

The transmission and impact of paratuberculosis infection in domestic and wild ruminants

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ABSTRACT: *Mycobacterium avium* subsp. *paratuberculosis* (*M. paratuberculosis*) infects domestic cattle, sheep, goats, deer, camelids and wild ruminants leading to chronic enteritis known as paratuberculosis (Johne's disease). The infection is chronic, progressive and unresponsive to treatment. Most infected animals do not develop clinical disease but may excrete the bacteria. Clinically sick animals suffer emaciation and in some species diarrhoea, followed by eventual death. During the course of the disease, excretion of *M. paratuberculosis* in faeces and milk occurs, and the organism spreads through the blood and lymph vessels of infected animals to multiple internal organs. The infection disseminates to both the female and male reproductive organs. Though *M. paratuberculosis* is not classified as a human pathogen, current opinions on the possible role of this mycobacteria in public health is discussed. This article attempts to review the ways and circumstances by which *M. paratuberculosis* is transmitted within an animal population and the importance of the disease on animal production. Published reports concerning the transmission and epidemiology of the disease are reviewed herein, and preventive and control measures are summarised.

Keywords: Johne's disease; *Mycobacterium avium* subsp. *paratuberculosis*; milk; semen; domestic and wild ruminants

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1. INTRODUCTION

Paratuberculosis, also known as Johne's disease, is a specific infectious granulomatous enteritis of cattle, sheep, goats, deer, camelids and wild ruminants caused by a small, gram-positive, acid-fast and facultative anaerobic intracellular bacterium (Thorel *et al.*, 1990). A very

slow growth which requires incorporation of Mycobactin, a growth factor derived from mycobacteria, in to the culture media for *in vitro* cultivation of the organism is characteristics for *Mycobacterium avium* subsp. *paratuberculosis* (*M. paratuberculosis*) (Merkal and McCullough, 1982; Green *et al.*, 1989; Pavlík *et al.*, 1994b, 1999a). In 1895, Johne and Frothingham described the

Table 1. Hosts of paratuberculosis other than domestic ruminants

Species	Country	References
Fallow deer (<i>Dama dama</i>)	Czech Republic	Pavlik <i>et al.</i> , 1994b, 2000a
	Germany	Von Weber and Gürke, 1992a
	USA	Riemann <i>et al.</i> , 1979; Temple <i>et al.</i> , 1979
Axis deer (<i>Axis axis</i>)	USA	Riemann <i>et al.</i> , 1979
Tule elk (<i>Cervus elaphus nannodes</i>)	USA	Jessup <i>et al.</i> , 1981
European red deer (<i>Cervus elaphus</i>)	Czech Republic	Pavlik <i>et al.</i> , 2000a
	Scotland	Fawcett <i>et al.</i> , 1995
	Ireland	Power <i>et al.</i> , 1993
	New Zealand	De Lisle <i>et al.</i> , 1993a
White-tailed deer (<i>Odocoileus virginianus</i>)	USA	Chiodini and Van Kruiningen, 1983
Roe deer (<i>Capreolus capreolus</i>)	Czech Republic	Pavlik <i>et al.</i> , 2000a
Sika deer (<i>Cervus nippon</i>)	USA	Temple <i>et al.</i> , 1979
Elk (<i>Cervus elaphus</i>)	USA	Manning <i>et al.</i> , 1998
	Canada	Rohonczy <i>et al.</i> , 1996
Moose (<i>Alces alces</i>)	USA	Soltys <i>et al.</i> , 1967
Pudu (<i>Pudu pudu</i>)	Belgium	De Meurichy <i>et al.</i> , 1985
Antelope kudu (<i>Tragelaphus strepsiceros</i>)	Czech Republic	Pavlas <i>et al.</i> , 1997
Feral goats (unspecified)	New Zealand	Ris <i>et al.</i> , 1988
Pygmy goat (<i>Capra hircus</i>)	Germany	Von Weber <i>et al.</i> , 1992b
Rocky Mountain goat (<i>Oreamnos americanus</i>)	USA	Williams <i>et al.</i> , 1979
Capricorn (<i>Ibex ibex</i>)	Germany	Von Weber <i>et al.</i> , 1992b
Capricorn (<i>Capra cylindricornis</i>)	Czech Republic	Pavlik <i>et al.</i> , 1999b
Mouflon (<i>Ovis musimon</i>)	Czech Republic	Pavlik <i>et al.</i> , 2000a
	USA	Boever and Peters, 1974
	Germany	Von Weber <i>et al.</i> , 1992b
Aoudads (<i>Ammotragus lervia</i>)	USA	Boever and Peters, 1974
Bighorn sheep (<i>Ovis canadensis</i>)	USA	Williams <i>et al.</i> , 1979
Cameroon sheep	Germany	Von Weber <i>et al.</i> , 1992b
Jimela topi (<i>Damaliscus lunatus jimela</i>)	USA	Steinberg, 1988
Sicilian ass	USA	Dierckins <i>et al.</i> , 1990
Pygmy ass (<i>Equus asinus</i> form. <i>dom.</i>)	The Netherlands	Van Ulsen, 1970
Alpaca (<i>Lama pacos</i>)	Australia	Ridge <i>et al.</i> , 1995
Bactrian camel (<i>Camelus bactrianus</i>)	USA	Thoen <i>et al.</i> , 1977
Rabbit (<i>Oryctolagus cuniculus</i>)	UK	Greig <i>et al.</i> , 1997, 1999
Fox (<i>Vulpes vulpes</i>)	UK	Beard <i>et al.</i> , 1999
Stoat (<i>Mustela erminea</i>)	UK	Beard <i>et al.</i> , 1999
Stumptail macaques (<i>Macaca arctoides</i>)	USA	McClure <i>et al.</i> , 1987

presence of acid fast bacilli in the intestine of a cow (Johne and Frothingham, 1895). In 1906 John M'Fadyean, suggested the disease be called Johne's disease (M'Fadyean *et al.*, 1912). Twort and Ingram (1912), isolated the causative agent and named it as *M. enteritis chronicae pseudotuberculosis bovis johne*. Since 1923 the disease has been known as Johne's disease or paratuberculosis and recognised as being caused by *M. paratuberculosis* (Bergey *et al.*, 1923). The bacterium shares a common genetic and antigenic homogeneity with members of *M. avium* species: *M. avium* subsp. *avium* and *M. avium* subsp. *silvaticum* (Thorel *et al.*, 1990). The genotype of *M. paratuberculosis* is distinguished from other mycobacteria by the presence of 14 to 18 copies of an insertion element IS900 (Green *et al.*, 1989; Thoresen and Olsaker, 1994; Pavlík *et al.*, 1999a; Bull *et al.*, 2000). *M. paratuberculosis* persists outside the host, in the environment up to one year (Larsen and Miller, 1978). The organism is relatively susceptible to sunlight, drying, high calcium content and high pH of the soil. Continuous contact with urine and faeces reduces the longevity of *M. paratuberculosis* (Jørgensen, 1977; Chiodini *et al.*, 1984a).

The disease occurs in most parts of the world and the prevalence seems to be increasing in some countries. Paratuberculosis is predominant in cattle and sheep in temperate climates with adequate rainfall and ground waters and in some humid, tropical areas. The incidence of paratuberculosis is high in animals kept intensively under environmental and husbandry conditions which are conducive to the spread of the infection (Chiodini *et al.*, 1984a). Paratuberculosis has also been reported in horses and pigs. Experimental infection of pigs caused granulomatous enteritis and lymphadenitis (Larsen *et al.*, 1971; Larsen *et al.*, 1972; Thorel, 1989). Pigs running with affected cattle may develop enlargement of mesenteric lymph nodes from which the causative agent can be isolated, and these may resemble the lesions of tuberculosis. Mice and hamsters are susceptible and are used in experimental work (Rieman *et al.*, 1979). The infection also occurs in different wild life and exotic species (Table 1). Several species of claw-hoofed animals are susceptible to cattle and sheep strains of *M. paratuberculosis* (De Lisle and Collins, 1993; Pavlík *et al.*, 2000a).

There is mounting evidence for a much wider host range of the disease than had been previously recognised, including non-ruminants. Natural infection in macaque monkeys was reported by McClure *et al.* (1987). A strong statistical association was found between paratuberculosis in rabbits and a history of the disease in cattle on affected farms (Greig *et al.*, 1997, 1999). Recent studies have demonstrated the isolation of *M. paratuberculosis* from the tissues of foxes and stoats (Beard *et al.*, 1999). It is not known if these and other species act as a reservoir of infection, perpetuating the cycle of disease on farms.

The major economic losses of paratuberculosis are caused by decreased milk production, increased cow-re-

placement costs and shorter life expectancy of animals (Ott *et al.*, 1999). Cows with subclinical infection frequently have problems of infertility and mastitis (Doyle, 1954; Merkal *et al.*, 1975; Buergelt and Duncan, 1978). In young deer herds, kept under intensive management conditions, clinical paratuberculosis can lead to severe economic losses (Temple *et al.*, 1979; Fawcett *et al.*, 1995). The continuing uncertainty as to, whether or not *M. paratuberculosis* may be factor in the causation of Crohn's disease in humans increases the importance of this disease more than ever.

The aim of this paper was to review concisely the ways and conditions by which the infection spreads in an animal population; put a maximum vigilance on the source of infection, including natural and multi-species reservoirs in wild and prevent possible *M. paratuberculosis* contamination of premises, feeds and water. Though *M. paratuberculosis* is not recognised as a human pathogen and foodstuffs of animal origin derived from infected animals may harbour the organism, the possible public health significant of the disease is also addressed.

2. PATHOGENESIS

Cattle become infected as calves by ingestion of faeces, contaminated milk, feed and water (Merkal, 1984). Following oral ingestion, *M. paratuberculosis* localises in the mucosa of the small intestine, its associated lymph nodes and to a lesser extent in the tonsil and pharyngeal lymph nodes. The primary site of bacterial multiplication is the terminal part of the small intestine and the large intestine. *M. paratuberculosis* is phagocytised by macrophages which in turn proliferate in large numbers and infiltrate the intestinal submucosa resulting in decreased absorption and chronic diarrhoea and malabsorption (Hole, 1953; Gilmour, 1976). Thickening of the wall of the intestine and corrugation of the intestinal epithelium is also prominent (Seitz *et al.*, 1989). Unlike *M. tuberculosis*, *M. paratuberculosis* is highly resistant *in vivo* to most standard anti-tuberculosis drugs. The organism cannot be reliably detected by culture in the laboratory, particularly when present in low abundance or in spheroplast form without a bacillary cell wall (Hope *et al.*, 1996). Different strains of *M. paratuberculosis* from different preferred hosts (Pavlík *et al.*, 1999a; Bauerfeind *et al.*, 1996), range from very slow growing to uncultivable, although methods are improving. The disease in animals demonstrates a wide range of histopathological types, from a pluribacillary (lepromatous) form with millions of typical acid fast bacilli visible in the tissues, to a paucibacillary (tubercloid) form in which *M. paratuberculosis* cannot be seen in the tissues and cannot be detected by culture, but in which there is a chronic granulomatous inflammatory reaction (Chiodini *et al.*, 1984a; Clarke and Little, 1996).

Whitlock and Buergelt (1996), divide infected cattle on the basis of the severity of the clinical signs in to four stages:

1. Silent stage: This stage represents young animals (calves, heifers) to the age of 2 years without any clinical symptom of the disease. At this early stage of the infection animals shed the organism in undetectable level, thus *M. paratuberculosis* could be detected only by tissue cultures or histology examination of the intestine or lymph nodes.

2. Subclinical stage (adult animals without visible clinical signs of paratuberculosis): At this stage, antibodies and cell mediated immune responses (CMI) against *M. paratuberculosis* may be detected. Only 15–25% cases of infected animals are detectable by faecal culture. Most of the animals in this group are often culled due to cases other than paratuberculosis.

3. Clinical paratuberculosis: In several weeks of clinical manifestation of the disease animals loose weight and suffer intermittent diarrhoea. Some animals may recover to the second stage, while the majority progress to the fourth stage with persistent diarrhoea. Faecal culture and serologic examinations of these animals are positive.

4. Advanced stage of clinical paratuberculosis: Oedema of the throat, cachexia and persistent diarrhoea are characteristics of this stage. Most of these animals are sent to emergency slaughter or die of dehydration and cachexia.

3. CLINICAL SIGNS

Calves younger than four months of age are highly susceptible to infection, however clinical signs are not manifested until 2 or more years of age. But unlike calves, wild ruminants infected via milk, commonly manifest clinical signs at 8 to 12 months of age (Manning *et al.*, 1998). Factors such as poor nutrition, concurrent parasitic, viral or bacterial infection, heavy milk production, or transportation stress may influence the rate of development of clinical disease following infection (St-Jean and Jeringan, 1991). The pH of the soil may influence the severity of the clinical signs. Cattle raised on alkaline soils, especially in limestone rich areas, may have a high incidence of infection but little clinical disease. A high prevalence of infection is recorded in the United States of America on acidic soils in contrast to alkaline soils (Kopecky, 1977). In cattle the disease is characterised by chronic and intermittent diarrhoea that is not responsive to treatment, oedema of the throat and abdomen, loss of coat colour, emaciation and eventual death. The chronic nature of the disease entails the late clinical manifestation of paratuberculosis as late as 3 to 5 years after infection (Riemann and Abbas, 1983; Chiodini *et al.*, 1984a).

In sheep, goats, camelids and deer, clinical manifestation of paratuberculosis tends to prevail at younger age than in cattle. Chronic weight loss is the primary clinical sign of paratuberculosis in sheep and goats. Only 10 to 20% of clinical cases present with diarrhoea or clumping of faeces in the advanced stage of the disease (Stelman, 1996). Similar clinical signs occur in wild ruminants but acute paratuberculosis is often observed in young game animals followed by profuse diarrhoea leading to death in 2 weeks (Griffin, 1988).

4. PATHOLOGICO-ANATOMICAL ALTERATIONS

In cattle, lesions are commonly confined to the posterior part of the alimentary tract and its associated lymph nodes. However, in the advanced stage of the disease, lesions may extend from the rectum to duodenum. Thickening of the intestinal wall up to three or four times normal thickness, with corrugation of the mucosa, is also characteristic. The ileocecal junction is always involved, with reddening of the lips of the valve in the early stages to oedema with gross thickening and corrugation later. No ulceration or discontinuity of the mucosal surface occurs. Mesenteric lymph nodes are moderately large and oedematous. Focal necrosis and mineralisation of mesenteric and ileocecal lymph nodes can be present in all species with high prevalence in deer (Williams *et al.*, 1983). The serosal lymphatic vessels of the involved jejunum and ileum are dilated and appeared beaded.

Cattle

The ileum often has a severely thickened, corrugated appearance due to the granulomatous infiltrate. Histologically, *M. paratuberculosis* is found in macrophages which infiltrate into the lamina propria of the intestine (Kubo *et al.*, 1983). The macrophages contain phagocytised mycobacteria (Paliwal and Rehbinder, 1981; Kubo *et al.*, 1983). There is no caseation, calcification, or fibrosis associated with lesions of paratuberculosis in cattle (Hines *et al.*, 1995).

Sheep

Gross intestinal lesions are usually mild, and mucosal thickening and corrugations are not commonly observed (Hines *et al.*, 1995). There may be a diffuse yellow or orange discoloration of segments of intestine due to pigmentation associated with the ovine-caprine strains of *M. paratuberculosis* (Hines *et al.*, 1995; Clarke and Little, 1996).

Goats

In goats, as in some sheep, tubercle-like foci with calcification often develop in the mucosa, submucosa, serosa, lymphatics of the intestine and regional lymph nodes

(Hines *et al.*, 1995). Tafti and Rashid (2000) observed various sized epitheloid macrophage microgranulomas in the paracortical zone and subcapsular sinuses of mesenteric lymph nodes (*sinus histiocytosis*) which contained only a few mycobacteria. A small number of mycobacteria are present in granulomatous lesions of subclinical infection (Sigurdardottir *et al.*, 1999).

Histopathological lesions of *M. paratuberculosis* affected foxes and stoats showed single macrophage-like cells or discrete *granulomata* consisting of small numbers of cells with the appearance of macrophages, in the cortex and paracortex of the mesenteric lymph nodes. Small numbers of intracellular acid-fast bacteria were present within the macrophages, and Langhan's-type multinucleated giant cells, irregularly scattered in the *granulomata*, in all layers of affected intestine (Beard *et al.*, 1999). Granulomatous lesions of the small intestine were similar to that observed in cattle (Hallman and Witter, 1933; Buergelt *et al.*, 1978).

5. DISTRIBUTION OF *M. PARATUBERCULOSIS* IN ORGANS

As the disease advances the infection is disseminated in organs distant from the gastrointestinal system via the blood and lymphatic vessels. *M. paratuberculosis* can be found within macrophages in the *lamina propria* of the

intestine, mesenteric lymph nodes, foetus, mammary gland, and uterus (Merkal, 1984). Phagocytes containing intracellular mycobacteria disseminate infection to other parts of the body and also probably migrate back onto the mucosal surface to shed bacilli (Lugton, 1999). The bacteria are carried by macrophages to other sites particularly the uterus, the foetus, the mammary gland, the testes and semen of bulls. *M. paratuberculosis* was detected in blood, cow's milk, semen of bulls, lymph nodes, different parenchymatous organs like liver, kidney, spleen, lung, uterus, mammary gland, testes, epididymis and bulbourethral gland of infected animals (Table 2).

Isolation of *M. paratuberculosis* from udder tissue (Doyle, 1954; Taylor *et al.*, 1981), supramammary lymph nodes (Alexejeff-Goleff, 1929; Doyle, 1954) and milk (Alexejeff-Goleff, 1929; Doyle, 1954; Smith, 1960; Taylor *et al.*, 1981) of cows with clinical signs of paratuberculosis has been reported. However, isolation of the organism from asymptomatic infected cows outnumbering symptomatic cows in most of paratuberculosis infected herds (Abbas *et al.*, 1983; Merkal, 1984; Whitlock *et al.*, 1986), was not reported until the latest work of Sweeney *et al.* (1992a), which disclosed that supramammary lymph nodes harbour *M. paratuberculosis* and direct shedding of the organism into the milk of asymptomatic infected cows occurs.

Disseminated infection have been documented also in sheep (Carrigan and Seaman, 1990), pygmy ass (Van Ul-

Table 2. Distribution of *M. paratuberculosis* in organs, tissues and secretions of infected cattle

Category	Specimens	Authors
All categories	liver and hepatic Inn.	Collins, 1997; Whitlock <i>et al.</i> , 1997; Pavlík <i>et al.</i> , 2000b
	retropharyngeal Inn	Whitlock <i>et al.</i> , 1997; Pavlík <i>et al.</i> , 2000b
	mandibular Inn.	Whitlock <i>et al.</i> , 1997; Pavlík <i>et al.</i> , 2000b
	spleen	Collins, 1997; Whitlock <i>et al.</i> , 1997; Pavlík <i>et al.</i> , 2000b
	lungs and Inn.	Collins, 1997; Whitlock <i>et al.</i> , 1997; Pavlík <i>et al.</i> , 2000b
	blood	Collins, 1997
	kidneys	Collins, 1997
Cows and heifers	udder	Doyle, 1954; Merkal, 1984; Collins, 1997
	supramammary Inn.	Allexejeff-Goleff, 1929; Whitlock <i>et al.</i> , 1997; Pavlík <i>et al.</i> , 2000b
	milk	Doyle, 1954; Smith, 1960; Taylor <i>et al.</i> , 1981
	uterus	Merkal and McCullough, 1982; Merkal, 1984; Collins, 1997
	foetus	Pearson and McClland, 1955; Merkal, 1984; Seitz <i>et al.</i> , 1989; Sweeney <i>et al.</i> , 1992b
	foetal membranes	Doyle, 1958
	cotyledons	Hole, 1953; Pearson and McClland, 1955; Lawrence, 1956; Doyle, 1958;
	uterine lavage fluid	Rohde <i>et al.</i> , 1990
Bulls	testes	Tunkle and Aleraj, 1965
	bulbourethral gland	Tunkle and Aleraj, 1965
	epididymis	Collins, 1997
	semen	Tunkle and Aleraj, 1965; Larsen and Kopecky, 1970

Explanations: Inn. = lymph nodes.

sen, 1970), and deer (Power *et al.*, 1993; De Lisle *et al.*, 1993; Manning *et al.*, 1998). *M. paratuberculosis* was isolated from uterus and testes in deer (Manning *et al.*, 1998) and mammary gland in goats (Collins *et al.*, 1995).

6. INFECTION BY *M. PARATUBERCULOSIS*

Animals are exposed to paratuberculosis infection in circumstances where there is a favourable condition for the survival of the organism and in management practice permitting a close contact between infected and *M. paratuberculosis* free animals. Cattle infected with *M. paratuberculosis* shed tremendous number of the pathogen in their faeces and the organism remain viable for long time depending on the environmental conditions. This faecal contamination of the environment is the most common sources of infection for cattle (Collins, 1994). Though in different degree of intensity, infection occurs both in pasture and in confinement.

6.1. Prenatal infection

Although the widely known infection of new-born animals occur by oral ingestion of the pathogen, calves may acquire infection in utero (Sweeney, 1996). Many studies have been carried out to solve the issue of whether *M. paratuberculosis* could be acquired in the womb of the dam. Isolation of *M. paratuberculosis* from the uterine mucosa and tissue of the foetus was reported earlier (Table 2). The reproductive organs of cows are reported to be included in the many sites where *M. paratuberculosis* has been isolated. Congenital infection by paratuberculosis was first reported by Alexejeff-Goleff (1929). Similarly Hole (1953), reported isolation of *M. paratuberculosis* from cotyledons of a cow with paratuberculosis. Pearson and McClelland (1955) have examined the foetuses of two cows with paratuberculosis and isolated the organism from both the foetus and the uterine mucosa. Lawrence (1956) isolated *M. paratuberculosis* from 5 (20.8%) of 24 foetuses collected at slaughter. Four of positive foetuses were from cows with clinical paratuberculosis, but the fifth one was from a cow in an infected herd but without clinical signs of the disease. Doyle (1958), examined 24 foetuses and foetal membranes from clinically affected cows, and found *M. paratuberculosis* infection in 9 (37.5%) of the foetuses and 13 (54.2%) of cotyledons.

Kopecky *et al.* (1967), have examined the *endometrium* and the ileocecal valve of 148 culled cows for paratuberculosis and other reasons. Fourteen out of the 18 instances of uterine infection and all isolations of *M. paratuberculosis* from the uterus alone involved cows which had no clinical evidence of paratuberculosis. Many cattle having no signs of paratuberculosis exist in such a

herd and uterine infection frequently occurs sub-clinically. *M. paratuberculosis* was isolated from more than 10% of the examined specimens. Seitz *et al.* (1989) demonstrated that of 407 cows 34 (8.4%) were culture positive for *M. paratuberculosis*; of 34 culture positive cows 9 (26.4%) had foetuses that also were culture positive. These results estimated the risk of foetal infection with *M. paratuberculosis* to be 26.4%.

Culture examination of 5 foetal tissues from 58 cows, which were heavy shedders of *M. paratuberculosis*, revealed that foetal infection was found only in cows that were apparently healthy but heavy faecal shedders (Sweeney *et al.*, 1992b). All five (17.9%) culture-positive foetuses were from 28 cows that were classified as heavy shedders. According to this study, the difference in number of positive foetuses from high shedders vs. low shedders was significant ($P < 0.05$).

6.2. Postnatal infection

As calves are the most susceptible group in a herd, faecal contamination of teats and the presence of mycobacteria in colostrum and milk expose suckling neonatal animals to ingest large doses of the organism. Contaminated pasture, water and feed may also be responsible for infection (Chiodini *et al.*, 1984a). The risk of infection is prominent in loose housing system or at pasture, where calves are frequently in contact with cows shedding the organism via their faeces or milk. Streeter *et al.* (1995) carried out a study in a herd with high prevalence of paratuberculosis infection and isolated *M. paratuberculosis* from the colostrum of 8 (22.2%) cows and from the milk of 3 (8.3%) cows. They also have pointed out that heavy faecal shedders are also more likely to shed the organism in the colostrum than are light faecal shedders. Calves born from paratuberculosis free dams acquire infection in their early age by ingestion of *M. paratuberculosis* via contaminated feed, water and utensils (Chiodini *et al.*, 1984a).

In farmed deer, transmission of *M. paratuberculosis* via milk and colostrum increases the risk of paratuberculosis infection in the herd (Manning *et al.*, 1998). In cervidae species, cross-fostering is common and infected hinds can survive as a nursemaid to kids within the same group. This form of transmission, where infection of young animals born from healthy hinds found infected was confirmed by IS900 RFLP (Restriction Fragment Length Polymorphism) analysis of single strain isolated from two roe deer (Pavlik *et al.*, 2000a).

Faeces

As the main lesions of paratuberculosis occur in the lower part of the small intestine and corresponding lymph nodes, the organism is largely excreted via faeces of infected animals. Apparently healthy individuals of the

population are known to shed substantial amount of *M. paratuberculosis* but with advanced infection, the organism is shed in faeces often at concentrations approaching 10^8 colony forming units (CFU) per gram. The environment of an infected herd may therefore be heavily contaminated. Under field conditions the disease is transmitted principally by the ingestion of feed and water contaminated by the faeces of infected animals (Chiodini *et al.*, 1984a; Rosenberger *et al.*, 1992). Excretion of *M. paratuberculosis* in Merino sheep with multibacillary Johne's disease occurred daily, proving that environmental contamination can be continuous on farms with endemic ovine Johne's disease (Whittington *et al.*, 2000).

Milk and colostrum

Some studies have suggested that as many as 35% of clinically infected cattle (Sweeney *et al.*, 1992b) and 11.6% of asymptomatic carriers (Taylor *et al.*, 1981) have detectable quantities of *M. paratuberculosis* in their milk. Streeter *et al.* (1995) carried out a study in a herd with high prevalence of paratuberculosis infection and isolated *M. paratuberculosis* from the colostrum of 8 cows (22.2%) and from the milk of 3 (8.3%) cows. They also have pointed out that heavy faecal shedders are also more likely to shed the organism in the colostrum than are light faecal shedders.

Afterbirth

Isolation of *M. paratuberculosis* from the endometrium, cotyledons and fetuses increases the probability that *M. paratuberculosis* may be excreted via the afterbirth (Pearson and McClelland, 1955; Lawrence, 1956; Doyle, 1958). As it was stated previously, contamination of the environment by the excretion of infected cows at parturition, especially in a loose housing system, may expose new-born calves to the risk of infection.

Semen

Isolation of *M. paratuberculosis* from the reproductive organs of bulls has been reported (Table 2). As *M. paratuberculosis* has been detected in the genitalia and the semen of infected bulls and survives antibiotics and freezing during semen conservation, intrauterine infection occurs commonly (Larsen and Kopecky, 1970; Larsen *et al.*, 1981). Therefore, infected semen thus may contribute to the association of *M. paratuberculosis* to the new zygote at the early stage of embryonic development rendering the foetus infected.

7. INFECTION IN ADULT ANIMALS

Only a small dose of organisms may be required to establish infection in a new-born calf, and overwhelming age-related resistance by introduction of a large dose of

organisms to an adult cow is probably possible (Collins, 1994; Sweeney, 1996; Toman *et al.*, 1999). The outcome of infection in adults is not well understood but some animals exposed for the first time as adults may develop clinical disease while others develop only a sensibility to Johnin (antigen extracted from *M. paratuberculosis* used for skin testing) for short periods although they may become carriers of the organism without manifesting clinical signs (Larsen *et al.*, 1975).

7.1. Faecal oral route of infection

The primary route of infection in cattle population occur by oral ingestion of *M. paratuberculosis* from contaminated feed and water. In an intensive farming system, where animals are kept indoor, the most common problem is faecal contamination of feed by use of common equipment for faeces and feed handling or feed bunk designs that allow faecal contamination. Although adults are considered refractory to *M. paratuberculosis* infection, a sufficient dose can probably cause infection and disease. In an extensive farming system, usually at pasture, animals concentrate in areas of water, feed and mineral supplements, where close contact of individuals increases the chance of infection.

M. paratuberculosis contamination of feed, water, and soil represents the major risk factor for the spread of the disease in farmed deer and wild ruminants in zoological gardens (Boever and Peters, 1974; Steinberg, 1988; Stehman, 1996; Manning *et al.*, 1998). Free ranging wild ruminants can be infected at pasture, temporarily or previously used by infected cattle (Riemann *et al.*, 1979; Jessup *et al.*, 1981; Pavlík *et al.*, 2000a). Greig *et al.* (1999), demonstrated the concept of inter-species transmission of *M. paratuberculosis* between livestock and rabbits running in pasture. In this case IS900 RFLP analysis was employed to identify the livestock and rabbit isolates of *M. paratuberculosis* (Pavlík *et al.*, 1995, 1999a).

7.2. Artificial insemination

Although bulls are the least in number in a given animal population, they can be significant sources of infection. In grazing herds, they may be mated to cows with susceptible unweaned calves. They also have direct contact with breeding cows by natural mating or indirectly by artificial insemination. Amstutz (1984) has pointed out that the prevalence of paratuberculosis is higher in bulls than cows. *M. paratuberculosis* organism may be incorporated in to the cow via semen collected from a shedder bull or semen contaminated during collection (Larsen and Kopecky, 1970). Faecal contamination of semen by *M. paratuberculosis* has been also reported by

Edmondson *et al.* (1948). Bacterial disease such as paratuberculosis, campylobacteriosis, leptospirosis and other species of bacterial agents residing in the bull's genital tract are infectious and are transmitted between animals via the venereal route, by the use of semen or early embryos in commercial artificial insemination or embryo transfer (Philpott, 1993).

According to the study carried out by Merkal *et al.* (1981a) small number of *M. paratuberculosis* in the semen of a bull might be sufficient to establish hypersensitivity in a recipient cow. Such hypersensitivity conceivably could lead to abortions when such cattle are skin tested with Johnin. Lymphocytic cell infiltration observed in intrauterine *M. paratuberculosis* inoculated cows, in this report, represents an inflammatory response to the bacteria which may be responsible for the decreased fertility (Merkal *et al.*, 1981a).

M. paratuberculosis injected into the mammary gland was transported to the supramammary lymph nodes in five of six cows and to the intestine of one cow. The bacillus caused hypersensitivity to Johnin and stimulated the production of complement-fixing antibodies (Larsen and Miller, 1978).

8. A POSSIBLE ROLE IN CROHN'S DISEASE

Crohn's disease is an inflammatory bowel disease (IBD), the general name for diseases that cause inflammation in the intestines of humans. It is a chronic granulomatous ileocolitis of unknown aetiology. The disease was first diagnosed by Dalziel in 1913 (Dalziel, 1913). The current name of the disease is originated from an article published by Crohn *et al.* in 1932 (Crohn *et al.*, 1932). Because of the unknown aetiology of the disease, many viruses and bacteria have been examined and considered as possible causative agent of the disease. In recent years, there has been an interest in the possible association of paratuberculosis and human Crohn's disease.

In 1901 Thomas K. Daziel, a surgeon at the Western Infirmary in Glasgow, operated on a colleague with chronic inflammation of the intestine. As he was aware of John's disease at that time, he collected other cases and published his observation of "Chronic Interstitial Enteritis" in the British Journal in 1913 (Dalziel, 1913). He wrote that the histological characters of the disease he had described in humans were so similar to John's disease thus justifying a proposition that the disease may be the same. The question Dalziel could not resolve was that he could not see acid fast mycobacteria in the diseased intestine in humans.

The possible etiological association between these two diseases has largely been prompted by the isolation of *M. paratuberculosis* from human patients with Crohn's

disease in the United States, Australia, The Netherlands, and France (Chiodini, 1989). In recent years, there has been an interest in the possible association of paratuberculosis and human Crohn's disease. Of the 3 primary groups currently engaged in the culture of human tissues, *M. paratuberculosis* has been isolated from 20, 33, and 38% of patients with Crohn's disease, but from only 0.8% (1 in 121) of controls (Chiodini, 1992). These human isolates have been shown to be of the bovine-type rather than of ovine-caprine origin (Collins *et al.*, 1990a; Whipple *et al.*, 1990) and indistinguishable from strains isolated from cattle (McFadden *et al.*, 1987; Chiodini, 1990, 1992; Fixa *et al.*, 2000).

The first report on isolation of *M. paratuberculosis* from the tissue of Crohn's disease patients increased the speculation about the possible role of this bacterium in the aetiology of Crohn's disease (Chiodini *et al.*, 1984b). Later subsequent studies demonstrated that the isolates were genetically identical to strains of *M. paratuberculosis* originated from cattle, and were able to develop paratuberculosis after oral administration to infant goats (Van Kruiningen *et al.*, 1986). Similar isolation of the organism from Crohn's disease patients was described by Chiodini (1989), Pavlík *et al.* (1994), and Thompson (1994). *M. paratuberculosis* has also been cultured in the laboratory from humans with chronic inflammation of the intestine of the Crohn's disease type, but in very rare cases (Coloe *et al.*, 1986; Gitnick *et al.*, 1989; Thorel, 1989; Haagsma *et al.*, 1991).

The European Union member states asked the Scientific Committee on Animal Health and Animal Welfare for an opinion on this issue. The report ended with the conclusion that currently available evidence is insufficient to confirm or to disprove that *M. paratuberculosis* is a causative agent of at least some causes of Crohn's disease in man (European Commission, 2000). Development of efficient diagnostic methods that would confirm the incrimination of this bacterium as the causative agent of the disease is highly required and will encourage the establishment of eradication and control programmes of the disease.

8.1. Milk and milk products

M. paratuberculosis is an intracellular pathogen that colonises and multiplies in white blood cells of cows (Chiodini *et al.*, 1984a). Such white blood cells are filled with *M. paratuberculosis*. Since the milk of cattle, like that of all mammals, contains white blood cells, milk from cattle infected with paratuberculosis is certain to contain white blood cells which are infected with *M. paratuberculosis*.

Five independent research groups, in USA (Chiodini and Hermon-Taylor, 1993; Meylan *et al.*, 1996; Sung and

Collins, 1998) Northern Ireland (Grant *et al.*, 1996, 1998), and Australia (Hope *et al.*, 1996) have reported that *M. paratuberculosis* may be capable of surviving commercial milk pasteurisation, and thus may be present in retail milk supply. The Australian study has shown that milk and colostrum taken from cattle with paratuberculosis and treated by the condition of commercial pasteurisation is considered unsafe to feed to new-born calves (Meylan *et al.*, 1996). Live *M. paratuberculosis* has been shown capable of surviving HTST (high temperature short time 71.7°C for 15 seconds) pasteurisation, as conducted by a commercial-scale HTST milk pasteurisation unit in research conducted in Australia (Hope *et al.*, 1996). Therefore people may be exposed to it by the consumption of pasteurised milk (Nauta and Van der Giessen, 1998). Thus, the possible participation of *M. paratuberculosis* in the Crohn's disease is related to consumption of milk from an infected cow.

According to Sung and Collins (1998), *M. paratuberculosis* is more heat resistant than *Listeria monocytogenes*, *Salmonella thyphimurium*, *Coxiella burnetti* and *M. bovis* and thus may be able to survive the conditions of commercial milk pasteurisation. Grant *et al.* (1996), published studies substantiating the higher heat resistance of *M. paratuberculosis* in raw milk than *M. bovis*, which could survive HTST. *M. paratuberculosis* is more robust than tuberculosis, and the risk that is conveyed to human populations in retail milk and in domestic water supplies is high (Hermon-Taylor *et al.*, 2000).

Though *M. paratuberculosis* is proved to survive laboratory simulated HTST pasteurisation, many authors criticise this practice as inadequate to represent commercial pasteurisation technology. The laboratory simulations give rise to much argument, because of the parameters that researchers choose to implement in their experiment. For example some of the laboratory drawback are stated as follows: lack of a homogenizer and a straight holding tube which results in laminar flow of milk particles rather than turbulent flow; artificially high mycobacterial loads used in the experiments; possible differences in the thermosusceptibility of laboratory cultured mycobacteria; and features of the small-scale unit (Hope *et al.*, 1996; Grant *et al.*, 1996; Stabel *et al.*, 1997). Laboratory cultured mycobacteria may have greater thermotolerance compared with *in vivo* mycobacteria (Merkal *et al.*, 1981b).

Stabel (2000), however, demonstrates that high-temperature short-time pasteurisation of milk inoculated with macrophages, containing ingested *M. paratuberculosis*, resulted in no viable *M. paratuberculosis*. Similar conclusion also has been made by Keswani and Frank (1998), that low levels of *M. paratuberculosis*, as might be found in raw milk, will not survive pasteurisation treatment.

Inasmuch as the recovery of *M. paratuberculosis* after commercial pasteurisation is suggested by some authors, the current knowledge about this issue is controversial. These authors seem to agree that *M. paratuberculosis*

survives laboratory conditions simulating pasteurisation, but the studies were not sufficient to conclude that it would survive commercial pasteurisation (IDF, 2001). However, laboratory pasteurisation show that HTST pasteurisation was only completely effective when the number of mycobacteria per litre did not exceed 10 CFU (Grant *et al.*, 1998; Sung and Collins, 1998).

On the contrary the USDA (United States Department of Agriculture) researchers have conducted a research using a laboratory-scale pasteuriser. They found that *M. paratuberculosis* did not survive their simulation of HTST pasteurisation. Nevertheless, the parameters they have chosen for the simulation are criticised by PARA (Paratuberculosis Awareness and Research Association) as being highly controversial (<http://www.crohns.org/foodsafety/dairy.htm>). Some of these criticisms lie on how the bacteria were treated during the simulation.

The solution, however, to this food safety uncertainty is not only to rely upon cooking techniques to kill this pathogenic bacterium, but also to reduce the numbers of *M. paratuberculosis* infected milk and milk products from reaching the food chain in the first place. Moreover, the main concern of veterinarians and public health officials in dealing with this robust micro-organism should not rest on the point whether *M. paratuberculosis* is able to survive the standard milk pasteurisation temperature, but rather on the conditions whether farmers may able to prevent the infection and maintain paratuberculosis free status of their herd.

8.2. Transmission via water and insects

As clinically or sub-clinically infected animals may shed millions of *M. paratuberculosis* organisms in their faeces, it is likely that ground and river waters contaminated with animal waste may be a source of human exposure (Hermon-Taylor and Chir, 1993). Though further epidemiological studies should be required a highly significant correlation was observed between the incidence of Crohn's disease in Cardiff, a city of the coastal plain of South Wales, and the shedding of *M. paratuberculosis* by cattle and sheep grazing on the steep upland (Mayberry and Hitchens, 1978). Although water, meat and milk and their products may harbour *M. paratuberculosis*, the speculation about the casual agent of this disease is continuing. *M. paratuberculosis* was isolated from different diptera species (*Scatophaga* spp., *Lucilia caesar*, *Calliphora vicina*), which sucked faeces of infected animals in stable and pasture and the gastrointestinal content at emergency slaughterhouses (Fischer *et al.*, 2001). Since these insects also suck fruits and vegetables, mechanical and faecal contamination of foodstuffs in house should not be underestimated. Therefore, further study will be required either to rule out or recognise the incrimination of *M. paratuberculosis* as a human pathogen.

9. DIAGNOSIS

The major difficulty encountered in the diagnosis of paratuberculosis is the exact identification of subclinical cases. Infected animals may not show symptoms of the disease for 3 to 5 years after infection and by the time clinical signs are manifested animals have already enough time to contaminate the environment. Moreover, the intracellular and slowly progressive nature of *M. paratuberculosis* complicates the diagnosis process. These are largely responsible for the relatively low sensitivity of the currently available tests for Johne's disease. Bearing in mind the four stages of Johne's infection, current tests generally cannot detect early stage I infection and they fail to detect many of the subclinically infected animals in Stage II. On a herd basis the serological tests may indicate whether or not a herd is infected, and this can be followed up by culture to identify faecal shedders. For this reason, a combination of more specific and sensitive diagnostic methods should improve the accuracy of the test (Whitlock and Buergelt, 1996).

The varieties of currently applied diagnostic methods are stated herein, but the detailed description of each method is beyond the scope of this paper, therefore readers are referred to see indicated references.

9.1. Detection of the immune response

As paratuberculosis first triggers the immune response of the host animal in different stage of the disease, various cellular and humoral responses are observed in the course of the clinical development.

9.1.1. Cell mediated immunity (CMI)

Though various methods for the detection of CMI exist, such as intradermal test (Körmeny, 1988), the lymphocyte transformation test (Buergelt *et al.*, 1977), the migration inhibition test (Bendixen, 1977), and assays for interferon-gama (IFN- γ) production test (Wood *et al.*, 1989), only two of them will be stated here.

Skin testing (ST)

Tests for the delayed hypersensitivity (DTH), commonly referred to as skin tests have been used for many years for diagnosing bovine tuberculosis. For paratuberculosis this test is performed similarly to tuberculin test by intradermal inoculation of an extract of *M. paratuberculosis*. An increase in the thickness of the skin on the site of injection >4 mm within 24 to 72 hours is considered as positive. Nevertheless, this test is not recommended because of lack of specificity and poor correlation with the infectious status of the animal (Chiodini, 1984a; Cocito *et al.*, 1994; Collins, 1996). The sensitivity of the

Johne's skin test is about 54%; specificity, about 79% (Hermel, 1998).

Interferon-gama detection (IFN- γ)

This method is similar to that of skin test except that IFN- γ test is performed in vitro. IFN- γ is released by lymphocytes after their exposure to antigens. Animals that are, or have been infected with *M. paratuberculosis* have cells circulating in their blood that have been "trained" to recognise the antigens of this bacterium and respond by releasing significant amounts of IFN- γ (<http://www.johnes.org/general/diagnosis.html#interferon>). Two assays known as a bioassay (Wood *et al.*, 1989) and sandwich enzyme immunoassay (EIA) have been evaluated (Rothel *et al.*, 1990). Results indicated that the IFN- γ preferred to bioassay. Studies have been performed to attain diagnosis of paratuberculosis in young animals by the detection of IFN- γ (Collins and Zhao 1995; McDonald *et al.*, 1999). However, these results indicated that non specific reactions and uncertain interpretation of assay limited the use of IFN- γ EIA in young animals.

9.1.2. Humoral immune response

Intravital diagnosis of *M. paratuberculosis* infection is difficult because of both the micro-organism's slow growth pattern and the immune response it elicits. In the initial stages of infection, *M. paratuberculosis* induces a cell-mediated response, which keeps the infection confined to the intestinal wall. It doesn't, however, produce antibodies in the bloodstream that serology tests could detect. At stage the animal isn't shedding bacteria, so even a faecal culture wouldn't detect an infected animal. As the infection progresses to clinical disease, that cell-mediated response drops off and a humoral response, which produces antibodies, predominates (Hermel, 1998). Humoral immunity emerges 10 to 17 months after infection (Lepper *et al.*, 1989) thus testing before this age should not be recommended.

Three serological tests to detect serum antibodies of cattle infected with *M. paratuberculosis* are being used in most diagnostic laboratories. The AGID (Agar Gel Immunodiffusion), ELISA (Enzyme-Linked Immunosorbent Assay) and CFT (Complement Fixation Test) are easy to perform though lacking sensitivity.

AGID

The AGID test has a high specificity (> 90%) in cattle with clinical signs compatible with Johne's disease (in late stages III and IV). Infected cattle without clinical signs are less often positive on AGID. The sensitivity is estimated to be 30% in pre-Stage IV infections (Hermel, 1998). The AGID test was among the first serological tests developed for the diagnosis of paratuberculosis. In the first half of the 1990s this test was used as a supplementary method where all animals older than 18 months

tested positive for AGID were subject to faecal culture and consequent culling from the farm (Pavlík *et al.*, 2000c). However this test is considered less sensitive than both the ELISA and the CF (Nielsen *et al.*, 2001).

CFT

The CFT detects complement-fixing antibodies to *M. paratuberculosis* in the blood serum. The specificity of the CFT is considered to be lower than that of both the AGID and ELISA. Moreover, this test is reported to detect antibodies 1 to 5 months later than the ELISA (Ridge *et al.*, 1991). The CFT, which is required by many countries for export or import, is intermediate in sensitivity and specificity to AGID and ELISA. With many false positives and false negatives, the CFT isn't recommended for routine diagnostic use. Antigens used in the assays in different countries vary in composition depending on the method of preparation (Hermel, 1998).

ELISA

The ELISA has been most widely used for screening herds. Detection of infection by ELISA techniques appears to be dependent upon the disease stage of the animal tested. ELISA sensitivity for clinical cases has been reported to be 85%, while the sensitivity is about 15% for subclinical cases (Hermel, 1998). Absorption of serum samples using *M. phlei* is done to remove most non-specific antibodies to related bacteria such as other mycobacteria, *Nocardia asteroides* and other closely related bacteria (Nielsen *et al.*, 2001). ELISA has been most widely used for screening purpose of herds. Most experts on paratuberculosis recommend any animal testing positive for Johne's based on ELISA be confirmed by faecal culture.

9.2. Detection of *M. paratuberculosis*

This method implies to the direct detection of the bacterium that causes the infection. Two culture methods and one genetic method are used in this method:

Conventional culture

Faecal and tissue culture is the most widely used diagnostic test for *M. paratuberculosis* (Nielsen *et al.*, 2001). Standard bacteriological method has been used for almost 100 years and is based on the culture of *M. paratuberculosis* on a media containing a growth factor Mycobactin: HEYM (Herrold's Egg Yolk Media) (Whipple *et al.*, 1991) or modified Löwenstein-Jensen medium are the preferred media used in many diagnostic laboratories (Jørgensen, 1982). Isolation of the organism on solid growth media is recommended by Whitlock and Rosenberger (1990).

The problem associated with this test is that the strain of *M. paratuberculosis* isolated from sheep frequently fail to grow on standard culture media, a long incubation period (5–16 weeks) and moderately expensive cost. The advantage of this method over serological methods (CFT

and AGID) is its high specificity (100%). Merkal (1970), reported that culture will detect infected animals shedding more than 100 CFU/g of faeces, and the reported diagnostic sensitivity of faecal culture is roughly 50% (Shin, 1989).

Radiometric culture (BACTEC)

This method is a radioactive-based detection method adapted from the one used to isolate *M. tuberculosis* in humans. Collins *et al.* (1990b) demonstrated that the BACTEC system, if modified, could also be used to diagnose paratuberculosis. The culture media is commercially available but requires supplementation with additional nutrients to enable the grow of *M. paratuberculosis*. The main advantage of this method over the standard one is that it can detect low numbers of *M. paratuberculosis* and can detect the bacterium faster (in 7 weeks) than standard culture methods. The other advantage is the BACTEC method can grow *M. paratuberculosis* from a wide varieties of animal species, including sheep. Disadvantages are that the BACTEC method is more expensive, requires an instrument to read the culture vials, and involves handling of radioisotopes (Sockett *et al.*, 1992).

DNA probe (non-culture detection)

The application of molecular biology methods as diagnostic tool for identification of paratuberculosis in cattle is currently under development and evaluation. The insertion sequence IS900, discovered in the late 1980s, is the only genetic marker so far used for specific detection of *M. paratuberculosis* (Collins *et al.*, 1989). About 15 to 20 copies of this sequence are integrated into the genome of *M. paratuberculosis* (Green *et al.*, 1989). A DNA test based on the 5'-region of IS900, can specifically distinguish *M. paratuberculosis* from other mycobacteria, including members of the *M. avium* species: *M. a. silvaticum* and *M. a. avium*. (Moss *et al.*, 1991). DNA probes enable detection of *M. paratuberculosis* without having to grow the bacterium, hence are faster (in less than three days). The main disadvantage of the DNA probe is cost. Moreover, the presence of PCR (Polymerase Chain Reaction) inhibitions in clinical specimens (esp. in faecal samples) limited the successful routine use of this diagnostic method on clinical samples.

10. CONTROL AND PREVENTION

M. paratuberculosis is more or less resistant to chemotherapeutic agents *in vitro* and treatment of infected animals is not successful. Although treatment may result in clinical improvement, and in some cases remission, animals continue to shed *M. paratuberculosis* in faeces and, upon withdrawal of chemotherapy, clinical disease recurs (Chiodini, 1991). The very chronic and slow nature of the disease combined with the current international animal movement requirements, stated in the

International Animal Health Code of the OIE (OIE, 2000), recommending certification of a negative herd history of paratuberculosis for the previous five years, may discourage herd owners from joining voluntary control or eradication programmes. Therefore, a veterinarian has to play the most part of the control programme by informing herd owners about the insidious nature of the disease, the major economic losses and the resulting animal marketing sanctions.

The lack of universally accepted and sensitive enough tests to detect infected animals in the earliest stages of the disease is a serious obstacle to eradication of paratuberculosis. Especially infected young animals can easily escape detection (Whitlock and Buergelt, 1996). Although the choice of currently available diagnostic tests is complicated by economic considerations, the best test is the one that detects faecal shedders of *M. paratuberculosis*. These cattle are in more advanced stage of the disease, and more likely to transmit the infection to their calves *in utero* or through their milk (Collins, 1994). Therefore, effective disease control programmes depend on a clear understanding of the sources of infection and the routes of transmission (Greig *et al.*, 1999), and early detection of infected animals, thereby allowing removal of carrier individuals from the herd (St-Jean and Jeringan, 1991; Pavlík *et al.*, 2000c).

Conventional culture, radiometric culture, and a DNA gene probe are techniques available to detect faecal shedders (Hietala, 1992). Nevertheless, the hitherto available diagnostic methods are able to detect *M. paratuberculosis* only after the disease has progressed certain stage of development and animals already started to shed the organism via their faeces. Clinical paratuberculosis is merely the tip of the iceberg in terms of the total number of infected animals on the farm. In US dairy herds Whitlock and Buergelt (1996) found that for every clinically infected animal that was born on the farm, a minimum of 25 other animals are probably infected and less than 30% of those were detected by currently available tests.

Difficulty in controlling the disease is also attributed to its long incubation period (several months to 15 years), chronicity, difficult diagnosis and the resistance of the organism in the barn and environmental conditions (MacIndoe, 1950; Chiodini *et al.*, 1984a).

Paratuberculosis will spread slowly and insidiously within and between herds and flocks or regions unless an effective method of control is established. Prior to establishing paratuberculosis prevention and control programme, a systematic evaluation of the occurrence and the prevalence of the disease in a given herd, district, zone or region is essential. Kennedy and Benedictus (2001) demonstrate a systematic approach to paratuberculosis by the use of surveillance strategy such as:

- notification of suspicion,
- investigation of suspected clinical cases,

- investigation of high-risk herds and flocks,
- movement testing,
- accreditation testing.

In the paratuberculosis control strategy used by these authors, zoning was an important tool in managing animal diseases in general and paratuberculosis in particular. For example Australia formally declared zones for sheep and cattle types of paratuberculosis in 1999 under the provisions of the nationally agreed standards for control of ovine and bovine Johne's disease. Western Australia was declared officially free of both infections on the basis of the disease control history and targeted surveillance (Ellis *et al.*, 1998; Higgs and Hawkins, 1998). Zones with rare or no infection were declared protected zones, while control zones were established in areas where the disease was more common. Although zoning and hence restriction of animal movement may interfere with animal marketing and can be abused as trade barrier, scientifically justified zoning and restrictions are consistent with the International Agreement on the Application of Sanitary and Phytosanitary Measures and on Technical Barriers to Trade (World Trade Organisation, 1995).

10.1. Prevention of new infection

Paratuberculosis control programme is time consuming and economically relatively costly, hence prevention of a herd or flock from new infection is the first option to be adopted. This practice could be achieved by maintaining the disease free status of a herd and stock replacement animals. Maintaining the paratuberculosis free status of a farm by a closed herd system or introduction of cows from a tested negative herd combined with a careful feeding of all cattle is an essential step to reduce the risk of new infection (Allenstein, 1994; Collins, 1994; Stehman and Shulaw, 1996). Kennedy *et al.* (2001), demonstrate the necessary management practice involved in the prevention of new infection to a herd:

- managing a close herd,
- avoiding unplanned introductions from herds of unknown status,
- managing risks associated with grazing land that has been grazed by other herds and susceptible species,
- introducing replacement females from low-risk regions or herd,
- introducing low-risk replacement cattle from other herds,
- managing risks when transporting cattle and at exhibitions and sales through separation and hygiene,
- using artificial insemination and embryo transfer,
- manage water and effluent flows from neighbouring land,
- manage disposal of manure.

10.1.1. Manure and milk management

The predominant source of *M. paratuberculosis* is faeces (manure). In sub-clinically infected cows *M. paratuberculosis* may also be secreted in the milk. Susceptible calves may acquire these bacteria early in life, either through their mothers' milk or contaminated teats and skin or by ingestion of water, grass or hay contaminated with faeces. When possible, young stock animals should be weaned as early as possible and removed to clean pasture without contact with adult manure. For dairy cattle the minimum time for complete separation is the first 6 months of life, the "window" of maximum susceptibility. Pasture rotation and avoidance of overgrazing will decrease contact with faeces on pasture. Livestock comingling with other ruminant species should be avoided if their paratuberculosis status is unknown and water sources must also be kept free of contamination (Stehman and Shulaw, 1996). Fertilising pastures and fodder crops with fresh manure and effluent from other farms is a hazardous practice (Kennedy and Benedictus, 2001).

Allenstein (1994) and Collins (1994) recommend the following steps in preventing infection of new-born calves and young animals at critical stage of susceptibility to paratuberculosis:

- prevent infection in calves (clean calving area, remove the calf immediately from the mother),
- never let a positive cow to calve on the farm,
- use colostrum from negative cows,
- feed milk replacer from clean utensils after colostrum,
- keep all adult manure away from calves,
- care full feeding of all cattle, avoid using manure loaders in feeding.

10.1.2. Culling progeny of infected animals

M. paratuberculosis infection can be transmitted from mother to offspring by contact with the mother's infected faeces, through infected colostrum or milk from the mother, or intrauterine into the foetus before the calf is born (Table 2). Depending on the extent to which manure management and milk/colostrum management recommendations listed above can be implemented, there is a moderate to high probability that offspring born to *M. paratuberculosis*-infected mothers will acquire the infection. Consequently, on a case by case basis, it may be wise to cull offspring born to infected mothers. If not culled, it may take two or more years to determine if the young animal become infected, and this will be time lost in pursuit of control or eradication of the *M. paratuberculosis* in the herd or flock (<http://www.johnes.org/> – Johnes' disease information center).

10.2. Identify and cull infected animals

Where individual animals are of such value that complete disposal of all stock is impracticable for eradication, a "culture and cull" program can reduce the prevalence of infection. Animals are re-tested at 6-month intervals until two consecutive negative herd tests are available (Moyle, 1975). This two faecal culture test performed at 6-month interval supplemented with serological tests has shown an effective result in controlling paratuberculosis in the Czech Republic (Pavlík *et al.*, 2000c). Thoen and Moore (1989), demonstrated that reduced economic loss and increased income of farms with a paratuberculosis control programme was attributed to improved milk production and the increased market value of slaughter animals. The risk of faecal/oral transmission of *M. paratuberculosis* to calves is minimised through concurrent management changes that correct potential avenues for the transmission (Allenstein, 1994; Collins, 1994).

10.3. Movement restriction

Paratuberculosis primarily spreads between premises by the movement of animals, and spreads within premises mainly by direct and indirect contact between adults and young animals. Infected animals may enter herds and flocks as planned introductions or by uncontrolled movements of stray domestic stock or of wild animals. Movements of animals due to auction, for breeding purpose and straying are the potential source of environmental contamination resulting in infection of susceptible animals (Kennedy and Benedictus, 2001). According the study conducted by Pavlík *et al.* (2000a), a heifer from infected herd has escaped and strayed for seven months. Upon recapture the heifer with enteritis and emaciation was proved faecal culture positive for *M. paratuberculosis*. On the following years the same RFLP type was identified from previously non-infected wild ruminants and cattle used to graze on the same pasture contaminated by the faeces of the heifer. Therefore, any intentional or accidental movement of animals among herds in districts, zones and regions should be conducted in accordance with the regulations and epidemiological criteria required by the paratuberculosis control principles.

10.4. Vaccination

Vaccination has been demonstrated to reduce the incidence of clinical disease in cattle, sheep and goats and to reduce or delay the excretion of high concentrations of bacteria (Saxegaard and Fodstad, 1985; Van Schaik *et al.*, 1996; Lopez Cruz *et al.*, 1999).

Vaccination of cattle against paratuberculosis is not routinely recommended. Although it may have served a purpose in the past, recent information indicates it is of limited value in controlling *M. paratuberculosis* infections, causes a false sense of security in owners, is a serious health risk for veterinarians and prevents use of serologic tests in a herd (Collins, 1994). However, vaccination has been used to control paratuberculosis in sheep in Britain (Cranwell, 1993) and Iceland (Sigurdsson, 1960), where the mortality rate due to paratuberculosis was reduced by 93%.

The importance of vaccination is generally considered in sheep and goats. For example, in Spain, New Zealand and in an endemic region in Australia killed vaccine is currently being trailed for use in massively infected sheep flocks suffering high mortality (Kennedy and Benedictus, 2001). In Norway, vaccination of kids at the age of 2 to 4 weeks with an attenuated live vaccine has improved the paratuberculosis control in goats, which was failed after several years of unsuccessful efforts by husbandry measures and the isolation and slaughter of clinically affected animals. The prevalence of infection was reduced from 53% to 1%, based on post-mortem examination. Moreover, infection occurred almost exclusively in goats that had been vaccinated at over four weeks of age, or not vaccinated at all (Saxegaard and Fodstad, 1985).

As herd immunity develops and environmental contamination declines over time, vaccination may significantly reduce the incidence of infection and clinical disease and the associated economic losses. If possible, vaccination should be used with other management procedures to reduce the exposure of susceptible animals (Kennedy and Benedictus, 2001). Nevertheless, vaccinated animals continue to shed mycobacteria, react to serological tests for paratuberculosis (Spangler *et al.*, 1991; Körmeny, 1994). Because these animals respond to the tuberculin used to perform intradermal test against *M. bovis*, permanent identification of these animals will be necessary.

11. CONCLUSIONS

Paratuberculosis remains a challenge for cattle producers and veterinarians. Effective disease control programmes depend on early diagnosis of infection and clear understanding and management of the sources of infection and the routes of transmission. Because paratuberculosis is largely subclinical, the economic importance of the disease is usually downplayed by farm owners. Therefore, veterinarians should provide the most accurate current knowledge about paratuberculosis. Isolating new-born calves from cows and from sources of faecal contamination has promoted the practice of lowering the incidence of paratuberculosis. But this practice, though widely used and showing promising results, cannot pre-

vent the possibility that some individuals may still acquire prenatal infection. Heifers and bull-calves from heavy shedder cows should not be retained as replacement animals in the herd. Equally it is very important to buy bulls, their semen and replacement heifers only from regions/farms which are declared paratuberculosis free. Avoiding any source of infection from manure contaminated water, feed, soil, and a thorough understanding of potential reservoirs in wild is important in developing an effective control programme. Although it is not known whether or not *M. paratuberculosis* causes illness in people, livestock diseases that are transmissible to human beings are currently affecting the confidence of consumers more than ever. Therefore paratuberculosis infection in food animals should be controlled as a precaution.

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