWP. Return of animals from forest grazing attributes to the onset of seasonal outbreaks in these areas. Thus, the seasonal preventive vaccination programme to protect the village herds is vital.

Movement of cattle especially for slaughter contributed to the spread of infection into many areas especially to the Western Province. Transport of animals with inadequate health precautions played an important role in introducing FMD into Central and Southern Provinces. Thus, it is imperative to make all the agencies involved in livestock related activities to be aware of national animal health regulations.

The 1997, epidemic was caused by foot and mouth virus which belongs to ME-SA topotype of sero type “O”. It attacked all the Provinces in the country and remained active until August 1999 recording 34,472 cases in the island. Mortality rate of this epidemic was 290 in one million.

REFERENCES

CHARACTERIZATION OF FOOT AND MOUTH DISEASE VIRUS TO STRENGTHEN DISEASE CONTROL IN SRI LANKA

R. Hettiarachchi\textsuperscript{1} B.V.Sc., Dip.TVM., M.Sc., M. Kodituwakku\textsuperscript{1} B.V.Sc. and J. Hammond\textsuperscript{2} B.V.Sc., Ph.D

\textsuperscript{1} Department of Animal Production and Health, Peradeniya, Sri Lanka
\textsuperscript{2} World Reference Laboratory for Foot and Mouth disease, Pirbright, U.K

Summary: Foot and mouth disease has been recognized as one of the most economically important disease affecting livestock industry in Sri Lanka. It is a notifiable disease by law and annual mass-scale vaccination campaigns have been carried out by the government authorities since 1984. The serotype 'O' of the foot and mouth virus has been identified as the only serotype circulating in the country since 1985. A molecular epidemiological study on clinical specimens from an outbreak in 2009 confirms that the virus belongs to ME-SA topotype outside the Pan Asia-2 subtype.

INTRODUCTION

Foot and mouth disease (FMD) is economically the most important animal disease of livestock world-wide. It is a highly contagious viral disease affecting virtually all cloven-footed domesticated mammals including cattle, buffalo, sheep, goats and pigs. Wild herbivores such as bison, deer, antelopes, reindeer, llamas, giraffes, and elephants are also susceptible and develop clinical signs (Anonymous, 2009). The disease is characterized by the formation of painful, fluid-filled vesicles (blisters) on the tongue, lips and other tissues of the mouth and on parts of the body where the skin is thin as on the udder and teats, between the two toes of the feet, and around the coronary band above the hoof. Laboratory tests are needed to confirm the diagnosis because several other diseases can produce similar lesions and moreover the specific strain of the foot and mouth disease virus (FMDV) has to be identified in order to initiate appropriate disease control programme.

In Sri Lanka FMD has been recognized as one of the earliest cattle diseases present even before 1842 (Cattle disease commission report of 1869-70, quoted by Fernando, 1969). Since then it has been regularly occurring and at present it is considered as endemic in selected areas in the country. Despite the annual mass-scale vaccination campaigns introduced in 1984, the disease continues to cause adverse effect on the development of livestock industry.

Among the seven distinguished serotypes of FMDV only one serotype namely ‘O’ has been identified in Sri Lanka since 1985. This is also the most prevalent serotype of FMDV, which affects many parts of the world (Samuel and Knowles, 2001). In each serotype of FMDV a number of subtypes and strains have been identified based on the degrees of infectivity, virulence and pathogenicity. Paton et al (2005) revealed that immunity to one subtype does not necessarily ensure adequate protection against other subtypes of same serotype. Therefore, the identity of the circulating field virus has to be determined as a preliminary step in an effective immunization programme. The strains or the subtypes of the field FMDV in Sri Lanka have not been established well or documented and unknown to the national animal health authority. This paper was intended to describe the FMDV strains present in the country to assist in improving national disease control capabilities.

MATERIALS AND METHODS

Epithelial tissue from ruptured FMD vesicles were collected in 20 ml universal bottles containing appropriate FMD specimen transport medium at two different outbreaks. These outbreaks were detected in two separate locations; one in endemic zone (Lankapura in year 2009) and the other in a disease free zone (Gampola in 2008). Two samples were obtained from each location; the affected animals were buffaloes at Lankapura and cattle at Gampola. The collected specimens were suspended in the specific medium which is a mixture of equal amounts of glycerine and 0.04 M phosphate buffer at pH 7.2-7.6 with antibiotics and stored at -20°C. Samples were repackaged according to PI650 requirement for UN3373 category B classification using the standard International Air Transport Arrangement (IATA) sample shipment boxes provided by the Food and Agriculture Organization (FAO) of the United Nations and dispatched to the World Reference Laboratory for FMD (WRLFMD), Pirbright, U.K. in August 2009.

Initially FMDV serotype was identified following virus isolation in cell culture and antigen enzyme linked immunosorbert assay (ELISA). Then FMDV RNA was extracted using a standard protocol used at WRLFMD. Thereafter reverse transcription polymerase chain reaction (RT-PCR) to detect FMD viral RNA was performed. Finally molecular epidemiological testing was
carried out using BTy1 and nucleic acid sequencing at VP1 region. Primers used for the RT-PCR were O-1C244F/EUR-2B52R and O-1C272F/EUR-2B52R.

RESULTS

Three samples showed positive reactions to RT-PCR whereas the virus isolation was successful with only one sample as shown in Table 1. The serotype identified from this sample was 'O'.

The molecular epidemiological analysis by gene sequencing (564-639nt) was performed using the positive sample and then ten viruses that are closely related to the Sri Lankan field virus were determined (Table 2).

Furthermore, comparisons were made to ascertain the relationship between SRL/1/2009 and Reference strains of FMDV belonging to serotype O (Table 3).

DISCUSSION

FMDV is a member of the Aphthovirus genus of the family Picornaviridae which comprises non-enveloped viruses with single-stranded RNA genome. FMDV exists in the form of seven different serotypes: A, O, C, Asia1, and South African Territories 1 (SAT1), (SAT2), and (SAT3) and at least 80 subtypes affecting ruminants, pigs, hedgehogs and elephants (Blood, Studdert and Gay, 2007). Serotype O which is the most prevalent serotype in the world has much greater genetic diversity that allows the classification of eight distinct lineages which fall into endemic cycles of disease that maintain the genogroup.

The use of the most appropriate vaccine as part of an understand and adhere to the recommended procedures in submitting the sample to local or reference laboratories. Required procedures were followed during this study and thereby the Sri Lankan authorities gained the experience and confidence in achieving the above task.

Field Specimens are usually checked for the presence of FMDV antigen by ELISA which is done directly on the clarified samples or after the virus has been grown on cell culture. It has been attempted to identify FMDV serotypes following virus isolation in cell culture and antigen ELISA. The virus has been detected in only one sample and the serotype was found to be 'serotype O'. Other three samples did not have live virus of vesicular diseases namely FMD, swine vesicular disease and vesicular stomatitis. But, it should not be interpreted that the samples were negative for FMDV since further tests could be carried out to confirm the previous test findings.

PCR can be used to detect and type the FMD viral RNA and to differentiate with other vesicular diseases (Reid et al., 2001). RT-PCR technique produces a detection system that is sensitive and considerably more rapid than multiple passages on tissue culture (Callen et al., 1998). RT-PCR detected the FMD viral RNA in three of the four samples and thereby provided evidence to confirm the outbreaks of FMD.

FMDV has been characterized not only to identify suitable vaccine strains but also to study epidemiological links. It helps to trace back and determine the origin of outbreaks (Locher et al., 1995). Nucleotide sequencing reveals the genomic relationship and thus identifies relationships between strains collected from the same or different areas within the country or regions and also at different time period. It reflects both the predominant animal movement patterns in the areas and localized endemic cycles of disease that maintain the genogroup. The nucleotide sequence of the field strains are compared with the sequences of a range of field and reference strains which are maintained in a central database at WRLFMD, Pirbright, U.K. Since only one isolate (O/SRL/1/2009) has been obtained from the study samples, the molecular epidemiological analysis was confined to only one strain. It has been found that the nucleotide sequence is closely related to the field isolates obtained from the outbreaks in 1999 (O/SRL/5/99, O/SRL/6/99) and 1997 (O/SRL/2/97) in the country. The next closely related field isolates in line have been O/IND/413/98, O/IND/77/00, O/IND/115/01, O/IND/156/00 and O/IND/27/95, the isolates obtained from outbreaks in India. Despite the high rate of mutation within the FMD virus genome, the field strain appears to be very closely related to the local isolates in the past and the isolates from India.

The virus isolate resulted from the 2009 outbreak has also been compared for nucleic acid sequences with the reference FMDV strains maintained at WRLFMD to determine the first ten closely related strains. It is interesting to notice that the reference virus of O Manisa group has 86% sequence homology whereas O BFS group virus has 81% sequence homology to SRL/1/2009.

CONCLUSION

The use of the most appropriate vaccine as part of an effective FMD control programme relies on an in-depth knowledge of the virus strains circulating in the country. The selection or development of an appropriate vaccine strain depends totally on the comparison of field isolates at
characterization of foot and mouth disease virus

Table 1. FMDV Serotypes identified from Sri Lankan samples from 2008-2009 outbreaks

<table>
<thead>
<tr>
<th>Location</th>
<th>Sample No.</th>
<th>Animal</th>
<th>VI/ELISA</th>
<th>RT-PCR</th>
<th>Final Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gampola^1</td>
<td>SRL/1/2008</td>
<td>Cattle</td>
<td>NVD</td>
<td>Negative</td>
<td>NVD</td>
</tr>
<tr>
<td></td>
<td>SRL/2/2008</td>
<td>Cattle</td>
<td>NVD</td>
<td>Positive</td>
<td>FMDV GD</td>
</tr>
<tr>
<td>Lankapura^2</td>
<td>SRL/1/2009</td>
<td>Buffalo</td>
<td>O</td>
<td>Positive</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td>SRL/2/2009</td>
<td>Buffalo</td>
<td>NVD</td>
<td>Positive</td>
<td>FMDV GD</td>
</tr>
</tbody>
</table>

1. Samples collected on 08.02.2008; 2. Samples collected on 18.02.2009

VI/ELISA: virus isolation in cell culture and antigen ELISA
RT-PCR: reverse transcription polymerase chain reaction on epithelial suspension for FMD viral genome.
NVD: no foot-and-mouth disease, swine vesicular disease or vesicular stomatitis virus detected
FMDV GD: foot-and-mouth disease virus detected.

Table 2. Molecular details of ten viruses that are closely related to the Sri Lankan Virus (SRL/1/2009)

<table>
<thead>
<tr>
<th>Virus name</th>
<th>Number of nucleotide compared</th>
<th>Number of nucleotide matched</th>
<th>Number of ambiguities</th>
<th>Percentage identified</th>
<th>Percentage difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>O/SRL/5/99</td>
<td>564</td>
<td>517</td>
<td>3</td>
<td>91.67</td>
<td>8.33</td>
</tr>
<tr>
<td>O/SRL/6/99</td>
<td>631</td>
<td>577</td>
<td>8</td>
<td>91.44</td>
<td>8.56</td>
</tr>
<tr>
<td>O/SRL/2/97</td>
<td>601</td>
<td>548</td>
<td>3</td>
<td>91.18</td>
<td>8.82</td>
</tr>
<tr>
<td>O/IND/413/98(PD-FMD)</td>
<td>639</td>
<td>582</td>
<td>0</td>
<td>91.08</td>
<td>8.92</td>
</tr>
<tr>
<td>O/IND/77/00(PD-FMD)</td>
<td>639</td>
<td>582</td>
<td>0</td>
<td>91.08</td>
<td>8.92</td>
</tr>
<tr>
<td>O/IND/115/01(PD-FMD)</td>
<td>639</td>
<td>581</td>
<td>0</td>
<td>90.92</td>
<td>9.08</td>
</tr>
<tr>
<td>O/BHU/2/2002(DQ165037)</td>
<td>638</td>
<td>580</td>
<td>1</td>
<td>90.91</td>
<td>9.09</td>
</tr>
<tr>
<td>O/IND/156/00(PD-FMD)</td>
<td>638</td>
<td>580</td>
<td>1</td>
<td>90.91</td>
<td>9.09</td>
</tr>
<tr>
<td>O/IND/27/95(1994;PD-FMD)</td>
<td>639</td>
<td>580</td>
<td>0</td>
<td>90.77</td>
<td>9.23</td>
</tr>
<tr>
<td>O/VIT/17/2005</td>
<td>639</td>
<td>580</td>
<td>0</td>
<td>90.77</td>
<td>9.23</td>
</tr>
</tbody>
</table>

Table 3. Comparison of SRL/1/2009 and Reference Virus Strains of FMDV serotype O

<table>
<thead>
<tr>
<th>Virus name</th>
<th>Number of nucleotide compared</th>
<th>Number of nucleotide matched</th>
<th>Number of ambiguities</th>
<th>Percentage identified</th>
<th>Percentage difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>O/TAW/2/99(AJ294927)</td>
<td>639</td>
<td>573</td>
<td>0</td>
<td>89.67</td>
<td>10.33</td>
</tr>
<tr>
<td>O/ISR/2/88(DQ164899)</td>
<td>639</td>
<td>563</td>
<td>0</td>
<td>88.11</td>
<td>11.89</td>
</tr>
<tr>
<td>O/MOR/1/91</td>
<td>639</td>
<td>560</td>
<td>0</td>
<td>87.64</td>
<td>12.36</td>
</tr>
<tr>
<td>O/IND/R2/75(AF204276)</td>
<td>639</td>
<td>553</td>
<td>0</td>
<td>86.54</td>
<td>13.46</td>
</tr>
<tr>
<td>O/IND/53/79(AF292107)</td>
<td>639</td>
<td>550</td>
<td>0</td>
<td>86.07</td>
<td>13.93</td>
</tr>
<tr>
<td>O1/Manisa/TUR/69(AJ251477)</td>
<td>639</td>
<td>548</td>
<td>0</td>
<td>85.76</td>
<td>14.24</td>
</tr>
<tr>
<td>O/TAI/189/87(TRRL)</td>
<td>639</td>
<td>547</td>
<td>0</td>
<td>85.6</td>
<td>14.4</td>
</tr>
<tr>
<td>O/PHI/5/95(DQ164946)</td>
<td>639</td>
<td>522</td>
<td>0</td>
<td>81.69</td>
<td>18.31</td>
</tr>
<tr>
<td>O/HKN/6/83(AJ294919)</td>
<td>637</td>
<td>519</td>
<td>2</td>
<td>81.48</td>
<td>18.52</td>
</tr>
<tr>
<td>O1/BFS/1860/UK/67(J2185)</td>
<td>639</td>
<td>517</td>
<td>0</td>
<td>80.91</td>
<td>19.09</td>
</tr>
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</table>
a reference laboratory such as WRLFMD and matching with vaccine strains. Virus characterization remains as the initial and essential step in this process. Molecular epidemiological studies on a field isolate obtained from an outbreak during early 2009 in the endemic zone of Sri Lanka have confirmed that the virus strain belongs to ME-SA topotype of Serotype O FMDV. Furthermore the most closely related field isolates and reference strains have been established. Vaccine matching will be carried out for O/SRL/1/2009 isolate in the near future to further strengthen the national disease control programme for progressive control of FMD.

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We would like to thank Drs. B.D.R. Wijewardena and L.W.B. Epakanda for collecting the clinical specimens. We greatly appreciate the encouragement of Dr. H.M.A. Chandrasoma, Director Animal Health of Department of Animal Production and Health to make this study successful.

REFERENCES


DIAGNOSIS AND TREATMENT OF CONGESTIVE HEART FAILURE DUE TO DILATED CARDIOMYOPATHY IN DOGS: THREE CASE STUDIES


Veterinary Teaching Hospital, Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya

SUMMARY: Dilated cardiomyopathy (DCM) should be considered in the differential diagnosis in dogs presented with clinical signs of congestive heart failure (CHF), which includes dyspnoea and abnormal breath sounds, cough, tachypnoea, tachycardia, arrhythmia, electrocardiography (ECG) abnormalities (P wave & QRS complex), syncope, pleural or pericardial effusions, ascites and/or dependent oedema, with other signs, such as, depression, exercise intolerance, inappetance and muscle wasting. DCM could be easily suspected by measuring the vertebral heart score (VHS) in a thoracic radiograph and confirmed by echocardiographic findings of poor myocardial contraction, low ejection fraction, left ventricular (LV) fractional shortening (<16%) and/or histology of myocardium.

INTRODUCTION

The most common causes of CHF in dogs are valvular disease or myocardial disease (Fuentes and Swift, 1998). In CHF, the heart is unable to pump an adequate supply of blood for the metabolic needs of the body. The drop in the effective forward stroke volume (SV) of the LV triggers a series of compensatory responses in order to maintain the systemic arterial blood pressure and cardiac output (CO) for months or years. This compensation is achieved by rising LV diastolic pressure, mean left atrial pressure and pulmonary venous pressure (PVP), resulting in transudation of fluid in body cavities (pleural effusion, ascites) and oedema. Clinical signs of CHF are due to 'backward' failure (congestion and oedema) and 'forward' failure (reduced CO).

Disease of the myocardium can be without inflammation (cardiomyopathy) or with inflammation (myocarditis) (Dukes-McEwan et al., 2003). Dilated cardiomyopathy (DCM) in dogs is the most common form of cardiomyopathy representing an end-stage of myocardial failure (Fuentes and Swift, 1998), which eventually affects the lungs, liver, and other body systems. The DCM is a progressive disorder characterised by reduction of the systolic myocardial contractility leading to a drop in CO and subsequent development of CHF. DCM causes a gradual increase in end-systolic diameter of one (typically the left) or both ventricles, (Fuentes and Swift, 1998; Richardson et al., 1996). The reduction in myocardial contractility leading to a fall in CO activates compensatory salt and water retention to increase blood volume which cause compensatory ventricular hypertrophy. The resulting cardiomegaly initially allows the SV and end-diastolic pressure to be maintained. Subsequently, the continuous deterioration of myocardial contractility, the increasing blood volume and the limitation in the ability to hypertrophy contribute to progressive CHF. Eventually the Atrio-Ventricular rings (A-V rings) become stretched, compromising the valvular function leading to mitral and/or tricuspid regurgitation. This in turn will increase the atrial pressure, thereby dilating the atria and increasing pressure in the pulmonary vein and capillaries causing the development of pulmonary oedema. Finally, when the CO becomes very inadequate ('forward' cardiac failure), it will cause congestion in the lungs, liver, and other organs and thromboembolic events.

The degree of cardiomegaly in dogs can be quantified by the vertebral heart score (VHS) measured in lateral radiographs (Bavegems et al., 2005; Buchanan, 1995). The lengths of the long and the short axes of the heart are scaled against the length of vertebrae dorsal to the heart, beginning with T4. The adult reference range for VHS in dogs is 9.7 ± 0.5 vertebrae (Buchanan, 1995; Gülanber et al., 2005) and these standards are similar in puppies and adult dogs (Sleeper and Buchanan, 2001).

Causes of DCM are many and include Taurine and L-carnitine deficiencies (particularly in cats, and also in dogs), sustained ventricular tachycardia, viral infections, Doxorubicin toxicity and immunological abnormalities (Fascetti et al., 2003; Novotny et al., 1994). Thromboembolic events are common in dogs and cats given taurine deficient diets (Fascetti et al., 2003; Falk et al., 1992). The offending primary cause may no longer be apparent at presentation (Fuentes and Swift, 1998). Idiopathic DCM is not fully understood but genetic factors and cellular/sub-cellular defects have been proposed. The clinical presentation of DCM may vary from case to case and according to breed.
DCM is prevalent in certain dog breeds, is rare in crossbreeds (Bond and Tilley, 1980), and is a heritable disease in some dog breeds, including the Boxer, Dobermann, Great Dane, Irish Wolfhound, St Bernard and Cocker spaniels (Oyama and Chittur, 2005; Nelson and Couto, 1998).

Clinical examination commonly reveals dyspnoea, tachypnoea, rales, crackles and increased breath sounds, tachycardia, arrhythmia, and systolic murmur. Further findings may include diastolic gallops, audible third heart sound (S3), weak pulse, pulse deficit, ascites, distension of jugular veins, pale mucosae, weight loss and muscle wasting, and sometimes hyperthermia. A long phase that is not associated with clinical signs may be common (Dukes-McEwan, et al., 2003; Fuentes and Swift, 1998). In general, the ECG is of limited value in the diagnosis of DCM, and may include abnormal amplitude or duration of P wave or the QRS complex indicating conduction abnormalities due to cardiac chamber enlargement. Routine biochemical analysis and haematology could be within reference ranges in majority of dogs. Pre-renal azotemia, when present, reflects poor perfusion resulting from low CO and congestion (Dukes-McEwan, et al., 2003).

This publication describes detailed history, diagnosis, treatment and pathological changes in three dogs with DCM, presented to the Veterinary Teaching Hospital, Department of Veterinary Clinical Sciences of the University of Peradeniya, Sri Lanka.

DETAILS OF THE THREE CASES

Dog A

Signalment, history and complaint

A nine year old male Golden Retriever with a complaint of dyspnoea, lethargy, poor appetite, exercise intolerance, constantly in standing posture and refusing to sleep, and an inadequate history of 3 months of treatment given in Colombo, for ascites.

Clinical examination

On admission he was restless, hypothermic (99.2°F), with limb oedema and whitish discharge from both eyes.

The cardiorespiratory systems revealed weak pulse (rate 120/min), CRT =2 sec, arrhythmic heart sounds with heart rate (HR) of 150/min, a pulse deficit, dyspnoea (respiratory rate (RR) 30/min), open-mouth breathing, crackles on lung auscultation, pleural effusion and cardiomegaly (VHS of 12.25 vertebrae) on thoracic radiography.

Abdominal examination revealed pain, hepatomegaly, splenomegaly, malena and ascites. Hematogram and serum chemistry revealed 26.6% PCV, 8,990/µl WBC count (90% neutrophils), thrombocytopenia (159,000 platelets/µl), azotemia [265 mg/dl blood urea nitrogen (BUN) and 8.3 mg/dl serum creatinine (SC)], marginal total plasma protein concentration (TP) of 5.1 mg/dl with hypoalbuminemia (2.24 mg/dl) and 8 IU/dl serum ALT. Urinalysis showed proteinuria (100 mg/dl), pyuria and hematuria. He was treated with diuretics to reduce the transudates, and fluid therapy, amino acids, vitamin supplements, lactulose as supportive treatment. Prognosis was judged as very poor.

On Day 2, echocardiography revealed LV dilatation with thinned walls, reduced ejection fraction and an intraventricular mass in the RV (2.09 x 1.66 cm) (Figure 1). About 1,000 ml red colour pleural effusion (without clots) was removed by thoracocentesis. Abdominal ultrasound revealed small amount of ascitic fluid and fibrotic splenic blood vessels. Hematuria, pyuria and proteinuria persisted on urinalysis, and the same treatment was continued.

On Day 3, hemorrhagic patches appeared in the oral cavity which was attributed to azotemia which worsened with SC elevating to 10.5 mg/dl. Open mouth breathing, tachypnoea, hematemesis, malena, pyuria, hematuria and proteinuria were also observed. Anemia (PCV 20.7%) and thrombocytopenia (57,000/µl platelets) worsened in comparison to day 1, and erythrocyte morphology revealed schistocytes indicating fragmentation of erythrocytes due to intravascular coagulation. The leukocyte count (11,640/µl with 96% neutrophils) and TP (5.18 mg/dl) remained in the normal ranges. The serum albumin improved to 3.51 mg/dl while globulins dropped to 1.67 mg/dl. Aminophyllin (bronchodilator & mild diuretic action) and promethazine (sedative) were added to the treatment schedule.

On Day 4, the patient succumbed to the illness. Necropsy examination revealed a dilated and globoid heart with thin ventricle walls (Figures 2&3) suggesting DCM. Each ventricle had a spherical, white colour mass with broad bases tightly attached to the endocardium (Figure 3) and histopathology revealed alternate deposition of fibrin and leukocytes (Figures 4A & 4B) confirming them to be thrombi. Degeneration and fragmentation of cardiac muscle fibres with moderate hemorrhages seen on histopathology were suggestive of degenerative myocardial disease. Pleural and peritoneal effusions were also seen.

Confirmed diagnosis

The pleural and peritoneal effusions and oedema were due to rising venous pressure as a result of cardiac compensation by dilation of ventricles. His refusal to sleep and preferring to be constantly in standing posture was due to breathing discomfort caused by the pleural effusion. The reduction of the systolic myocardial contractility (reduced SV and CO) leading to hypoperfusion of tissues and organs was evident by dyspnoea, tachypnoea, progressive anemia, pre-renal azotemia, lethargy, poor appetite and exercise intolerance. The effect of increased venous pressure on the liver would have caused hepatic congestion leading to hypoalbuminemia, which would have aggravated the transudation of fluid in to body cavities. Intraventricular thrombosis would have been facilitated due to disturbance in the forward flow in cardiac chambers as a consequence of reduced myocardial contractility and
valvular regurgitation, which was also evident by the pulse deficit and fragmented erythrocytes (schistocytes) on blood smear.

**Dog B**

**Signalment, history and complaint**

A 7 year old female German Shepherd (25 Kg bw) with a complaint of lethargy, exercise intolerance, dyspnoea and swollen hind limbs. She had a previous history (2½ m ago) of ascites and Babesiosis.

**Clinical examination**

On admission, the cardiorespiratory systems revealed a distended Jugular vein (Figure 5), tachycardia, systolic arrhythmia (tachyarrhythmia) with a systolic murmur, and a pulse deficit with HR of 154-185/min and PR of 140/min. The ECG recordings were not remarkable, other than a short P wave. Lateral and VD thoracic radiographs (Figure 7A & 7B) showed a VHS of 12.25 vertebrae and a globoid shaped heart (cardiomegaly) suspecting right heart enlargement and/or pericardial effusion. An enlarged RA was revealed on angiography (Figure 6). The lungs were congested with a RR of 64/min, distended pulmonary trunk (Figure 7A) and pleural effusion (Figure 7B). Limb oedema was detected and approximately 5 litres of transudate ascitic fluid was removed through paracentesis (1 g/dl protein, specific gravity of 1.005).

The differential diagnosis included right heart failure, liver impairment and renal impairment. Hypoalbuminemia (0.89 g/dl) and anemia (PCV 20.5%, RBC 2.78 x10^6/μl, Hb 6.8 g/dl) which would have become aggravated by Babesia gibsonii; WBC 12.930 x10^3/μl, platelets 290,000/μl, TP 5.52 g/dl and BUN 13.62 mg/dl and ALT 17 mg/dl, ruled out liver impairment.

The findings suggested CHF leading to venous congestion causing ascites and limb oedema, and normal renal and liver functions. He was treated with diuretics (frusemide and spironolactone) to reduce the transudation, digoxin (0.0055-0.011 mg/kg bid) to improve myocardial contractility, and fluid therapy, amino acids, vitamins supplements as supportive treatment.

On Day 4, 25 ml of a transudate pleural effusion (straw coloured, turbid, protein 1.26g/dl, albumin 0.75 g/dl, no WBC) was removed through thoracocentesis. On Day 6, cardiomegaly reduced to a VHS of 10.75 vertebrae, and HR dropped to 120-130/min. By Day 9, oedema reduced, but she showed lethargy, diarrhea and vomiting and died.

Necropsy examination revealed a globoid shaped heart with vegetative growths (Figure 8) and a mass in the mitral valve, and pleural effusion indicating endocardiosis and DCM. Histopathology of the mitral valve mass revealed alternate deposition of fibrin and leukocytes (Figures 9A & B), confirming it to be a thrombus.

**Confirmed diagnosis**

The pleural and peritoneal effusions and oedema were due to rising venous pressure as a result of cardiac compensation by dilation of ventricles. Hypoalbuminemia due to hepatic congestion would have aggravated the transudation of fluid into body cavities. The reduction of systolic myocardial contractility (reduced SV and CO) leading to hypoperfusion of tissues and organs was evident by dyspnœa, tachypnœa, and progressive anemia, lethargy and exercise intolerance. Endocardiosis would have impaired valvular and atrioventricular functions leading to DCM, reduced myocardial contractility, CHF and death.

**Dog C**

**Signalment, history and complaint**

A 7.5 year old male Cocker spaniel with a chronic cough of 23 days. As the owner was a physician, a detailed history of the case was available on admission. On Day 1 of the sickness he had been coughing throughout the day and had been treated for microfilariosis with ivermectin, antibiotic, and prednisolone for 6 days at a veterinary clinic in Colombo. On Day 9 he had become cyanotic with abdominal breathing and had been treated with antibiotic, piriton, prednisolone by the same veterinary clinic for 8 days and the cough had stopped. On Day 16, ARV and DHL vaccinations had been given and coughing had recurred that night, and the following day again he had been treated with antibiotics, piriton, salbutamol. Radiographs taken on Day 22 had suggested cardiac involvement and he had been treated with frusemide and was referred to the VTH on Day 24.

**Clinical examination**

On admission (Day 24), the cardiorespiratory systems revealed cardiomegaly, a globoid shaped heart with the VHS of 13.0 vertebrae, arrhythmia (HR of 72-95/min), biventricular enlargement and normal atria (deep Q wave, tall and wide QRS, slanted ST on ECG), tachypnoea (RR of 30-60/min), hoarse breathing, prominent pulmonary vessels in thoracic radiograph (no indication of pneumonia or congestion) and SPO, was 94-98%. Echocardiography revealed an enlarged LV, with no defects in valves, no pericardial effusion, no abnormal masses. He showed intermittent cough which aggravated when excited, such as during meal time and oral medication. The only significant change seen on laboratory testing was hypocalcaemia (7.74 mg/dl). He showed exercise intolerance throughout his stay at the VTH with normal PCV of 28%. The urine output was 55.3% indicating poor tissue perfusion.

Based on clinical experience gained from the previous two cases, a provisional diagnosis of DCM with potential CHF was made and prognosis was considered as guarded. He was treated with diuretics (frusemide, addi K), digoxin, and Kalzana (calcium supplement). The urine output improved to 68.4% on day 26 indicating improvement in tissue perfusion. The VHS dropped to 11.3 vertebrae by day 28, and the vasodilator/antihypertensive angiotensin receptor blocker (ARB) 'Losartan' 25mg q24h was added to the treatment schedule till the day of discharge.

The initial cause of the DCM was not clear. It was presumed that the vaccination had been done during a
Figure 1. Dog A - Echocardiograph on the second day of treatment. Interpretation - LV dilatation with normal or thinned walls and reduced ejection fraction, and an intraventricular mass (RV) of 2.09 x 1.66 cm.

Figure 2. Dog A Dilated and rounded heart

Figure 3. Dog A Mural thrombi in the RV and LV, thin ventricular wall

Figure 4. (A: 10x, B: 100x) Dog A Histopathology of the ventricular mass. Alternate deposition of fibrin and leukocytes are shown by arrows. (H & E staining)

Figure 5. (Dog B) Distended jugular vein (arrow) on physical examination

Figure 6. Dog B Angiograph demonstrating RA enlargement and distended anterior vena cava (arrow)

Figure 7. Dog B - VD (7A) and Lateral (7B) thoracic radiographs showing generalised cardiomegaly (globoid heart shape), pericardial effusion. VHS = 12.25 vertebrae

Figure 8. Dog B Endocardiosis - vegetative growths (black arrow) and thrombus (white arrow) in the mitral valve

Figure 9. Dog B Histopathology of the thrombus in the mitral valve. Deposition of fibrin and leukocytes are shown by arrows. [10X (9A) and 40x (9B)]
Dilated cardiomyopathy in dogs

Figure 1. Dog A - Echocardiograph on the second day of treatment. Interpretation - LV dilatation with normal or thinned walls and reduced ejection fraction, and an intraventricular mass (RV) of 2.09 x 1.66 cm

Figure 2. Dog A - Dilated and rounded heart

Figure 3. Dog A - Mural thrombi in the RV and LV, thin ventricular wall

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Figure 6. Dog B - Angiograph demonstrating RA enlargement and distended anterior vena cava (arrow)

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Figure 8. Dog B - Endocardiosis - vegetative growths (black arrow) and thrombus (white arrow) in the mitral valve

Figure 9. Dog B - Histopathology of the thrombus in the mitral valve. Deposition of fibrin and leukocytes are shown by arrows. [10X (9A) and 40x (9B)]
period when the dog was convalescing from an ongoing respiratory involvement, which would have progressed to a chronic cough, leading to reduced tissue oxygenation and hypoxia. This situation would have further weakened the myocardial contractility leading to reduced CO, cardiac compensation and DCM. Therefore, antibiotics were added as a prophylactic measure to the treatment schedule.

By day 30 an improvement was seen by the cessation of cough, a reduction in VHS to 10.75, elevation of PCV to 37% and urine output to 90%, but the hoarse breathing continued. This revealed that gradual reduction of the cardiac enlargement had improved the SV and CO and enhanced perfusion and oxygenation of tissue. Digoxin was tapered down during the last 2 days of hospitalization and Kalzana was continued. The bronchodilator theophyllin (Quibron) was given to facilitate breathing. He was discharged on day 32.

**Confirmed diagnosis**

Cardiomegaly and ECG abnormalities revealed cardiac compensation by biventricular dilatation resulting in elevated PVP as evident by prominent pulmonary vessels and breathing difficulties. The reduction of the systolic myocardial contractility (reduced SV and CO) leading to hypoperfusion of tissues and organs, was evident by poor urine output, cough induced by excitement, lethargy and exercise intolerance. Following the treatment for DCM, cardiomegaly reduced indicating the return of the myocardial contractility and perfusion to normalcy, which was evident by the increase in PCV and urine output.

**DISCUSSION AND CONCLUSION**

All three cases of DCM were older dogs between 7-9 years of age. The common complaints at the time of admission included dyspnoea, ascites, lethargy and exercise intolerance. Additional complaints made on individual cases were swollen hind limbs, cough, preferring to be in standing posture, and refusing to sleep.

The clinical phase of DCM is usually associated with CHF, which includes dyspnoea, cough, tachypnoea, rales, crackles and increased breath sounds, depression, exercise intolerance, inappetence, syncope, muscle wasting, abdominal distension, polydipsia, tachycardia and arrhythmia (Dukes-McEwan, et al., 2003; Fuentes and Swift, 1998). Further findings may include diastolic gallops, audible third heart sound (S3), weak pulse, pulse deficit, ascites, pale mucosa and sometimes hyperthermia (Dukes-McEwan, et al., 2003). In advanced cases, narrow pulse pressure, elevated jugular venous pressure, mitral or tricuspid regurgitation, presented by systolic murmurs, may occur. The progression of heart failure is associated with LV remodeling, which manifests as gradual increases in left ventricular end-diastolic and end-systolic volumes, wall thinning, and a change in chamber geometry to a more spherical, less elongated shape. This process is usually associated with a continuous decline in ejection fraction (EF). The concept is termed 'cardiac remodeling' which is the common mechanism for the progression of cardiac dysfunction.

The clinical signs of the 3 cases described included general findings such as restlessness, emaciation, limb oedema and hypothermia. Ventricular remodeling was evident by cardiomegaly (biventricular enlargement) with elevated VHS (12.25 to 13.00) and globoid cardiac silhouettes. The abnormal myocardial activity leading to CHF was evident by arrhythmia and tachycardia (150 85/bpm) with weak pulse, poor pulse rate, systolic murmur, and distended jugular vein. Cardiac catheterization and coronary angiography could be used to exclude ischemic heart disease. Reduced systolic activity and ventricular EF led to thrombolytic events and venous congestion evident in radiographs as distended pulmonary trunk, causing dyspnoea, tachypnoea, open mouth breathing, cough, and also as transudate effusions in body cavities and the sub cutis (pleural effusions, limb oedema and/or ascites). Thoracocentesis relieved respiratory distress in two dogs.

Prediction of survival times and identification of influencing factors are of interest for dog owners as well as veterinarians (Dukes-McEwan, et al., 2003). Predictive factors of survival include the degree of cardiomegaly, the degree (or class) of heart failure, presence of pleural effusion and pulmonary oedema, echocardiographic parameters, presence of ventricular premature complexes (VPCs), biventricular heart failure and atrial fibrillation. Polymorphisms in genes encoding cytoskeletal, contractile, or other cardiac proteins can be important prognostic information of DCM (Mestroni, 2003).

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**REFERENCES**


Confined diagnosis the concept of canine idiopathic dilated cardiomyopathy. This process is usually associated with gradual increases in left ventricular end-diastolic and end-systolic volumes, wall thinning, and a change in shape. This can lead to Laennecian collateral pathways in left ventricular cavity in acute myocardial infarction, which was evident by prominent pulmonary congestion evident in radiographs as distended pulmonary vessels and breathing difficulties. The reduction of the degree (or class) of heart failure, presence of pleural effusions, limb oedema, and arrhythmia (Dukes-McEwan, Silva et al., 2003). In advanced dog and cat cases, narrow pulse pressure, elevated jugular venous pressure, and gallops, audible third heart sound (S3), weak pulse, pulse hemostasis, and emaciation. Physical examination of the legs should reveal firm, dry, and hot skin and limbs, increased breath sounds, depression, Beppu, S., Izumi, S., Miyatake, K., Nagata, S., Park, Y.D., Falk, R.H., Foster, E. and Coats, M.H. (1992). Ventricular thrombi and thromboembolism in dilated cardiomyopathy: a prospective follow-up study. *American Heart Journal.* 123(1): 136-142.


Effect of a radiant energy-treated lysozyme antimicrobial blend on the control of clostridial necrotic enteritis in broiler chickens

A cage study was conducted to demonstrate the effect of Entegard REV, a lysozyme-based antimicrobial blend, on the performance of broiler chickens and necrotic enteritis (NE) disease reduction of birds that were challenged with *Eimeria maxima* and *Clostridium perfringens*. In the experiment, challenge by the infectious agents without medication resulted in impaired feed consumption, weight gain, and feed conversions and caused high incidence of gross NE lesions and NE mortality rate. Entegard REV included in feed at 200 g/metric ton (MT) was very effective in reducing negative health effects in the birds after NE challenge, and its ability to control the disease was not statistically different from a commonly used antibiotic growth promotant, bacitracin methylene disalicylate, at 55 g/MT.


Detection of *Coxiella burnetii* in placenta and abortion samples from British ruminants using real-time PCR

A real-time PCR was developed to detect *Coxiella burnetii* (the cause of Q fever) in ruminant placentas and aborted fetuses. Primer and probe sets previously developed for human tissue studies were used to target the insertion sequence IS1111 gene for *C. burnetii*. The assay was highly sensitive, with a limit of detection of 10 copies of template, theoretically equating to a single bacterium, and did not cross-react with a panel of other bacteria. To determine sensitivity on field samples submitted for the diagnosis of abortion, results using the IS1111 PCR assay were compared with a com1 PCR assay. When applied to ruminant abortion material, including placental cotyledons and fetal samples, the IS1111 and com1 assays yielded positive results in 23 (25 per cent) of 93 and 19 (20 per cent) of 93 samples, respectively. One infected goat herd was monitored for 31 months: 57 (92 per cent) of 62 placental cotyledon samples from aborting and non-aborting goats, and 10 (30 per cent) of 33 fetal samples were positive by the IS1111 PCR assay.


Selenium enrichment of table eggs

Selenium is an essential trace element with a recommended dietary allowance for human adults of 55 ig/d. However, there is evidence that greater dietary intakes may have possible health benefits, including a reduction in the risk of cancer. Several studies have shown the feasibility of enriching eggs using organic Se and that Se-enriched eggs are an effective way to supplement human diets. However, few studies have examined the response of egg Se concentration to high (>1 ig/g) dietary organic Se intake by the laying hens. The objective of the current study is to examine the effect of higher dietary organic Se levels on production, egg mass, and egg Se levels. These were assessed by feeding 3 breeds of laying hens (Barred Plymouth Rock, Lohmann Brown, Lohmann White) a basal diet containing 0.3 ig of Se/g of diet as Na2SeO3. Into this diet, Se yeast (SelenoSource AF 600), an organic source of Se, was added at 1.0, 2.4, or 5.1 ig of Se/g of diet for 4 wk. Feed consumption, egg production, and egg mass were not affected by the dietary Se concentration in all 3 breeds. Within the range of Se levels employed in the laying hens’ diet, egg Se content increased linearly as dietary levels of Se increased. The results of this study indicate that feeding up to 5.1 µg/g of Se will not affect egg production and the welfare of the laying hen and is a practical way of producing Se-enriched eggs for the consumers.


Developments in poultry genetic research 1960-2009

Major advances in the study of inheritance were made in the early 20th century that became the basis for the systematic improvement of chickens for specialised egg or meat production in the last 50 years. The developing sciences of applied poultry breeding are reflected in publications in British Poultry Science during this period and are illustrated by a selection of papers in five areas: (i) papers on the measurement of new phenotypes (disease resistance and egg shell strength); (ii) undesirable correlated responses to selection (reproductive efficiency and bone strength); (iii) genetic interactions (gene-gene and gene-environment); (iv) selection for production efficiency (feed conversion efficiency and residual feed intake); and (v) papers illustrating the potential role of technology based on knowledge of variation at the level of the DNA (Quantitative Trait Loci for ascites resistance and polymorphisms in the growth hormone receptor gene).


The demand for veterinary services in western Canada

The objective of this study was to determine the number of hours veterinarians in western Canada work per week, how they apportion their time by species, and clinics’ hiring intentions for new veterinary associates. Of 1099 clinics contacted, 706 (64%) responded to the survey, representing 80% (1774/2227) of private practitioners in western Canada. Practitioners devoted 73% of their time to small animals (SA), 11% to beef practice, and 9% to horses. Sixty-four percent of clinics and 66% of practitioners were devoted exclusively to companion animal (SA and horses) practice; only 4% of clinics and 4% of practitioners were devoted exclusively to food animal practice. A total of 230 clinics were seeking to hire another veterinarian, representing 223 full-time equivalents (FTEs). When adjusted for clinics that did not respond, the total number of vacancies in western Canada could be as high as 347 FTEs with 57% of vacancies in companion animal practice. The survey, however, did not assess how determined the clinics were in their attempts to hire another associate.

Short communication

COMPETITIVENESS OF DAIRYING IN DIFFERENT SCALE OF OPERATIONS IN BAREILLY DISTRICT OF UTTAR PRADESH IN INDIA

K.A.C.H.A. Kothalawala1,2 B.V.Sc., M.V.Sc., M.Sc. and Sanjay Kumar1 B.V.Sc., Ph.D

1. Division of Livestock Economics and Statistics, IVRI, Izatnagar, 243 122, Bareilly, U.P. India
2. Division of Livestock Planning and Economics, Department of Animal Production and Health, Galmbe, Peradeniya, Sri Lanka

SUMMARY: A research was carried out to study the cost and profit of rural dairies in Bareilly district of Uttar Pradesh, India. A total of 120 sample households were selected using multistage stratified random sampling method and analysis was done on five categories on land size as landless, marginal, small, medium and large. The results revealed that buffaloes (4.1) dominated the herd followed by indigenous cattle (0.4) and then crossbred animals (0.1) in the area. The number of total animals, buffaloes, crossbred and indigenous cattle increased with the size of the land in contrast to the milking to milch animal ratio which decreased with the land size. The average milk yield ranged from 4.3 litres to 5.2 litres with an overall average of 4.8 litres per farm per day in the area. According to the results, cost of production of milk was lowest in small farmers (Indian Rupees (INR) 10.95) followed by landless farmers (INR. 11.10) and highest in large farmers (INR. 12.32). Thus, small farmers earn a higher profit (INR. 1.05) than large farmers. The profit excluding labour was highest in landless farmers (INR. 5.21) followed by marginal farmers (INR. 5.01). On an average a farmer can earn Rs. 4.09 from every litre of milk for their labour in this area.

INTRODUCTION

India is the single largest milk producer in the world (Dairy India Year Book, 2007). Indian economy is predominantly rural, based on agriculture in which dairying is an integral part. Livestock sector has consistently recorded a higher growth rate ranging from 4-5 percent in recent past in Indian economy. Dairy sector alone has given more than 65 percent share in livestock GDP and its increasing rate indicates the significance of dairying in India (Kumar and Jain, 2004). Sixty eight percent of cattle population in India is owned by small farmers with less than 2 ha of land. Recent advent of the world trade organization (WTO) regulations, opened up global competition in the dairy sector, exposing the smallholder producer to unfair market regime in India (Kurup, 2002). The competence of dairy farmers to stand in this market depends upon the ability to produce at a lower cost as a unit than their competitors. The competitiveness may vary with the scale of operation with availability of resources.

In this background an economical analysis of dairy enterprise in different scales (land size) in Bareilly district was carried out with an objective of studying cost effectiveness of dairying in different scales in the study area.

METHODOLOGY

The study was conducted in rural areas of Bareilly district in Uttar Pradesh in India. Sampling method was the multistage stratified random sampling. Development blocks were the first strata, gram panchayats were the second strata and household respondents were the third strata. Bhojipur and Bithariachinpur blocks were selected randomly for the study. Two gram panchayates (Trikunia and Sarkara) from Bitharichainpur block and other two (Dalpatpur and Ishapur) from Bhojipura block were selected randomly. Data were collected using pre-structured questionnaire during the period February to April, 2006 from 30 randomly selected households from each selected gram panchayat. Thus, a total of 120 households were interviewed to collect data on number of animals, their species, their crosses, average milk production per farm, dung production, quantity of feeding concentrates, green fodder, dry fodder and value of shed, animals, equipments and labour hours. Prevailing rates in the area for all the inputs such as concentrates, green fodder, dry fodder and out puts such as milk and dung were also obtained.

Since there were positive correlation between land holding and number of animals kept (Saran et al., 2002), the collected data were categorized according to the land area owned by the farmer as landless (no own land), marginal (< 1 ha), small (1-2 ha), medium (2-4 ha) and large (> 5 ha) farmers. Data were computed and analyzed using Microsoft excel to calculate average, SD, and percentage. In estimating the production cost and profit, the input cost was divided in to two groups as variable cost and fixed cost. The variable cost included cost of labor, green fodder, dry fodder, miscellaneous charges (veterinary and breeding service and other charges). Fixed cost included interest on fixed capital, depreciation on shed and equipments. Interest rate on capital assets (animals and shed) was worked out at the rate of 12.5 per cent per annum as charged by the State bank of India for such purposes. It was assumed that cattle owners used their own funds. The interest on working capital was not computed, as there was regular income flow from the sale of milk. Annual depreciation cost for chaff cutter and other
equipments was also calculated using straight-line method. The rate for machinery and chaff cutter was considered as 10% per annum (Baruah et al., 1996) and depreciation on cattle shed was worked out at the rate of 2% per annum for sheds in good condition and 5% for sheds of medium condition respectively (Shah, 1983; Gover, 1992). Appreciation or depreciation of animal was not included and animal value was taken as an average value.

Gross cost included all the expenditure incurred on the milch animals of the farm. It comprised of the expenditure on purchase of inputs from the market and imputed value of owned resources. A total of fixed cost and variable cost were added together to get gross cost. Net cost was estimated by deducting the value of dung from gross cost. Milk production cost to net cost [CMP (nc)] was estimated using following formula (Kotalawala, 2006).

\[
\text{CMP(nc)} = \frac{\text{TFC} + \text{TVC} - \text{VD}}{\text{LMP}}
\]

Cost of milk production to total cost [(CMP (tc)] was estimated using the following formula.

\[
\text{CMP(tc)} = \frac{\text{TFC} + \text{TVC}}{\text{LMP}}
\]

Where,

\[
\text{CMP(nc)} = \text{Cost of milk production per litre excluding value of dung}
\]

\[
\text{TVC} = \text{Total variable cost/ day}
\]

\[
\text{TFC} = \text{Total fixed cost/ day}
\]

\[
\text{VD} = \text{Value of dung/ day}
\]

\[
\text{LMP} = \text{Litres of milk produced/ day}
\]

Net return to total cost per litre of milk was calculated using following formula.

\[
\text{NR} = \frac{\text{TR} - \text{TGC}}{\text{TR}} - \frac{\text{TGC}}{\text{LMP}}
\]

Profit when labour cost excluded was calculated using following formula

\[
\text{NR (nc)} = \frac{\text{TR} - (\text{TGC} - \text{Labor Cost})}{\text{LMP}}
\]

Where,

\[
\text{TR} = \text{(Return from milk + Return from dung) per day}
\]

\[
\text{TGC} = \text{Total gross cost/ day}
\]

\[
\text{NR} = \text{Net Return}
\]

RESULTS AND DISCUSSION

Table 1 summarises the average number of animals per farm and average milk production per day in a given farm. According to the table 1, Buffalo were found to be the dominant milch animal in the area. In all the groups viz. landless, marginal, small, medium and large, number of

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Landless</th>
<th>Marginal</th>
<th>Small</th>
<th>Medium</th>
<th>Large</th>
<th>Overall</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total No. cattle &amp; buffalo</td>
<td>3.3</td>
<td>3.8</td>
<td>4.4</td>
<td>4.7</td>
<td>4.5</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>No. of milking animals</td>
<td>1.2</td>
<td>1.2</td>
<td>1.3</td>
<td>1.2</td>
<td>1.3</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>No. of indigenous cattle (milch)</td>
<td>0.1</td>
<td>0.5</td>
<td>0.5</td>
<td>0.7</td>
<td>0.0</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>No.of buffaloes (milch)</td>
<td>1.8</td>
<td>1.8</td>
<td>2.0</td>
<td>2.6</td>
<td>3.0</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Crossbred cattle(milch)</td>
<td>0.0</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.0</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>No. total milch animals</td>
<td>1.8</td>
<td>2.4</td>
<td>2.6</td>
<td>3.4</td>
<td>3.0</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>Milking /Milch Ratio</td>
<td>0.7</td>
<td>0.5</td>
<td>0.5</td>
<td>0.4</td>
<td>0.4</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Av. Milk production (l)</td>
<td>4.3</td>
<td>4.2</td>
<td>5.1</td>
<td>5.0</td>
<td>5.2</td>
<td>4.8</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Average cost and profit of production of milk

<table>
<thead>
<tr>
<th>Group</th>
<th>Cost of Production (INR/l)</th>
<th>Profit (INR/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cost (nc)</td>
<td>Cost (nc)</td>
</tr>
<tr>
<td>Landless</td>
<td>11.10</td>
<td>9.46</td>
</tr>
<tr>
<td>Marginal</td>
<td>11.46</td>
<td>9.96</td>
</tr>
<tr>
<td>Small</td>
<td>10.95</td>
<td>10.24</td>
</tr>
<tr>
<td>Medium</td>
<td>11.32</td>
<td>10.37</td>
</tr>
<tr>
<td>Large</td>
<td>12.32</td>
<td>10.84</td>
</tr>
<tr>
<td>Average</td>
<td>11.35</td>
<td>10.17</td>
</tr>
</tbody>
</table>
buffaloes was observed to be higher than the number of indigenous or crossbred cattle. The average number of milch buffalos per farm were 2.2 whereas the average number of milch indigenous and crossbred cattle were 0.4 and 0.13 respectively. In addition, it was observed that the average number of milch buffalos increases with the land size and there is no significant difference in number of cross bred or indigenous cattle across the land size. The average number of milch animals per farm was 2.7 and increased with the land size. Average number of milking animals was 1.2 per farm in the area and it was lower than the average number of milking animals (3 per farm) in Punjab (Gover et al., 1992). In contrary milking to milch ratio was highest in landless farmers and it decreased with the land size.

The average milk yield per day per farm ranged from 4.3 litres to 5.2 litres across the scales with an overall average of 4.8 litres per farm per day (Table 1).

Cost of production of 1 litre (COP$_{1l}$) of milk ranged from lowest of Indian Rupees (INR) 10.95 (small farmers) to highest of INR 12.32 (large farmers) with an average of INR 11.35 in the study area. The cost in the large farmers was marginally higher than the average pricing realized (INR 12.00) per litre of milk in the area. Though the total cost of milk production was highest in large farmers both the major cost components of feed and labour were highest in landless farmers explaining the high cost with low availability of resources (crop by products) at the farm yard.

The net cost was lowest in landless farmers (INR. 9.46) and highest in large farmers (INR. 10.84). The present findings were higher than the net cost of INR. 6.83 per litre of milk from crossbred cow and INR.7.58 from buffalo in Uttar Pradesh (Chandra and Agarwal, 2000). However, it was similar to the findings of Sharma and Singh (1993) in Himachal Pradesh where the net production cost of milk was INR. 10.01. Small farmers can earn the highest profit of INR. 1.05 while large farmers had slightly negative profit margin from dairying. On average, farmers in the area can earn about INR.0.65 per litre of milk. These findings were similar to the net profit per litre of Rs. 0.57 to Rs. 1.41 in Himachal Pradesh (Sharma and Singh, 1993). The profit when family labour was excluded ranged from INR. 5.21 to INR. 4.21 with an average profit INR. 4.74 per litre in the area.

**CONCLUSION**

The landless, marginal, small and medium farmers have advantage of gaining higher net profit from dairying than large farmers in the area. Therefore it can be concluded that especially farmers with land size of less than or equal to two hectare are more economic producers than their large scale counterparts. That could be due to the optimum utilization of resources.

**ACKNOWLEDGEMENT**

Authors would like to acknowledge the Council for Agricultural Research Policy (CARP), Sri Lanka for financing the project. Further, special thanks are to Dr.Triveni Dutt (Principal Scientist), Dr. Sunil Kumar (Research Associate), Dr.Ravi Rangan Sinha of the Indian Veterinary Research Institute, India for supporting in data collection. Further Department of Animal Production and Health is also acknowledged in facilitating the study.
Figure 1. Comparison of the cost of production, feed cost and labour cost in different scale of operation
Figure 1. Comparison of the cost of production, feed cost and labour cost in different scale of operation
SULPHATION OF ENVIRONMENTAL ESTROGENIC COMPOUNDS IN VIVO

J.G. Shirani Ranasinghe B.V.Sc., Ph.D
Department of Biochemistry, Faculty of Medicine, University of Peradeniya, Peradeniya

Summary: Sulfate conjugation represents a major pathway in vivo for the biotransformation and or excretion of xenobiotics or endogenous compounds. In the present study, we used Sprague Dawely rats as a model to investigate the occurrence of sulfation of Bisphenol A in vivo. The treated group and control group were treated subcutaneously with 300 g/Kg Bisphenol A and corn oil 0.1 ml respectively for 7 days. Serum samples were analysed using ODS 80 TM TSK-GEL column. The presence of bisphenol sulphate in the serum of the treated rats were identified by HPLC analysis and confirmed by photo diode array detector.

In conclusion the results obtained in the present study showed clearly that occurrence of the in vivo sulfation of bisphenol A.

INTRODUCTION

The chemicals with hormonal activity may have the potential to cause endocrine related diseases in human and wild life (Nagao et al., 1999). Environmental estrogen-like chemicals have been recognized as a potential hazardous factor for human health (Roy et al., 1997). The binding capacity of these compounds to estrogen receptors will lead to estrogenic action of the compound in the body. These estrogen like chemicals make their way into the food chain and the adverse effects have been already notified in many cases. The abnormal sexual development of reptiles and birds (Guillette, 1995), the decline in sperm quality of men (Fry, 1995; Yokoto et al., 1992) have been taken into consideration. Bisphenol A is a chemical with weak estrogen-like activity when evaluated in growth of the mammary gland of Noble rats (Hyoung et al., 2007). It is a compound of plastic used in dental filling that are often used to protect teeth in children (Olea et al., 1996). The toxicity of Bisphenol derivatives for human and in particular its potential for acute poisoning has been reported (Auger et al., 1996).

Sulfate conjugation represents a major pathway in vivo for the biotransformation and or excretion of xenobiotics or endogenous compounds such as steroid and thyroid hormones, catecholamines and bile acids (Jadwiga et al., 1999; Mulder and Jakoby, 1990; Falany and Roth, 1993). The cytosolic sulfotransferases catalyze the transfer of a sulfonate group from the active sulfate (Lipman, 1958). The sulfation may increase the water-solubility of these compounds facilitating removal from the body. The in vitro sulfation of Bisphenol A using recombinant human cytosolic sulfotransferases were reported (Suiko et al., 2000).

In the present study we used Sprague Dawely rats as a model to investigate the occurrence of sulfation of Bisphenol A in vivo.

MATERIALS AND METHODS

Animals
Eight weeks old female Sprague Dawely rats were used in this study. They were divided into 2 groups control (n=8) and treatment (n=8). The rats were housed in individual metal cages and kept in a room in which the temperature and humidity were controlled at 24±1°C and 50%±5% respectively with a 12 hr light- dark cycle. Food and water were available ad lib. The animal experiment was carried out according to the International Guiding Principles for Biomedical Research Involving Animals (International guiding principles, 1985).

Bisphenol A was obtained from Sigma Chemical. All HPLC grade reagents were purchased from Wako Pure Chemical Industries. Bisphenol sulphate was synthesized according to the method described by Jevons (Jevons, 1963).

Treatment
After 7 days adaptation period treatment and control groups were administered subcutaneously 300g/ Kg Bisphenol A and corn oil 0.1 ml respectively for 7 days.

Samples
Food intake and weight of individual rats were monitored throughout the experimental period. After 7 days of the treatment period, blood samples were collected in plastic tubes from tail veins, incubated at room temperature for 1.5 hr and centrifuged for 5 minutes at 5000 g. Serum samples separated were stored at -80°C.

Preparations of serum samples for HPLC analysis
Two ml of cold (-20°C) ethanol was added to 1 ml of serum sample, and the mixture was incubated at -20°C for 2 hrs and centrifuge at 5000g for 5 min (Sotter et al., 1996). The supernatant was collected, dried by
evaporation and the residues were redissolved in 100 µl buffer A (see below), filtered through a Cosmotic filter (0.45 µm pore size 4 mm diameter and subjected to HPLC analysis as described below.

**HPLC analysis**

A Hitachi L-600 HPLC system fitted with an ODS-80 TM TSK-GEL column (84.6 mm i.d; TOSOH, Tokyo) was employed. Buffer A was 0.002% formic acid (in water) and Buffer B was a mixture of 30% acetonitrile and 70% Buffer A. After injection of the sample, the column was eluted at 35°C with a linear gradient from 100% Buffer A and 60% Buffer B over 40 min, followed by a linear gradient to 100% Buffer B through 36 min. The spectrum of both the standard and serum peaks were examined by photo diode array detector.

**RESULTS**

As an attempt to investigate the in vivo sulfation of Bisphenol A, rats were treated with Bisphenol subcutaneously. Serum samples collected and analyzed by the HPLC procedures as described above. As shown in Figure 1, a distinct peak (peak a) with a retention time of 5.87 min was detected for the synthetic Bisphenol A sulfate. When the serum samples of rats treated with bisphenol A were analyzed, a peak with the same retention time was detected as shown in Figure 2 A. In untreated serum samples that peak was not detected (Figure 2 B). In order to confirm the presence of Bisphenol sulfate in the serum of treated group, the serum was hydrolyzed with 1N HCl by boiling for 15 minutes. In order to confirm the compound as Bisphenol sulphate, the spectra were examined using photo diode array detector. As shown in figure 3A and 3B when the spectra of those 2 peaks namely serum and Bisphenol sulphate standard were examined a similar spectra were Bisphenol A. The interaction of Bisphenol sulphate with their receptors can be further investigated to assess the toxicity by exposure to bisphenol A diglycidyl ether placental barrier at a late stage of gestation in rats.

**DISCUSSION**

The toxicity and metabolism of estrogenic compounds Bisphenol and to study the factors which facilitate their detoxification of Bisphenol A by sulfation. A radiolabeled cell culture study can be employed to measure the proportion of sulphate and glucuronide form of the enzyme. This enzyme is involved in the detoxification of Bisphenol F. Glucuronidation is a major detoxification pathway in all vertebrates, whereas it is rare in invertebrates (Dutton, 1970).

**CONCLUSION**

In conclusion, the results obtained in the present study showed clearly the occurrence of the toxicity by exposure to bisphenol A diglycidyl ether in vivo. The distribution of bisphenol F in pregnant and nonpregnant rats. Bisphenol A in HepG2 cells and the release of them out of the cell. The profiling of bisphenol F in pregnant and nonpregnant rats.

**REFERENCES**


**Figure 1.** HPLC chromatogram of Bisphenol A Sulfate (Synthetic)

**Figure 2A.** HPLC chromatogram of treated Rat Serum. Peak a and b

**Figure 2B.** HPLC chromatogram of untreated rat serum samples
spectra of those 2 peaks namely serum and Bisphenol diiod array detector. As shown in figure 3A and 3B when the Bisphenol sulphate, the spectra were examined using photo for 15 minutes. In order to confirm the compound as group, the serum was hydrolyzed with 1N HCl by boiling the presence of Bisphenol sulfate in the serum of treated that peak was not detected (Figure 2 B). In order to confirm detected as shown in Figure 2 A. In untreated serum samples were analyzed, a peak with the same retention time was detected for the synthetic Bisphenol A sulfate. Figure 1, a distinct peak (peak a) with a retention time of 5.87 min was detected for the synthetic Bisphenol A sulfate. Buffer B was a mixture of 30% acetonitrile and 70% Buffer A (see below), filtered through a Cosmotic filter (0.45 µm pore size 4 mm diameter and subjected to HPLC analysis as described below. As shown in

### RESULTS

In vivo sulfation of environmental estrogens occurs in vitro and sulfated Bisphenol A was found in the culture medium (Suiko et al., 2000). This demonstrated clearly the occurrence of the sulfation of Bisphenol A in HepG2 cells and the release of them out of the cell.

Glucuronidation is a major detoxification pathway in all vertebrates, whereas it is rare in invertebrates (Dutton, 1996). Bisphenol A sulphation by the rat liver UDP glucurondiase has recently has been reported (Yokoto, et al., 1999).

The distribution of bisphenol F (4, 4’-dihydroxy-diphenyl- methane, BPF) was studied in female Sprague-Dawley rats with a single dose of 7 or 100 mg/kg and BPF residues were detected in the uterus, placenta, amniotic fluid, and fetuses. Large amounts of radioactivity (8-10% of the dose) were still located in the digestive tract lumen at the end of the study (Cabaton et al., 2006)

Bisphenol is efficiently absorbed and distributed to the reproductive tract in female rats, and its residues pass the placental barrier at a late stage of gestation in rats.

In conclusion, the results obtained in the present study showed clearly the occurrence of the in vivo sulfation of Bisphenol A. The interaction of Bisphenol sulphate with their receptors can be further investigated to assess the detoxification of Bisphenol A by sulfation. A radio-labeled cell culture study can be employed to measure the proportion of sulphatae and glucuronide form of Bisphenol and to study the factors which facilitate their formation and excretion.

### DISCUSSION AND CONCLUSION

The toxicity and metabolism of estrogenic compounds in mammals attracted serious research during the last quarter of the 20th century (Shah and McLachian, 1976).

Sulphation, a major phase II detoxification pathway, is known to be utilized in vivo for the removal of xenobiotics. Incubation of bisphenol A and [\(^35\)S] in HepG2 cells culture medium was used as a model for investigating whether the sulfation of environmental estrogens occurs in vitro and sulfated Bisphenol A was found in the culture medium (Suiko et al., 2000). This demonstrated clearly the occurrence of the sulfation of Bisphenol A in HepG2 cells and the release of them out of the cell.

Glucuronidation is a major detoxification pathway in all vertebrates, whereas it is rare in invertebrates (Dutton, 1996). Bisphenol A sulphation by the rat liver UDP glucurondiase has recently has been reported (Yokoto, et al., 1999).

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### REFERENCES


New President

Prof. B.M.A. Oswin Perera

Prof. Oswin Perera graduated as a veterinarian from the University of Ceylon, Peradeniya, with First Class Honours in 1970 and was recruited to the teaching staff of the School of Veterinary Science of the same University. He was awarded a Commonwealth scholarship in 1972 for post graduate studies in the UK, and obtained a PhD in animal reproduction from the University of Glasgow in 1975. Thereafter, he returned to Sri Lanka and served the University of Peradeniya until 1987 in successive capacities as Lecturer, Senior Lecturer, Associate Professor and Head of the Department of Veterinary Clinical Studies. During this period he established field programmes supported by laboratory techniques such as radioimmunoassay to study the reproduction of buffaloes, Zebu cattle and goats, and developed methods for improving their productivity under practical farming conditions. He obtained international and bilateral funding for research and technology transfer projects from FAO/IAEA (Austria), SAREC (Sweden), ODA (UK) and ACIAR (Australia). He spent a year's sabbatical leave from 1983-84 at the University of Nottingham, UK, on a Commonwealth post-doctoral fellowship.

In 1988 Prof. Perera left Sri Lanka to work for the United Nations on two long-term assignments spanning a period of 14 years (1988-1995 and 1997-2004), as Animal Scientist/Regional Expert of the Joint FAO/IAEA Division of the International Atomic Energy Agency, Vienna, Austria. During this period he was engaged in planning, coordinating and implementing FAO/IAEA programmes in animal production and health in Asia, Africa, Eastern Europe and Latin America. His work involved assisting researchers, veterinarians and livestock personnel in developing countries to strengthen their capabilities for using modern laboratory techniques in combination with applied field studies to improve the productivity and health of farm livestock. He planned and conducted over 40 international group activities including training courses, workshops, meetings and seminars, and was presented with a Merit Award by the IAEA in 2004 for outstanding performance.

Prof. Perera returned to Sri Lanka in 2005 and re-joined the University of Peradeniya as Professor of Farm Animal Production and Health. He teaches courses in animal reproduction (Theriogenology) for BVSc and MVSc students, and conducts training workshops for continuing professional development of field veterinarians. His research includes studies on reproductive efficiency in livestock, application of reproductive biotechnologies, farmer-participatory research methods, reproductive biology and behaviour of elephants, and methods for mitigating human-elephant conflict (HEC). He served as Chairman of the Atomic Energy Authority of Sri Lanka from 2006 to 2007 and continues to serve as a member of its Board of Management.

Prof. Perera is a consultant and resource person to a number of national and international organizations in technical, scientific and policy-making capacities, and has visited over 50 countries for short-term assignments, conduct of workshops and training courses, and participation at congresses and symposia. He has published over 45 full-length research and review papers in international journals, 40 full papers in proceedings of scientific meetings, 25 chapters in books and monographs, 55 abstracts of presentations at meetings, and delivered over 15 invited keynote addresses and plenary papers at international meetings.

ASSOCIATION NEWS

61st Executive Committee of the Sri Lanka Veterinary Association (2008/2009)

President: Prof. B.M.A. Oswin Perera
President Elect: Dr. Athula Mahagamage
Vice Presidents: Dr. U.S. Bandara
Dr. D.R.T.G. Ratnayake
Secretary: Dr. Basil Alexander
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Prof. R. Sivakanesan
Prof. R. Sivakanesan
Edit SLVJ:
Ms. Sharma Nathaniels
Company Secretary:
FACULTY OF VETERINARY MEDICINE & ANIMAL SCIENCES

Workshops/Seminars/Training Programmes

A workshop to review the undergraduate training programme in Poultry Health and Management coordinated by Dr. N.U. Horadagoda was held on 03.02.10 at the Department of Veterinary Public Health and Pharmacology.

A workshop on IT based Syndromic Disease Surveillance which was sponsored by Teasdale-Corti Veterinary Public Health Project was held on 02.02.2010 at the IT Centre, University of Peradeniya. Dr. Tim De Jager, HRD Consultant from Canada was the resource person. Dr. Craig Stephen, Dr. Tim De Jager and Jenny Coates were at the Faculty of Veterinary Medicine and Animal Science from 17.01.10 - 23.01.10 as a part of the implementation of the above Project.

Prof. B.M.A.O. Perera (Department of Farm Animal Production & Health) participated as an invited expert at a Working Group Meeting of the Regional Cooperative Agreement for Asia-Pacific (RCA) held at the International Atomic Energy Agency, Vienna, Austria, from 22-26 February 2010, to assist with the Development of RCA Strategic Priorities for the period 2012-2017.

The Faculty of Veterinary Medicine and Animal Science conducted a training workshop on Basic Health Care and Management of Asian Elephants from 16 to 20 January 2010, for technical personnel of the Departments of Wildlife Conservation and National Zoological Gardens.

It was organized under the framework of an EU-Asia Link Programme with Dr. Anil Pushpakumara as Course Director and Prof. Oswin Perera, Dr. Niromi Jayasekera and Dr. Aruna Amarasinghe as resource persons, and was held at the Pinnawela Elephant Orphanage, Uda-Walawe National Park and the Elephant Transit Home.

Dr. A. Dangolla coordinated an intensive course on at the Post Graduate Institute of Science, University of Peradeniya during the period 12-14, March 2010 and also served as a resource person. The participants were teachers from the Faculties of Veterinary Medicine and Animal Science, Medicine, Science and staff from the Ministry of Health. Around 50 of the participants in this course were medical and veterinary graduates.

Dr. A. Dangolla also coordinated an ongoing at the Post Graduate Institute of Science, University of Peradeniya. The Course commenced on 23 January 2010 and is due to end in July 2010. He also serves as a resource person with the other resource personnel from the Faculties of Medicine and Science and also from the Ministry of Health and the Department of Animal Production and Health. Around 50 participants from the government and private sectors are presently following this course.

VETERINARY COUNCIL

Prof. R. Sivakanesan was elected as the President of the Sri Lanka Veterinary Council.

Drs. S.S.P. Silva, T.P. Wijayathilaka and Vipula Dharmawardena were elected as new members to replace the outgoing council members.

A series of meetings were held to review the existing Veterinary Surgeons and Practitioners’ act.
Disasters can be human-made, natural, or biological. Human-made disasters can be fires, environmental contamination, industrial or chemical disasters, war, and terrorism. Natural disasters can be hydrometeorological, such as, floods, storms, hurricanes, landslides, fire and droughts, or geophysical, such as, tsunamis and earthquakes. Epidemics, pandemics, insect infestations, rabies, foot and mouth disease, rinderpest, bird flu, bovine spongiform encephalitis are some biological disasters.

A disaster ("bad star" in Greek) is a tragedy of a natural, biological or human-made hazard that negatively affects society or environment. The UN Office for the Coordination of Humanitarian Affairs (UNOCHA) and the International Federation of Red Cross and Red Crescent Societies (IFRC) define disaster as an event that has occurred unexpectedly with destructive consequences or a calamitous event resulting in loss of life, great human suffering and distress, and large scale material damage (Sprayson, 2006). None of the international agencies have considered the effects of a disaster on animals in their definitions of a disaster (Sprayson, 2006). To some, the lives of animals may not be of equal importance to those of humans, but to dismiss the human-animal dynamic that exists at some level in all countries is to show a lack of understanding as to how most of the world lives.

The decision makers who elevate humans to a higher plane compared with animals are apt to ignore the close interdependence between these species and are not cognisant of the thousands of years of human-animal co-evolution, based on domestication around which entire industries and communities have been developed. Although the needs of animals are often thought to be of secondary importance to that of people in the aftermath of natural disasters or conflicts, the fate of people and animals is often intrinsically linked (Emergency Medicine news & records, Vet Rec, 2005). In many cultures, animals are as intrinsic a part of the family unit as children. The pet dog or cat could be the only surviving member of the household to comfort the victimized surviving humans following a catastrophe. Indeed, there are many examples where domesticated animals have been responsible for the survival of humans by directing assistance to injured people. On September 11, 2001 attack on the World Trade Center (WTC) in the USA, a guide dog named Roselle skillfully guided her blind owner from his office in the WTC down 78 flights of stairs after the building was struck (AVMA Collections 2008). During the tsunami disaster, the mongrel dog Selvakumar in Chinnakalapet, India, has pulled his 7 year old young master out of water to safety. Similar stories of a Doberman who rescued his young master, and a captive elephant who rescued a kid by grabbing the kid from his trunk and on to his back, were reported from the southern province of Sri Lanka.

A single animal may often be responsible for putting food on the table for an extended family. The produce of farm animals could be the only available food source immediately after a massive disaster involving total destruction of agricultural fields. Failing to appreciate the fundamental role that animals play in the lives of people in many countries can create further problems in situations where life is already difficult. Hence, focusing assistance only on humans following a disaster, without considering the needs of the animals upon which the community depends for food, milk, clothing, money and also comfort means that only half the societal needs are met.

The management of a disaster may be approached in phases (Sprayson, 2006, Emergency Medicine news & records, Vet Rec, 2005)

- **Mitigation** to reduce the impact before it strikes, by building defenses and implementing appropriate policies. Mitigation can be the most cost-effective intervention of all.
- **Preparedness** - planning and public education in the face of pending disaster.
- **Response/emergency relief** - the emergency phase within the first days or weeks following the actual event.
- **Recovery** - actions are taken to restore the pre-disaster status quo and social structure. Long-term planning is needed to secure a sustainable future for both people and animals.

Veterinary professionals can play two major roles in emergency management (Sprayson, 2006). Firstly, they can offer traditional rescue and emergency services after disasters. They can join with humanitarian relief efforts and follow up with recovery programmes. In the 2004 Tsunami, veterinary professionals in Sri Lanka joined in the relief efforts for animals, such as, feeding misplaced pets, spay/neuter and vaccination campaigns to mitigate rabies (Figures 1 and 2). Sea turtles, a protected species in Sri Lanka, were at risk of being wiped out after 2004 tsunami when the waves killed adult turtles, and >20,000 hatchlings and eggs each, at the Benthota beach. This made turtles more endangered, since even in ideal conditions only one hatching in a thousand survives to adulthood in the wild. Sri Lankan veterinarians helped to establish turtle watch huts at Kosgoda beach to prevent poaching and encourage turtles to return to our beaches to lay their eggs (Figures 3 and 4), and they also treated injured turtles (Figure 5).
Figure 1. A mobile neutering (sterilization) camp to mitigate a rabies outbreak after the 2004 Tsunami at Kosgoda, Sri Lanka. Veterinarians, veterinary students and support staff assisting the camp funded by the IFAW (International Fund for Animal Welfare)

Figure 2. A veterinarian and a welfare worker in an anti-rabies vaccination campaign to mitigate rabies in the post-tsunami period

Figure 3 and 4. Sri Lankan veterinarians established turtle watch huts in the Kosgoda beach in the southern province to prevent poaching and encourage turtles to return to our beaches to lay their eggs

Figure 5. A veterinarian and veterinary students attending to an injured turtle in the southern beach of Sri Lanka
Secondly, veterinarians can get involved in mitigation and preparedness phases of a disaster. Veterinarians, more than any other professionals, can make a vast contribution to improving the quality of life for both animals and their owners, with our knowledge of the epidemiology of endemic and post-disaster diseases, animal health and husbandry, nutrition and behavior. Due to our role as veterinarians in societies in which we work, we can see how disasters affect all sectors of a community. We are in a unique position to appreciate the true scope of a disaster, as we deal with animals and their owners on a day-to-day basis. Veterinarians can contribute in many ways by combining their professional expertise during an emergency.

The fate of animals in a disaster will have other impacts on the human population in addition to the loss of food sources, transport and livelihood (Emergency Medicine news & records, Vet Rec 2005). Contamination of flood water and water resources with animal carcasses, and zoonotic diseases (those transmitted between humans and animals) can be major hazards in post-disaster situations. Disease outbreaks are more likely in situations, such as overcrowding following population displacement after a disaster.

Rabies (mainly from dogs) and leptospirosis (from rats) are two devastating post-disaster zoonotic diseases. Leptospirosis was assessed by the World Health Organization to be a real risk due to fears that, following the floods, a proliferation of rats would cause pollution of water. It is important to ensure that a second disaster of an infectious disease outbreak do not result in further deaths. Anti-rabies vaccination campaigns for dogs will prevent the spread of rabies, while spaying/neutering of animals in the affected areas will prevent them from producing unwanted litters. The dog eradication programs have poor success rates because catching all stray dogs in an area is never possible as they immediately flee at the first sign of danger, and will populate other areas, where they will continue to breed and pose dangers.

A massive biological disaster of veterinary importance in Sri Lanka was the three rinderpest (cattle plague) outbreaks. Leonard Woolf, who was appointed as assistant government agent to the Southern Province of Sri Lanka in 1908, in his autobiography 'Growing' has described the terrible catastrophe of rinderpest that occurred in the island as early as 1909 (Woolf 1961). He had described the destruction and devastation to domestic cattle and buffaloes which were the most valuable property of the people at that time. This was because the prosperity of the farming districts depended upon cattle and buffaloes for ploughing and threshing, and the bullock carts were the only form of transport available to the villagers. The outbreak had affected the economy negatively as farmers could not plough their paddy fields and the transport of salt in bullock carts to the tea estates in central hills were hindered. The devastation was equally bad in the wild causing death to almost all cloven footed animals, such as, wild buffaloes, sambhur, pigs, etc.

During the Second World War in 1943 rinderpest was reintroduced to Sri Lanka through import of goats from India (Department of Animal Production and Health, 1993). In 1944, large scale vaccination using the caprinized rinderpest vaccine together with slaughtering of animals infected and in-contact led to the eradication of this disease. Thus Sri Lanka was declared free of rinderpest in 1946 until the third outbreak in 1987 which had reportedly commenced when goats were imported from India without adequate quarantine for the Indian Peace Keeping Force (IPKF) (Hettiarachchi and Sritharan, 1996, Vadivelu, 1989). The island-wide average prevalence of this disease reached 3.5% during 1992-1995, with the highest prevalence of 9% in the dry zone (Silva, Dangolla & Allen, 1998). Up to 1993, approximately 40,000 sick animals and 20,000 deaths had been reported, which devastated the farming community in the affected areas. The role of veterinary professionals and other animal health personnel in controlling this disease by mass vaccination campaigns was evident by the diminishing number of outbreaks, with the last fresh case observed in June 1993 (Asian Livestock 1994).

Biological terrorism, described as the use of microorganisms or toxins derived from living organisms to induce death or disease in human beings, animals, or plants (Fitzpatrick and Bender 2000) is another area that needs attention. As terrorism is on the rise throughout the world, the relatively low cost, ease of production, low volume of material needed, and potentially slow onset of action of biological weapons have been described as ideal for use by terrorists. Veterinarians have an important role in the surveillance for potential biological weapon agents through prompt reporting of foreign animal diseases, zoonotic or other reportable diseases, or any unusual disease occurrence. The ability of veterinarians to recognize the signs and symptoms of the likely disease agents is vital. The importance of veterinarians in public health has since become much more widely understood (AVMA Collections 2008).

The Centers for Disease Control and Prevention (CDC, Atlanta) has organized potential agents of bioterrorism into 3 categories (A, B, and C) according to degree of risk to public health (AVMA Collections 2008). Category A agents are those deemed most critical to public health; Categories B and C agents are less likely to cause large-scale illness and death, but still have potential for widespread dissemination and, in some cases, massive economic disruption and human deaths if used for bioterrorism.

**Disaster/Emergency preparedness**

A hazard is a situation which poses a level of threat to life, health, property or environment. Once a hazard becomes 'active', it can create an emergency situation. Mitigation efforts attempt to prevent hazards from developing into disasters altogether, or to reduce the effects of disasters when they occur. The mitigation phase differs from the other phases because it focuses on long-term measures for reducing or eliminating risk.
Emergency management (or disaster management) is the discipline of dealing with and avoiding risks that are involved in preparing for disaster before it occurs, disaster response (e.g. emergency evacuation, quarantine, mass decontamination, etc.), as well as supporting, and rebuilding society after natural or human-made disasters have occurred (Haddow and Bullock, 2004).

The USA was overwhelmed when hurricanes Katrina, Rita, and Wilma struck in succession in 2005, and the disaster response systems proved to be inadequate (AVMA Collections 2008). Response efforts directed at helping animals achieved real success, but they also were confounded by many factors. Some of these factors were common to general rescue efforts surrounding these disasters, while others were specific to animal management and handling. Many factors were involved in failure to evacuate pets in a rapid-onset disaster when owners have only a few hours notice to evacuate. Failures were common in situations where the owners thought the evacuated area was safe for pets, in households with many animals, low pet attachment, and low levels of preparedness. Pet management practices prior to the disaster, such as dogs being kept outdoors or owners not having cat carriers for their cats, also increased the risk of failure. Mitigation of pet evacuation failure should focus on reinforcing responsible pet ownership and strengthening the human-animal bond.

Recent outbreaks involving both animals and humans, such as severe acute respiratory syndrome (SARS), West Nile virus, monkey pox, and avian influenza virus, are reminders of the need to view diseases not as affecting only one species, but globally, integrating animal and human health surveillance, epidemiology and laboratory systems and to creating new strategic partnerships among the global public health community (Parker 2009). Avian Influenza (“Bird flu”) is a rapidly spreading, high mortality disease infecting a wide variety of bird species including domestic poultry, caused by the Avian Influenza A virus HPAI (H5N1). Migratory birds carry the pathogenic form of this same virus. The carnage of poultry in the eastern Indian state of West Bengal in 2007, in which 3.7 million birds were culled, is a striking testament to the failure of the global response to the bird flu crisis (www.grain.org). In a flash, one of the world’s most dynamic areas of poultry farming has been practically ruined, a priceless stock of biodiversity wiped out, and the livelihoods of millions of poor families pushed to the brink. This has been caused not so much by bird flu as by the response to it. During the past 2 years in Sri Lanka, district level preparedness teams have been established with veterinarians, MOHs & PHIs to prevent this disease which devastated the south-east Asian countries. The World Bank has only recently agreed to fund large-scale training programs as a preventive strategy (Wijayathilaka et al., 2007).

Veterinary assistance may also be required to identify safe food sources following an emergency, disaster or catastrophic event. Veterinarians can conduct inspections within impacted communities to ascertain whether or not recommended storage conditions have been maintained to ensure the safe distribution and consumption of food products. In addition, inspection of food products for disaster-related contaminants, rodents, insects and other pests is essential.

Another important risk factor that has impact on human health in the aftermath of a disaster is loss of control of disease vectors. A disaster can distract attention away from measures to control vectors of diseases such as malaria and dengue (Emergency Medicine news & records, Vet Rec 2005).

Pandemic preparedness

A disease epidemic occurs when there are more cases of that disease than normal. A pandemic is a worldwide epidemic of a disease (WHO 2009). An influenza pandemic may occur when a new influenza virus appears against which the human population has no immunity. With the increase in global transport, as well as urbanization and overcrowded conditions in some areas, epidemics due to a new influenza virus are likely to take hold around the world, and become a pandemic faster than before.

WHO definitions of phases of an influenza pandemic provide a global framework in pandemic preparedness (WHO 2009). Pandemics can be either mild or severe in the illness and death they cause, and the severity of a pandemic can change over the course of that pandemic.

Phase 1 - no virus circulating among animals reported to cause infections in humans.
Phase 2 - when an animal influenza virus circulating among domesticated or wild animals is known to have caused infection in humans, and is therefore considered a potential pandemic threat.
Phase 3 - when an animal or human-animal influenza reassortant virus has caused sporadic cases or small clusters of disease in people, but has not resulted in human-to-human transmission sufficient to sustain community-level outbreaks.
Phase 4 - verified human-to-human transmission of an animal or human-animal influenza reassortant virus able to cause community-level outbreaks which marks a significant upwards shift in the risk for a pandemic.
Phase 5 - human-to-human spread of the virus into at least two countries in one WHO region.
Phase 6 (pandemic phase) - community level outbreaks in at least one other country in a different WHO region in addition to the criteria defined in Phase 5. Designation of this phase will indicate that a global pandemic is under way.

Post-peak period, pandemic disease levels in most countries with adequate surveillance will have dropped below peak observed levels.

The 2005 hurricanes in the US, created intense focus and activity on improving local and national preparedness and response planning for biosecurity and the handling of animals in disasters (AVMA Collections 2008). Some of the areas that were taken into consideration were;
A method for decontamination of animals involved in floodwater disasters

Epidemiologic features and risk factors for pet evacuation failure

The veterinarian’s role in preparedness and response.

The veterinarians’ duty of care in response to disasters and food animal emergencies.

Psychologic first aid skills to be learned by field veterinarians to mitigate the effects of depopulation on farm families and rural communities, including the potential for posttraumatic stress disorder. Such skills would enable them to help their communities in the event of livestock depopulation.

Veterinary accreditation and some new imperatives for national preparedness

Public health roles for small animal practitioners - as small animals may serve as sentinels for human disease, surveillance of zoonotic disease is one important role for practitioners.

Biological terrorism against animals and humans - veterinarians should know baseline disease prevalence and incidence rates, as these are prerequisite to any effective surveillance system.

Medical and behavioral surveillance and deployment morbidity among search-and-rescue dogs used in the disaster areas. The number of morbidities found in search-and-rescue dogs used in the rescue operations during October 2001 to June 2002, after the terrorist attack on the World Trade Center and the Pentagon, emphasized the need for veterinary care for these working dogs.

Roselle, the guide dog who skillfully guided her blind owner to safety after the 9/11 attack, had been trained at Guide Dogs for the Blind, where a team of professionals including veterinarians, guide dog instructors, nurses, psychologists, and social workers contributed to her ability to serve when needed.

In Sri Lanka, the SLVA scientific sessions in 2007 reported the successful treatment and recovery of a war casualty dog of the Sri Lanka Army, which suffered serious injuries from a grenade explosion (Weerasingehe, Wijesinghe, Wickramasinghe, Thalagala, de Silva, Dangolla and Silva, 2008). The research sessions of the University of Peradeniya recorded the treatment and recovery of an explosive sniffer police dog intoxicated following ingestion of explosives (TNT, C-4 and plastic explosives) which revealed important clinical information to veterinarians (Wijesinghe, Weerasingehe and Silva 2007).

Veterinarians fill important roles in national biosecurity and preparedness by representing the government in regulatory activities such as animal inspection, control of animal movement, and foreign animal disease surveillance. Although most veterinarians possess expertise in national biosecurity and biological risk assessment, many do not recognize their capabilities or do not realize how their expertise may be applied in the areas of preparedness and response (AVMA Collections 2008). Therefore, specialized groups of veterinarians should be accredited with skills, knowledge, and aptitude and the additional areas of knowledge likely to be expected in the future.

The Overseas Group of the British Veterinary Association (BVA) had appealed veterinary professionals with significant overseas experience to become involved in supporting a veterinary disaster management resource for members of the profession and organizations involved in crisis work overseas (Bowen and Sprayson, 2006). The BVA through individual responses and contacts with their sister organizations (the Commonwealth Veterinary Association (CVA) and World Veterinary Association (WVA), maintain database of details of individual veterinarians and healthcare professionals as ‘point of contact’ in countries throughout the world (BVA Off the Record, 2007). With up-to-date information on current development policies, programs and projects within the country, these contact veterinarians will be in a position to assess in-country needs at a time of crisis and advise interested parties accordingly. Veterinarians who may become involved in the organized response to any disaster or emergency must understand the legal, legislative, and policy issues surrounding disaster preparedness and response. Those involved in the resource, act as advocates for the health and welfare needs of livestock within disaster management work and increase awareness of the role played by animals in a sustainable future for the affected communities.

Recently there had been many natural disasters in other countries where animal welfare organizations have assisted the affected communities and animals. In 2006, the eruption of Tungurahua volcano in Ecuador’s Sangay National Park has showered the local region with ash, contaminating water, crops and animal feed. The ash pollution has killed animals and caused suffering to many survivors (Edwards 2008). Thousands of animals were stranded during severe floods in Bolivia in 2007 and around 13,000 cattle were estimated dead (Edwards 2008). Door-to-door searches and animal rescue operations were implemented after hurricanes Katrina and Rita hit the US Gulf Coast in 2005, where evacuees were forced to leave their pets behind (IFAW Disaster Relief). These hurricanes of 2005 called attention to one of the most important problems associated with natural disasters: that preplanning failed to take into account the human-animal bond (AVMA Collections 2008). Lack of coordination and control of response activities was the most significant problem encountered by emergency relief workers. The American Veterinary Medical Association (AVMA) has recognized that it could facilitate a meeting of all groups involved in animal disasters, allowing those actually involved in relief efforts to identify hindrances to their efforts and
collectively find ways to address those problems. Due to the increasing threats of epidemics, it would be timely for the Sri Lanka Veterinary Council with the Sri Lanka Veterinary Association to organize veterinary accreditation programs on disaster/emergency management with the objective of establishing a veterinary preparedness teams in Sri Lanka.

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Prepartum feeding behavior is an early indicator of subclinical ketosis

Cows diagnosed with subclinical ketosis (SCK) after calving are at increased risk of developing other diseases and compromised reproductive performance. The objective of this study was to determine whether changes in feeding and social behaviors during the transition period were associated with SCK during the week after calving. Feeding behaviors of 101 Holstein dairy cows were monitored from 3 wk before to 3 wk after calving. Ten otherwise healthy animals were identified as having SCK by serum beta-hydroxybutyrate levels >or=1,000 micromol/L taken during wk +1. These animals were matched by parity with 10 healthy animals. During the week before calving and the 2 wk after calving, animals with SCK had lower dry matter intake, had fewer visits to the feeder, and spent less time at the feeder than healthy animals. For every 10-min decrease in average daily time spent at the feeder during the week before calving, the risk of SCK increased by 1.9 times. During the same week, a 1-kg decrease in average daily dry matter intake increased the risk of SCK by 2.2 times. The largest increase in risk of SCK was associated with a 1-kg increase in the change in average daily intake from wk -2 to -1. During the week before calving, animals with SCK initiated fewer displacements at the feed bunk compared with animals that remained healthy after calving. The results of this study provide evidence that time spent feeding, dry matter intake, and social behavior play an important role in transition cow health. These results indicate that special consideration should be given to management and social factors that can negatively affect dry matter intake and feed bunk attendance during the transition period.


The effect of dystocia on the dry matter intake and behavior of Holstein cows

Dairy cows that have a difficult calf delivery (dystocia) are more likely to develop health complications after calving, reducing productivity and welfare. Understanding the behavioral cues of dystocia may facilitate prompt obstetric assistance and reduce the long-term effect of the challenging delivery. The aim of this study was to describe the effects of dystocia on dairy cow behavior during the period around calving and to assess the use of these behaviors as potential indicators of dystocia. Individual dry matter intake, water intake, feeding and drinking time, meal size, standing time, and number of transitions from standing to lying positions (bouts) were recorded during the 48-h period before and after the time of calf delivery for 22 Holstein cows [11 cows with dystocia and 11 cows with unassisted delivery (eutocia)]. Cows with dystocia consumed 1.9 kg less during the 48 h before calving compared with cows with eutocia (14.3 +/- 1.0 vs. 16.2 +/- 1.0 kg, respectively), and this difference increased to 2.6 kg in the 24 h before calving (8.3 +/- 0.7 vs. 10.9 +/- 0.7 kg/d). There were no differences in drinking time between the groups, but cows with dystocia consumed less water 24 h before calving (22.4 +/- 4.4 vs. 36.2 +/- 4.4 kg/d, respectively) and consumed more water during the 24-h period after calving (56.9 +/- 3.1 vs. 48.7 +/- 3.1 kg/d) compared with cows with eutocia. Cows with dystocia transitioned from standing to lying positions more frequently than cows without dystocia beginning 24 h before calving (10.9 +/- 0.7 vs. 8.3 +/- 0.7 bouts/d). Dry matter intake and standing bouts in the 24 h before calving were the most accurate variables in discriminating between cows with and without dystocia, suggesting that cows with dystocia begin to alter their behavior beginning 24 h before calving.


Using gait score, walking speed, and lying behavior to detect hoof lesions in dairy cows

The objective was to determine whether changes in the different components of gait, walking speed, and lying behavior were associated with hoof pathologies in lactating Holstein cows. In experiment 1, 53 cows had their gait scored, their walking speed estimated, and their lying behavior monitored before clinical assessment of the hooves. Multiparous cows with ulcers scored higher than cows without ulcers for overall gait score [numerical rating score (NRS); 3.3 +/- 0.2 vs. 2.8 +/- 0.2], back arch, joint flexion, asymmetric steps, and reluctance to bear weight. Although cows with ulcers did not walk more slowly than cows without ulcers (1.4 m/s), they spent more time lying down (827.8 +/- 29.1 vs. 738.2 +/- 15.5 min/d) because of longer lying bouts (93.3 +/- 5.9 vs. 79.7 +/- 3.4 min). In experiment 2, 47 cows were monitored for hoof health and changes in gait score from wk 4 before to 24 wk after calving. Differences were found after calving between cows that developed an ulcer and cows that did not for NRS (3.1 +/- 0.1 vs. 2.35 +/- 0.1), back arch, joint flexion, asymmetric steps, and reluctance to bear weight. Numerical rating score, back arch, and asymmetric steps were able to discriminate cows with ulcers at least 4 wk before the diagnosis. Cows that developed a sole ulcer had a faster decline in lying time during the periparturient period and a faster increase beginning in wk 2 after calving. The NRS was a more consistent predictor of sole ulcers than lying behavior or speed. The NRS was able to discriminate cows with ulcers across studies at a high intraobserver accuracy and reasonable specificity and was able to predict the presence of ulcers at least 4 wk before diagnosis. Abduction/adduction of the rear legs, head bob, and tracking-up did not consistently discriminate cows with ulcers, and we suggest that these measures are less useful for on farm gait assessment. Compared with the other gait attributes, back arch, joint flexion, asymmetric steps, and reluctance to bear weight best predicted the presence of sole ulcers.

INSTRUCTIONS TO CONTRIBUTORS

The Sri Lanka Veterinary Journal is the official publication of the Sri Lanka Veterinary Association. It is intended for the publication of original works and reviews in the field of veterinary and allied sciences. Articles of clinical interest and research notes too are considered for publication. Submission of a paper implies that the results reported have not been published by the author and is not being considered for publication elsewhere. A section of the journal is devoted to report the activities of the Veterinary profession in Sri Lanka and elsewhere.

General layout: The Journal will consist of two sections. Section A will be devoted to research articles, reviews, short communications and clinical communications. Section B will include updates on new developments in veterinary science, new policy decisions and legislation affecting the animal sector, local disease situation, new drugs, education, annual sessions- scientific and technical matters, abstracts of published articles and correspondence.

Manuscript: Scripts should be type written on one side of the paper with double spacing and with ample margins. The sequence: Title page, Summary, Text, Acknowledgement, References, Tables and Legends. Pages should be numbered consecutively beginning with the title page.

The title page should carry (i) the title of the article, (ii) short running title (not exceeding 40 words typed at the foot, (iii) surname and initials of all the authors, (iv) address where work was carried out, and (v) the name and address of the author for correspondence, typed as a foot note.

Electronic Manuscripts: If accepted for publication the authors will be requested to submit an electronic manuscript stored in 3.5 inch disc in IBM compatible format and an exactly matching print out. The word processing package used should be specified in the covering letter.

Title: This should be brief and consistent with the work reported.

Text: The work should be presented in concise English. Presentation may be divided into sections such as introduction, materials and methods, results and discussion.

The text of the leading articles should be approximately 5000 words. Review articles may be based on detailed study of the literature related to the authors study area where the author has published. The text should not exceed 7500 words including the list of references. Case reports should be a concise account, where detailed investigations have been carried out and contributing to new knowledge. The text should not exceed 1500 words, three figures or tables and 5 pertinent references.

Summary: The summary should state the scope of the work, the principal findings and a brief conclusion. It should appear at the beginning of the text.

Tables: They should be numbered, and each accompanied by a brief and clear title. The tables should be self explanatory. The text should not repeat what the tables already convey.

Illustrations: The original and two copies of each illustration should be submitted. The original must be drawn in Indian ink on white tracing paper, and should not be lettered; copies should be lettered clearly. Illustrations should be larger than the size intended for reproduction, and the percentage reduction should be stated. Computer aided illustrations should be stored in separate files in the disc when submitting.

References: References in the text should be cited thus: Carter (1979) or (Carter, 1979). When reference is made to more than two authors, all the names must be listed at the first instance [eg. Taylor, Walker and Rowe (1979)], and abbreviated, eg. as Taylor et al (1979) in subsequent citing. They should be listed thus: Hagster, I., Grant, R.J., Combs, G.E. and O’kelly, R. (1978). Effects of bambermycin on performance of growing swine. Journal of Animal Science, 47: 1235-1238. No editorial responsibility can be taken for the accuracy of the references.

The original manuscript and two copies must be submitted to the Editor, Sri Lanka Veterinary Journal, Department of Biochemistry, Faculty of Medicine, Peradeniya, Sri Lanka.

The editor has the right to accept or reject an article, and in all matters concerning the journal, the editor’s decision shall be final.