Paratuberculosis

Johne's Disease

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Importance

Paratuberculosis is a chronic mycobacterial disease characterized by irreversible wasting, diarrhea and death from cachexia in ruminants. This disease is caused by the obligate pathogen *Mycobacterium avium* subsp. *paratuberculosis.* Infection generally occurs early in life, and many infected animals become chronic carriers. Unless testing is done, paratuberculosis can exist undetected in a herd for years. Only a few carriers develop overt disease, usually after several years, and the symptoms can be confused with other diseases. Paratuberculosis also causes production losses in asymptomatically infected animals. Subclinical carriers are estimated to produce 15-16% less milk, with losses of 1,300-2,800 pounds of milk per lactation. There is no effective treatment. Unless measures are taken to control or eradicate the organism, the prevalence of infection gradually increases in the herd and greater numbers of animals become clinically ill.

Recently, *M. avium* subsp. *paratuberculosis* has been isolated from many nonruminant species, including both mammals and birds. Little is known about these infections; however, some species could act as reservoirs for paratuberculosis in domesticated ruminants, and some might develop clinical disease. *M. avium* subsp. *paratuberculosis* has also been implicated as a possible cause of Crohn's disease in humans; this connection is still controversial and unproven. Control programs for paratuberculosis have been established in some nations including Australia, Norway, Iceland, Japan, the Netherlands and the United States.

Etiology

Paratuberculosis results from infection by *Mycobacterium avium* subsp. *paratuberculosis*, an acid-fast rod previously known as *Mycobacterium paratuberculosis* and *M. johnei*. *M. avium* subsp. *paratuberculosis* is a member of the *M. avium-Mycobacterium intracellulare* (MAI) complex of organisms.

Some strains of *M. avium* subsp. *paratuberculosis* preferentially infect specific hosts. The two main types, which can be distinguished by restriction fragment length polymorphisms, are the Type II or C strains, found in cattle, and type I or S strains, found in sheep. The C strains have a broad host range including cattle, goats, camelids and both ruminant and nonruminant wildlife. The S strains seem to mainly infect sheep and other small ruminants, but they can also be found in other species including red deer. Cross-species transmission of the S and C strains can occur between sheep and cattle, although this appears to be uncommon. Evidence also exists for a goat-specific strain in Norway and a unique strain in bison.

Species Affected

Paratuberculosis affects domesticated and wild ruminants including cattle, sheep, goats, llamas, alpaca, camels, moose, elk, bighorn sheep, buffalo, deer and reindeer. In addition, *M. avium* subsp. *paratuberculosis* has been isolated from many nonruminant species including rabbits, cats, foxes, stoats, weasels, badgers, bears, raccoons, armadillos, opossums, wood mice and Norway rats. It has also been found in jackdaws, rooks and crows. Disease has been reported in wild rabbits, nonhuman primates and pigs. Horses and dogs can be infected experimentally.

Geographic Distribution

Paratuberculosis can be found worldwide. Only Sweden and some states in Australia are proven to be free of this disease.

Transmission

In ruminants, *M. avium* subsp. *paratuberculosis* is mainly transmitted by the fecal–oral route. Infected animals can shed large numbers of organisms in the feces; this shedding can begin before the onset of clinical signs. Asymptomatic carriers may shed the bacteria intermittently. *M. avium* subsp. *paratuberculosis* has also been isolated from colostrum, milk, udder, and the male and female reproductive tracts. Transmission can occur on fomites, and insects may act as mechanical vectors.

the Center for Food Security & Public Health
Iowa State University®
College of Veterinary Medicine
Iowa State University
Ames, Iowa 50011
Phone: 515.294.7189
Fax: 515.294.8259
cfsph@iastate.edu
www.cfsph.iastate.edu

Institute for International Cooperation in Animal Biologics
Iowa State University
College of Veterinary Medicine
www.cfsph.iastate.edu/IICAB/

Young animals are most susceptible to infection. Animals usually become infected when they nurse from an udder soiled with feces or are housed in contaminated pens. They may also be infected when they drink milk or colostrum. In one study, 3-19% of asymptomatic cows shed *M. avium* subsp. *paratuberculosis* in the milk and 9-36% in the colostrum. Up to 35% of symptomatic cows shed bacteria in the milk. *In utero* infections may also be seen. The estimated risk of fetal infection from culture positive cows is 26%, but it may be much lower in asymptomatic animals that shed small numbers of bacteria. In addition, horizontally transmitted infections have been documented between calves housed in the same pen. The importance of transmission in semen and embryos is unknown.

Little is known about the transmission of *M. avium* subsp. *paratuberculosis* in nonruminant species, but fecal-oral spread is likely to be important. In rabbits, this organism has been isolated from the feces, testes, uterus, placenta, fetuses and milk. Both horizontal and vertical transmission seems to take place in wild rabbit populations. Predation might be a route of transmission to carnivores or omnivores. Genetic analyses and experimental studies suggest that transmission is possible between ruminants and nonruminant species. Wildlife may be able to spread *M. avium* subsp. *paratuberculosis* between farms.

M. avium subsp. *paratuberculosis* is resistant to environmental conditions and can survive on pastures for more than a year. Viable bacteria have also been found for up to a week in bovine urine, and for up to 8-11 months in bovine feces. In one study, this organism survived for as long as nine months in pond, tap or distilled water; in another study, it survived up to 19 months in tap water. Sunlight helps to inactivate *M. avium* subsp. *paratuberculosis*. Some studies also suggest that it is inactivated by drying and alkaline soils; however, soil moisture and pH do not influence survival in other studies. Recent data suggest that *M. avium* subsp. *paratuberculosis* could become dormant for a limited time in the environment.

Incubation Period

The incubation period is usually months or years; periods ranging from 4 months to 15 years have been reported. Calves generally become infected soon after birth but rarely show clinical signs before they are two years old.

Clinical Signs

In cattle, the main symptoms of paratuberculosis are diarrhea and wasting. Most cases are seen in 2 to 6 year old animals. The initial symptoms can be subtle and may be limited to weight loss, decreased milk production or roughening of the hair coat. The diarrhea is usually thick, without blood, mucus or epithelial debris, and may be intermittent at first. Tenesmus is not seen. As the disease progresses, the diarrhea becomes more constant and severe over weeks or months, and intermandibular or ventral edema may occur. The temperature and appetite are usually normal and animals are alert. Paratuberculosis is progressive; affected animals become increasingly emaciated and usually die as the result of dehydration and severe cachexia.

The clinical signs are similar in other ruminants. In sheep and goats, the wool is often damaged and easily shed, and diarrhea is less common than in cattle. In captive deer, paratuberculosis can be rapidly progressive.

The impact of infection on nonruminant hosts is still unknown. Intestinal lesions and/or disease have been reported in rabbits and non–human primates. Histological lesions have been reported in other species including foxes and stoats; however, gross lesions are usually mild in species other than rabbits. Few or no lesions have been reported in wild birds and rodents.

Post Mortem Lesions

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In cattle, the carcass may be thin or emaciated. Dependent edema can sometimes be seen, and fluid may be found in the body cavities. The characteristic lesion is a thickened, often corrugated, wall in the distal small intestine. In more advanced cases, the lesions can extend from the duodenum to the rectum. The mucosa is not ulcerated. Discrete plaques may be seen early in the disease; these plaques can sometimes be detected by holding the intestine up to a light source. The mesenteric lymph nodes and other regional nodes are often enlarged and edematous. Gross lesions are usually absent in subclinical carriers, and may also be absent or minimal in some symptomatic animals. Histologically, the lesions are characterized by diffuse granulomatous enteritis, with the accumulation of epithelioid macrophages and giant cells in the intestinal mucosa and submucosa. Acid-fast organisms may be found inside macrophages.

Similar lesions occur in sheep and goats. The mucosa is often only slightly thickened in these species, but caseated or calcified nodules are sometimes found in the intestines and associated lymph nodes. Some (S) strains of *M. avium* subsp. *paratuberculosis* produce a pigment that stains the intestinal lesions brownish–yellow.

The lesions in other ruminants resemble those found in cattle, sheep and goats. In South American camelids, the gross lesions are most similar to those found in cattle; however, lymph node necrosis and mineralization can also occur. Multiorgan dissemination has been reported in these species. In deer, the lesions resemble those seen in small ruminants.

Limited information is available for nonruminant species. Thickened intestines and enlarged lymph nodes have been reported in rabbits. Few or no gross lesions have been reported in other species of wildlife, even when organisms could be isolated from the intestines and lymphoid tissues.

Morbidity and Mortality

The incidence of paratuberculosis seems to be increasing worldwide. Among ruminants, infections are most common in dairy cattle. Approximately 20-50% of the herds are infected in many dairy-producing countries. In the United States, the herd infection rate for dairy cattle has been reported as 24.2% in the Midwest, 23.5% in the West, 17.2% in the Southeast and 16.1% in the Northeast. The prevalence appears to be lower in beef cattle; 8% of U.S. beef herds were infected in one survey. Up to 31% of all zoos in the U.S. have reported at least one case of paratuberculosis in exotic ruminants.

In an endemic herd, only a minority of the animals develops clinical signs; most animals either eliminate the infection or become asymptomatic carriers. In a newly infected herd, the infection usually spreads for years before the first symptomatic cases appear. If no preventative measures are taken, the number of infected animals in the herd gradually rises, young animals are exposed to increasing doses of bacteria, and clinical cases appear – first in older animals and later, as the exposure level increases, also in younger animals. In herds where the organism is widespread, clinical signs can be seen in second- and first-calf cows, and even springers or bred heifers. The percentage of asymptomatic carriers that develops overt disease is unknown. The mortality rate is approximately 1% in most herds, but up to 50% of the animals may be asymptomatically infected, resulting in losses in production. Subclinical carriers are estimated to produce 15-16% less milk, with a loss of 1,300-2,800 pounds of milk per lactation. Once the symptoms appear, paratuberculosis is progressive and affected animals eventually die.

The importance of wildlife reservoirs for domesticated animals is not known. There is some limited epidemiological evidence of natural transmission from wildlife, including red deer, to cattle and vice versa. Rabbits may serve as reservoirs in the U.K., due to the high rate of excretion of organisms in the feces and the high prevalence of infection. In some parts of the U.K., up to 63% of the rabbits may be infected. Known foci of infection in the U.S. include Rocky mountain bighorn sheep and mountain goats in Colorado, and tule elk in California. Infections in other wild ruminant and nonruminant species have also been reported in the U.S., but the incidence appears to be much lower. A prevalence of 2.7% to 8.3% was reported in wild animals in Wisconsin, and 0% to 6.0% in Georgia. Surveillance in wild deer found *M. avium* subsp. *paratuberculosis* in one of 313 wild deer (0.3%) tested in twelve southeastern states. Six deer (2%) were seropositive by ELISA but seronegative by agar gel immunodiffusion.

Treatment

There is no satisfactory treatment for paratuberculosis. Theoretically, some combinations of antibiotics might be successful; however, long-term treatment would probably be required and it is not likely to be economically feasible. In addition, *M. avium* subsp. *paratuberculosis* is susceptible to relatively few drugs, and the chance of a complete cure is low.

Diagnosis

Clinical

Paratuberculosis can resemble many other diseases. A diagnosis should be confirmed with laboratory tests, particularly on farms where the disease is not known to exist. In herds known to be infected, paratuberculosis is sometimes diagnosed clinically.

Differential diagnosis

The differential diagnosis includes gastrointestinal parasitism, peritonitis, renal amyloidosis, lymphosarcoma, kidney failure, chronic salmonellosis and other chronic infectious diseases, copper deficiency and starvation.

Laboratory tests

Paratuberculosis can be diagnosed with a variety of tests; the choice of test varies with the stage of disease. Early "silent" infections can be detected only by culturing the organisms from postmortem tissues or, rarely, by histopathology. Some subclinical carriers can be identified with serology, delayed–type hypersensitivity (DTH) reactions, polymerase chain reaction (PCR) assays or fecal culture. Bacteria are usually shed intermittently by carriers. Clinical cases can be diagnosed by culturing *M. avium* subsp. *paratuberculosis* from the feces or tissues, or by demonstrating the organism with microscopy, DNA probes or PCR. Pathology, and histopathology can also be used in clinical cases, and serology may be useful in some animals.

Microscopy

Ziehl–Neelsen stains can be used to detect *M. avium* subsp. *paratuberculosis* in the feces; clumps of small, strongly acid-fast bacilli are diagnostic. Organisms may also be found in smears from the intestinal mucosa or the cut surfaces of lymph nodes. Organisms may be sparse or absent in some infected animals.

Culture

Bacteria can be cultured from the feces, thickened areas of the intestinal wall, and ileal, mesenteric and ileocecal lymph nodes. Suitable media include Herrold's egg yolk medium, modified Dubos's medium and Middlebrook 7H9, 7H10 and 7H11 media; mycobactin is necessary for bacterial growth. *M. avium* subsp. *paratuberculosis* grows slowly; colonies may take 5 to 14 weeks to appear. On Herrold's medium, the colonies are initially very small, colorless, hemispherical and translucent, with round, even margins and a smooth, glistening surface. With time, the colonies become larger, more opaque, rough and mammilate. Sheep strains are reported to be smooth, uniform and mostly pigmented. Some strains, particularly some sheep strains, can be difficult to grow. Radiometric culture can detect *M. avium*

subsp. paratuberculosis more rapidly than standard culture methods. This technique may be able to detect some isolates that are more difficult to grow.

Serology and tests for cell–mediated immunity

Humoral immunity usually develops 10 to 17 months after infection. Serology can be used for the presumptive identification of infected animals, as well as to estimate the prevalence of infection in a herd or confirm paratuberculosis in animals with clinical signs. Animals that have cleared the infection can be seropositive. A variety of serological tests are available, including complement fixation, enzyme-linked immunosorbent assays (ELISAs) and agar gel immunodiffusion. A milk ELISA has recently been licensed in the U.S.

Intradermal testing with johnin or avian purified protein derivative tuberculin can detect delayed-type hypersensitivity (DTH) reactions to *M. avium* subsp. *paratuberculosis*; however, this test is insensitive and nonspecific reactions are common DTH reactions may diminish or disappear as the disease progresses. Exposure to other mycobacteria, including environmental saprophytes, can result in false positives. *In vitro* tests that detect cell-mediated immunity to *M. avium* subsp. *paratuberculosis* include a gamma interferon assay and a lymphocyte transformation test.

Both antibody levels and cell-mediated immunity can diminish or be negative in advanced disease.

PCR and DNA probes

PCR and DNA probes can detect *M. avium* subsp. *paratuberculosis* and distinguish it from other species and subspecies of mycobacteria. A test kit is available for fecal samples in some countries. Pulsed-field electrophoresis and restriction fragment length polymorphisms have also been used to distinguish C strains from S strains.

Samples to collect

Although the link is speculative, veterinarians collecting samples should keep in mind that this organism may be linked to Crohn's disease.

M. avium subsp. *paratuberculosis* can be isolated from the feces, mesenteric, ileal and ileocecal lymph nodes, and thickened areas of the intestinal wall. Smears may be taken from the intestinal mucosa or cut surfaces of the lymph nodes for microscopic examination. Multiple samples of the intestinal wall and mesenteric lymph nodes should be collected into fixative for histology. Serum samples may be taken for serology. Fresh, frozen or DHIA-preserved milk samples can be tested with the milk ELISA.

Biopsies of full-thickness sections of ileum and regional lymph nodes may occasionally be useful in very valuable animals.

Recommended actions if paratuberculosis is suspected

Notification of authorities

Paratuberculosis is a reportable disease in many states; state regulations should be consulted for more specific guidelines. Federal regulations prohibit culture–positive or DNA test-positive animals from being moved across state lines except to slaughter.

Federal: Area Veterinarians in Charge (AVIC):
www.aphis.usda.gov/animal_health/area_offices/

State Veterinarians:
www.usaha.org/Portals/6/StateAnimalHealthOfficials.pdf

Control

M. avium subsp. *paratuberculosis* is usually introduced into a herd in an infected animal, and persists in breeding stock. Farmers with uninfected herds should buy replacement animals from test-negative herds with good records and management practices. In the U.S., the voluntary Johne's Disease Herd Status Program identifies cattle herds at low risk for this disease. Herds that have been free of paratuberculosis for the last five years may also be an option. Cross-species transmission of the S and C strains can occur, but seems to be relatively uncommon. Therefore, the greatest risk of infection for cattle appears to be from other cattle, and for sheep from other sheep. All animals should be quarantined and tested before adding them to the herd. Live attenuated vaccines can decrease the severity of symptoms and the bacterial load but do not prevent infection or the shedding of bacteria. In many countries, vaccine use is restricted.

Once paratuberculosis has entered the herd, the basic control techniques are 1) to prevent young animals from becoming infected by decreasing their exposure to infectious manure, colostrum and milk and 2) to decrease exposure in the herd by separating infected animals and culling them as soon as it is economically feasible. In surveillance programs, the herd is re-tested periodically, and test-positive animals are separated from the rest of the herd and/or culled. Symptomatic animals and heavy shedders, in particular, should be isolated and culled as soon as possible. Calves from infected dams should also be considered for removal, as intrauterine infections are possible.

Although test and removal procedures can reduce the prevalence of infection, good management practices are also needed to reduce transmission within the herd. Ruminants should be born in clean areas free of manure. In a dairy herd, cows should calve in clean, dedicated maternity pens and the calves should be reared separately from the adult herd for at least the first year. Dairy calves should be fed colostrum collected from negative dams with thoroughly cleansed udders, then fed milk replacer or pasteurized milk. (Some studies suggest this organism can

sometimes survive pasteurization, and milk replacer is the safer source.) Similar measures may be used in beef cattle, small ruminants or other species, to the extent that it is practical. Pooled milk and colostrum should be avoided in all species.

Good manure management and disposal techniques are also important. Manure build-up should be prevented, and surfaces should be kept clean. Food and water troughs may be elevated to reduce contamination. Piped water is less likely to be contaminated than ponds. As much as possible, young animals should be prevented from contacting manure from adults. Contaminated pastures should be tilled and reseeded, or used for animals that will not become herd replacements. Because wildlife can also be infected, they should be kept from contact with livestock. Potential routes of transmission from wildlife to livestock include fecal contamination of pastures, water supplies and grain or hay.

M. avium subsp. *paratuberculosis* is resistant to most disinfectants. Contaminated surfaces should be thoroughly cleaned with soap and water, followed by "tuboricidal" disinfectants if needed. This organism may survive drinking water treatments, including chlorination. It also seems to survive pasteurization in some cases.

Public Health

Paratuberculosis vaccines can cause severe local reactions including sloughing of tissues, chronic synovitis and tendonitis, if they are accidentally injected into humans. Some cases may require surgery.

Limited data suggest that *M. avium* subsp. *paratuberculosis* may be involved in Crohn's disease, a chronic enteritis of humans. Crohn's disease is characterized by periods of malaise, abdominal pain, chronic weight loss and diarrhea, with remissions and relapses. The disease often begins between the ages of 16 and 25 years, and persists lifelong. There is no cure. The cause of Crohn's disease is unknown; however, it may be the result of several interacting factors including a genetic predisposition, an abnormal immune response, and environmental factors including responses to intestinal microorganisms. *M. avium* subsp. *paratuberculosis* can be found in some patients; however, isolation is rare and studies to date have not been able to determine whether this organism has a causative role or is simply an "innocent bystander" that can grow in the inflamed intestinal wall.

Internet Resources

Diagnosis and Control of Johne's Disease.
National Academies Press
http://www.nap.edu/books/0309086116/html

International Association for Paratuberculosis
http://www.paratuberculosis.org/

The Merck Veterinary Manual
http://www.merckvetmanual.com/mvm/index.jsp

University of Wisconsin School of Veterinary Medicine
Johne's Information Center
http://www.johnes.org/

World Organization for Animal Health (OIE)
http://www.oie.int

OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals
http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/

OIE Terrestrial Animal Health Code
http://www.oie.int/international-standard-setting/terrestrial-code/access-online/

References

Antel BioSystems Inc. Review of technical studies: Johne's milk ELISA. 4 p. Available at: http://www.antelbio.com/Testing/Milk.pdf. Accessed 6 Apr 2007.

Antel BioSystems Inc. Press release: Parachek® is the first USDA approved Johne's disease test for bovine milk. February 13, 2007. Available at: http://www.antelbio.com/Press_Room/Parachek%20is%20the%20first%20USDA%20approved%20Johnes%20disease%20test%20for%20bovine%20milk.htm. Accessed 6 Apr 2007.

Beard, P. M., M. J. Daniels, D. Henderson, A. Pirie, K. Rudge, D. Buxton, S. Rhind, A. Greig, M. R. Hutchings, I. McKendrick, K. Stevenson, and J. M. Sharp. 2001. Paratuberculosis infection of nonruminant wildlife in Scotland. J Clin Microbiol. 39:1517-1521.

Beard, P. M., K. Stevenson, A. Pirie, K. Rudge, D. Buxton, S. M. Rhind, M. C. Sinclair, L. A. Wildblood, D. G. Jones, and J. M. Sharp. 2001. Experimental paratuberculosis in calves following inoculation with a rabbit isolate of *Mycobacterium avium* subsp. *paratuberculosis*. J Clin Microbiol. 39:3080-3084.

Collins DM, De Zoete M, Cavaignac SM. *Mycobacterium avium* subsp. *paratuberculosis* strains from cattle and sheep can be distinguished by a PCR test based on a novel DNA sequence difference. J Clin Microbiol. 2002;40:4760-4762.

Collins M, Manning E. Johne's information center [online]. The University of Wisconsin–School of Veterinary Medicine;2007. Available at: http://www.johnes.org. Accessed 9 Apr 2007.

Cook WE, Cornish TE, Shideler S, Lasley B, Collins MT. Radiometric culture of *Mycobacterium avium paratuberculosis* from feces of tule elk. J Wildl Dis. 1997;33: 635-637.

Corn JL, Manning EJ, Sreevatsan S, Fischer JR. Isolation of *Mycobacterium avium* subsp. *paratuberculosis* from free-ranging birds and mammals on livestock premises. Appl Environ Microbiol. 2005;71:6963-6967.

Crawford GC, Ziccardi MH, Gonzales BJ, Woods LM, Fischer JK, Manning EJ, Mazet JA. *Mycobacterium avium* subspecies *paratuberculosis* and *Mycobacterium avium* subsp. *avium* infections in a tule elk (*Cervus elaphus nannodes*) herd. J Wildl Dis. 2006;42:715-723.

Daniels MJ, Hutchings MR, Beard PM, Henderson D, Greig A, Stevenson K, Sharp JM. Do non-ruminant wildlife pose a risk of paratuberculosis to domestic livestock and vice versa in Scotland? J Wildl Dis. 2003; 39:10-15.

Davidson WR, Manning EJ, Nettles VF. Culture and serologic survey for *Mycobacterium avium* subsp. *paratuberculosis* infection among southeastern white-tailed deer (*Odocoileus virginianus*). J Wildl Dis. 2004;40:301-306.

Dohmann K, Strommenger B, Stevenson K, de Juan L, Stratmann J, Kapur V, Bull TJ, Gerlach GF. Characterization of genetic differences between *Mycobacterium avium* subsp. *paratuberculosis* type I and type II isolates. J Clin Microbiol. 2003;41:5215-5223.

Grant IR, Hitchings EI, McCartney A, Ferguson F, Rowe MT. Effect of commercial-scale high-temperature, short-time pasteurization on the viability of *Mycobacterium paratuberculosis* in naturally infected cows' milk. Appl Environ Microbiol. 2002;68:602-607.

Greig A, Stevenson K, Henderson D, Perez V, Hughes V, Pavlik I, Hines ME, McKendrick I, Sharp JM. Epidemiological study of paratuberculosis in wild rabbits in Scotland. J Clin Microbiol. 1999; 37:1746-1751.

Greig A, Stevenson K, Perez V, Pirie AA, Grant JM, Sharp JM. Paratuberculosis in wild rabbits (*Oryctolagus cuniculus*). Vet Rec. 1997;140:141-143.

Hendrick SH, Kelton DF, Leslie KE, Lissemore KD, Archambault M, Duffield TF. Effect of paratuberculosis on culling, milk production, and milk quality in dairy herds. J Am Vet Med Assoc. 2005;227:1302-1308.

Judge J, Kyriazakis I, Greig A, Davidson RS, Hutchings MR. Routes of intraspecies transmission of *Mycobacterium avium* subsp. *paratuberculosis* in rabbits (*Oryctolagus cuniculus*): a field study. Appl Environ Microbiol. 2006;72:398-403.

Kahn CM, Line S, editors. The Merck veterinary manual [online]. Whitehouse Station, NJ: Merck and Co; 2003. Paratuberculosis. Available at: http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/55900.htm. Accessed 5 Apr 2007.

Kopecna M, Ondrus S, Literak I, Klimes J, Horvathova A, Moravkova M, Bartos M, Trcka I, Pavlik I. Detection of *Mycobacterium avium* subsp. *paratuberculosis* in two brown bears in the central European Carpathians. J Wildl Dis. 2006;42:691-695.

Mackintosh CG, Labes RE, Clark RG, de Lisle GW, Griffin JF. Experimental infections in young red deer (*Cervus elaphus*) with a bovine and an ovine strain of *Mycobacterium avium* subsp *paratuberculosis*. N Z Vet J. 2007;55:23-29.

Manning EJB, Kucera TE, Gates NB, Woods LM, Fallon-McKnight M. Testing for *Mycobacterium avium* subsp. *paratuberculosis* infection in asymptomatic free-ranging tule elk from an infected herd. J Wildl Dis. 2003;39:323-8.

Motiwala AS, Amonsin A, Strother M, Manning EJ, Kapur V, Sreevatsan S. Molecular epidemiology of *Mycobacterium avium* subsp. *paratuberculosis* isolates recovered from wild animal species. J Clin Microbiol. 2004;42:1703-1712.

Palmer MV, Stoffregen WC, Carpenter JG, Stabel JR. Isolation of *Mycobacterium avium* subsp *paratuberculosis* (Map) from feral cats on a dairy farm with Map-infected cattle. J Wildl Dis. 2005;41:629-635.

Quist CF, Nettles VF, Manning EJB, Hall DG, Gaydos JK, Wilmers TJ, Roper RR. Paratuberculosis in Key deer (*Odocoileus virginianus clavium*). J Wildl Dis. 2002; 38: 729-737.

Rideout BA, Brown ST, Davis WC, Giannella RA, Huestan WD, Hutchinson LJ. Diagnosis and control of Johne's disease [online]. Washington, DC: National Academies Press; 2003. Available at: http://www.nap.edu/books/0309086116/html. Accessed 6 Apr 2007.

Rossiter CA, Hutchinson LJ. Hansen D, Whitlock RH. Prevention and control of Johne's disease in dairy herds. A workbook for veterinarians and producers [online]. 1st ed. United States Animal Health Association National Johne's Working Group (NJWG) Subcommittee on Education Prevention and Control of Johne's Disease in Dairy Herds; 2002 March. Available at: http://www.usaha.org/njwg/jddairym.html.* Accessed 5 Sept 2003.

Rubery ED (Judge Institute of Management, University of Cambridge, UK). A Review of the evidence for a link between exposure to Mycobacterium paratuberculosis (MAP) and Crohn's disease (CD) in humans. A report for the Food Standards Agency [online]. Food Standards Agency, United Kingdom; 2002 Jan 22. 52 p. Available at: http://www.foodstandards.gov.uk/multimedia/pdfs/mapcrohnreport.pdf. Accessed 13 Sept 2003.

United States Animal Health Association National Johne's Working Group [USAHA NJWG] Subcommittee on Education. Prevention and control of Johne's disease in dairy cattle. USAHA NJWG; 2002 March. Available at: http://www.usaha.org/njwg/jddairy.html.* Accessed 6 Sept 2003.

U.S. Department of Agriculture, Animal and Plant Health Inspection Service [USDA APHIS]. Uniform program standards for the voluntary bovine Johne's disease control program. USDA APHIS; 2002 Apr. APHIS 91-45-014. 25 p. Available at: http://www.aphis.usda.gov/vs/nahps/johnes/johnes-umr.pdf. Accessed 2 Apr 2007.

U.S. Department of Agriculture, Animal and Plant Health Inspection Service [USDA APHIS]. Preventing introduction of Johne's disease [online]. USDA APHIS; 2007 April. Available at: http://www.aphis.usda.gov/animal_health/animal_diseases/johnes/prevent-intro.shtml#prevent. Accessed 5 Apr 2007.

van Roermund HJ, Bakker D, Willemsen PT, de Jong MC. Horizontal transmission of *Mycobacterium avium* subsp. *paratuberculosis* in cattle in an experimental setting: Calves can transmit the infection to other calves. Vet Microbiol. 2007 Jan 23; [Epub ahead of print].

Whittington RJ, Marshall DJ, Nicholls PJ, Marsh IB, Reddacliff LA. Survival and dormancy of *Mycobacterium avium* subsp. *paratuberculosis* in the environment. Appl Environ Microbiol. 2004;70:2989-3004.

World Organization for Animal Health [OIE]. Manual of diagnostic tests and vaccines [online]. Paris: OIE; 2004. Paratuberculosis. Available at: http://www.oie.int/eng/normes/mmanual/A_00045.htm. Accessed 25 Mar 2007.

*Link defunct as of 2007