Bacterial Diseases of the Ruminant Alimentary Tract

ACTINOMYCOSIS (LUMPY JAW)

SYNOPSIS

Etiology *Actinomyces bovis,* normal inhabitant of the ruminant oral cavity.

Epidemiology Common but sporadic disease from infection through wounds to the buccal mucosa by feed or through dental alveoli.

Clinical findings Initially painless, hard, immovable bony swelling on mandible or maxilla. Eventually discharge of small amounts of pus through one or more openings in skin.

Clinical pathology Presence of "club" colonies containing gram-positive filaments.

Diagnostic confirmation Isolation of organism.

Treatment and control Surgical debridement. Iodides and/or antimicrobial orally or parenterally.

ETIOLOGY

Actinomyces bovis is the primary cause, but other bacteria may be present in extensive lesions including non-*bovis Actinomyces* spp.

EPIDEMIOLOGY

The disease is sporadic but common in cattle. Occasional cases occur in pigs, horses, goats, dogs, and humans. Although actinomycosis occurs only sporadically, it is important because of its widespread occurrence and poor response to treatment. It is recorded in most countries of the world.

A. bovis is a common inhabitant of the bovine mouth and infection is presumed to occur through wounds to the **buccal mucosa** caused by sharp pieces of feed or foreign material. Infection may also occur through **dental alveoli** and may account for the more common occurrence of the disease in young cattle when the teeth are erupting. Infection of the **alimentary tract** wall is probably related to laceration by sharp foreign bodies.

PATHOGENESIS

An initial trauma of the oral mucosa or gingiva caused by small but sharp penetrating feed particles creates a portal of entry for the causative agent. The ensuing infection causes periostitis and osteomyelitis.[1] In the jawbones a rarefying **pyelogranulomatous osteomyelitis** is produced.

The effects on the animal are purely physical. Involvement of the jaw causes interference with prehension and mastication, and when the alimentary tract is involved there is physical interference with ruminal movement and digestion, both resulting in partial starvation. Rarely, localization occurs in other organs, caused apparently by hematogenous spread from these primary lesions.

CLINICAL FINDINGS

Cattle

Actinomycosis of the **jaw** commences as a painless, **bony swelling** that appears on the mandible or maxilla, usually at the level of the **central molar teeth**. The enlargement may be diffuse or discrete and in the case of the mandible may appear only as a thickening of the lower edge of the bone with most of the enlargement in the intermandibular space. Such lesions are often not detected until they are too extensive for treatment to be effective.

The more common, discrete lesions on the lateral surfaces of the bones are more readily observed. Some lesions enlarge rapidly within a few weeks, others slowly over a period of months. The swellings are very **hard, immovable** and, in the later stages, painful to the touch. They usually break through the skin and discharge through one or more openings (Fig. 8-25).

The discharge of pus is small in amount and consists of sticky, honey-like fluid containing minute, hard, yellow-white granules. There is a tendency for the sinuses to heal and for fresh ones to develop periodically. Teeth embedded in the affected bone become malaligned and painful and cause difficult mastication with consequent loss of condition. In severe cases, spread to contiguous soft tissues may be extensive and involve the muscles and fascia of the throat. Excessive swelling of the **maxilla** may cause dyspnea. Involvement of the local lymph nodes does not occur. Eventually the animal becomes so emaciated that destruction is necessary, although the time required to reach this stage varies from several months to a year or more.

The most common form of actinomycosis of soft tissues is involvement of the **esophageal groove** region, with spread to the lower esophagus and the anterior wall of the reticulum. The syndrome is one of **impaired digestion**. There is periodic diarrhea with the passage of undigested food material, chronic bloat, and allotriophagia. Less common lesions of soft tissue include **orchitis** in bulls, the **trachea** causing partial obstruction, and abscess in the brain or lungs.

Pigs

Rare cases of wasting occur because of visceral actinomycosis but extensive granulomatous lesions on the skin, particularly over the **udder**, are more common.

CLINICAL PATHOLOGY

Smears of the discharging pus stained with Gram stain provide an effective simple method of confirming the diagnosis. In nondraining lesions tissue core biopsies or fluid aspirates provide suitable material to identify the causative agent. Gram-positive filamentous rods can be identified by staining the crushed yellow granules found in pus.

Fig. 8-25 **A,** Polled Hereford cow with actinomycosis of the left mandible. **B,** Brown Swiss cow with actinomycosis of the left mandible with a draining tract.

NECROPSY FINDINGS

Rarefaction of the bone and the presence of loculi and sinuses containing thin, whey-like pus with small, gritty granules are usual. An extensive fibrous tissue reaction around the lesion is constant, and there may be contiguous spread to surrounding soft tissues. The

presence of **"club" colonies** containing the typical, thread-like bacteria is characteristic of the disease. These formations may be seen on microscopic examination of smears made from crushed granules in pus or on histologic examination of section.

Granulomatous lesions containing pockets of pus may be found in the esophageal groove, the lower esophagus, and the anterior wall of the reticulum. Spread from these lesions may cause a chronic, local peritonitis. There may be evidence of deranged digestion with the rumen contents sloppier than usual, an empty abomasum, and a mild abomasitis and enteritis. Involvement of local lymph nodes does not occur, irrespective of the site of the primary lesion.

DIFFERENTIAL DIAGNOSIS

Abscesses of the cheek muscles and throat region are quite common when spiny grass awns occur in the diet. They are characterized by their movability and localization in soft tissues compared with the immovability of an actinomycotic lesion. Pus may be thin, fetid, or caseous depending on the duration of the abscess. Prompt recovery follows opening and drainage.

Foreign bodies or accumulations of dry feed jammed between the teeth and cheek commonly cause a clinical picture that resembles actinomycosis, and the inside of the mouth should be inspected if the enlargement has occurred suddenly.

The syndrome of indigestion caused by **visceral actinomycotic lesions** resembles that caused by chronic peritonitis.

Cutaneous and mammary lesions in sows closely resemble necrotic ulcers associated with *Borrelia suilla.*

TREATMENT AND CONTROL

Treatment is with surgical debridement and antibacterial therapy, particularly iodides. Oral or intravenous administration of iodides is the most common treatment approach, although less effective in cases of actinobacillosis. For intravenous treatment a 10% or 20% sodium-iodide solution is administered slowly intravenously at a dose of 70 mg/kg. This treatment may be repeated after 1 to 2 weeks. Oral treatment with potassium iodide at a dose of 6 to 10 g per animal daily for at least 10 days has also been proposed; reports of treatment efficiency are anecdotal. Another treatment recorded as being effective consists of isoniazid given orally at the rate of 10 to 20 mg/kg BW daily for about 30 days. Cessation of the growth of the lesion should occur, but response in advanced cases is poor. Repeated cryotherapy with liquid nitrogen is reported to be effective. For control, isolation or disposal of animals with discharging lesions may be advisable, although the disease does not spread readily unless predisposing environmental factors cause a high incidence of oral lacerations. In severe cases and cases unresponsive to iodide treatment, parenteral antimicrobial therapy using penicillin, ampicillin, tetracyclines, or florfenicol have been suggested.

TREATMENT AND CONTROL

Treatment

Sodium-iodide (as 10% or 20% solution) (70 mg/kg IV, may be repeated after 7–10 days) (R-2)

Potassium-iodide (6–10 g per animal orally every 24 h for 10 days) (R-2)

Isoniazid (2.5–5 mg/kg orally every 24 h for 30 days) (R-2)

Procaine penicillin (44,000 IU/kg IM every 24 h for 7 days) (R-2)

Florfenicol (20 mg/kg every 48 h IM) (R-2)

Oxytetracycline (10 mg/kg IM every 24 h for at least 7 days (R-2)

Oxytetracycline long-acting formulation (20 mg/kg IM every 72 h) (R-2)

Control

Isolation and disposal of cattle with discharging lesions (R-2)

IM, intramuscularly; IV, intravenously.

REFERENCE

1. Militerno G. *Vet Rec*. 2008;163:369.

ACTINOBACILLOSIS (WOODEN TONGUE)

SYNOPSIS

Etiology *Actinobacillus lignieresii.*

Epidemiology Organism is normal inhabitant of alimentary tract. Infection through abrasion of oral mucosa or skin. Site difference in sheep and cattle reflects differences in risk associated with prehension of food. Sporadic disease but outbreaks in which herd/flock predisposing factors are present.

Clinical findings Difficulty in prehension of food. Inflammation and abscessation of tongue and draining lymph nodes in cattle and of lips in sheep; Nodulary/proliferative skin lesions most common on head, neck, or lower limbs.

Clinical pathology and diagnostic confirmation Demonstration of organism.

Treatment and control Iodides, antibiotics, and hygiene. Avoidance of abrasive pastures.

Actinobacillosis refers to a sporadically occurring inflammatory process of soft tissue usually occurring in cattle, sheep, goats, and buffaloes. A similar condition has also been reported n horses and humans, where it was associated with animal bites. The condition manifests as a chronic pyogranulomatous inflammatory condition involving the tongue (wooden tongue), skin (skin actinomycosis), lymph nodes, and more rarely parts of the upper digestive tract including esophagus, rumen, and reticulum.

ETIOLOGY

The causative agent of actinobacillosis is *A. lignieresii,* a normal inhabitant of the upper digestive tract of ruminants that becomes an opportunistic pathogen once having penetrated into deeper soft tissue through an integumental or mucosal break. *A. lignieresii* may be recovered in pure culture from the lesions, but other pyogenic organisms may also be present. Recent investigations have shown that bacteria with phenotypic similarity to *A. lignieresii* isolated from horses are genotypically distinct from those isolated from ruminants and they have been designated as *Actinobacillus* genomospecies 1.

EPIDEMIOLOGY

Occurrence

The disease in **cattle** has a worldwide distribution and is usually of sporadic occurrence on individual farms. In **sheep**, the disease is recorded in most sheep-raising countries and is common in Scotland. In most instances, only occasional cases occur but in some flocks a morbidity rate of up to 25% may be encountered. Actinobacillosis also occurs, but is rare, in horses.

Source of Infection and Transmission

A. lignieresii is a normal inhabitant of the oral cavity and rumen of ruminants. The organism is susceptible to ordinary environmental influences and does not survive for more than 5 days on hay or straw. Infection in soft tissue results from damage to the oral mucosa or skin.

In **cattle**, infection most often occurs through ulcerating or penetrating lesions to the sulcus of the tongue, penetrating lesions in the apex, and lacerations to the side of the body of the tongue caused by the teeth. Abattoir surveys suggest that subclinical infections are common and have found small actinobacillary granulomas in the draining lymph nodes of the head and approximately 3% of tongues in slaughter cattle. Recently reports of outbreaks of **skin actinomycosis** in young beef cattle that were associated with skin lesions on lower limbs have been published. The underlying cause could not be determined but was assumed to be increased occurrence of skin lesions e.g., though abrasive surfaces that would have created portals of entry for this environmental pathogen.[1] An incidental report of a postoperative complication of a C-section in which actinobacillosis occurred in the wound is available.[2]

In **sheep**, the different nature of prehension of food leads to lesions predominantly in the lips and cheeks with occasional extension to the mucous membranes of the turbinates and the soft tissue of the head and neck.

Risk Factors

The disease is usually sporadic, but multiple cases in a herd and apparent outbreaks of the disease can occur when animals graze **abrasive pasture** species or pastures with **spiny awns** and transmission may be enhanced by infected discharges contaminating these pastures or feeds. A high prevalence is recorded in cattle grazing "burnt-over" peat pastures in New Zealand. These pastures contain a great deal of gravel and ash likely to cause oral injury. A similar high incidence has been observed in sheep fed prickly pear (*Opuntia* spp.). A severe outbreak has also been reported in heifers fed on very dry, stemmy, tough haylage and in cattle fed wheat straw from a specific thresher that produced straw with sharp edges. There is a higher prevalence of this disease in cattle in areas of copper deficiency.

Actinobacillosis granulomas may also occur at **atypical sites** in cattle, such as the external nares or the jugular furrow following infection of surgery wounds or **traumatic lesions** caused by nose grips or jugular venipuncture.[2] Reports of skin actinomycosis affecting several to many animals in a herd have been reported in recent years.[1,3] Infection of the cheeks resulting in bilateral facial enlargement is also recorded.

Zoonotic Implications

A. lignieresii is rarely associated with human disease but has been isolated from bite wounds inflicted by horses and ruminants.

PATHOGENESIS

Local infection by the organism causes an acute inflammatory reaction and the subsequent development of granulomatous lesions in which necrosis and suppuration occur, often with the discharge of pus to the exterior. Spread to regional lymph nodes with **ensuing lymphadenitis** is usual. Lingual involvement in cattle causes interference with prehension and mastication because of acute inflammation in the early stages and distortion of the tongue at a later stage. Visceral involvement is recorded and is identical with that described under actinomycosis.

CLINICAL FINDINGS

Cattle

The onset of **glossal actinobacillosis** is usually acute, and the affected animal is unable to eat for a period of about 48 hours. There is excessive **salivation** and **gentle chewing** of the tongue as though a foreign body were present in the mouth. On **palpation** the tongue is swollen and hard, particularly at the base, with the tip often appearing to be normal. Manipulation of the tongue causes pain and resentment. Nodules and ulcers are present on the side of the tongue, and there may be an ulcer at the anterior edge of the dorsum. In the later stages in which the acute inflammation is replaced by fibrous tissue, the tongue becomes shrunken and immobile and there is considerable interference with prehension.

Lymphadenitis is common and is often independent of lesions in the tongue. There may be visible and palpable enlargement of the submaxillary and parotid nodes. Local, firm swellings develop and often rupture with the discharge of thin, nonodorous pus. Healing is slow and relapse is common. Enlargement of the retropharyngeal nodes causes loud snoring respiration and interferes with **swallowing**.

Cutaneous actinobacillosis is also recorded with actinobacillosis granulomas occurring on atypical but visible areas such as the external nares, cheeks, skin or eyelid, and limbs. External trauma from abrasive materials in the environment is the usual initiating cause. Lesions are several centimeters in diameter and are pliable or firm and painful on palpation, red, and can bleed easily. Caseated small foci may be evident in the mass when it is debulked.

Sheep

In sheep the tongue is not usually affected. Lesions up to 8 cm in diameter occur on the **lower jaw, face, and nose**, or in the skin folds from the lower jaw to the sternum. They may be superficial or deep and usually extend to the cranial or cervical lymph nodes. Viscid, yellow-green pus containing granules is discharged through a number of small openings. Extensive lesions cause the formation of much fibrous tissue, which may physically impede prehension or respiration. Thickening and scabbiness of the lips may also be observed. Involvement of the nasal cavities may cause persistent bilateral nasal discharge. Affected sheep have difficulty in eating and many die of starvation. *A. lignieresii* is also an occasional cause of **mastitis** in ewes.

A similar involvement of the lips with abscessation in the area of the mandibular lymph nodes is recorded in camels. Incidental cases as well as outbreaks reported in **buffaloes** all were associated with cutaneous but not with glossal involvement.[4] In **horses** the disease is uncommon but intermandibular phlegmon, or infection of the tongue or of the muzzle can occur as well as infection at other body sites.

CLINICAL PATHOLOGY

Purulent discharges commonly contain "sulfur" bodies, which are granular in nature and, on microscopic examination, consist of **club-like rosettes** with a central mass of bacteria. These are not pathognomonic for *A. lignieresii* but can also be found in purulent exudate from granulomas associated with *A. bovis, Pseudomonas aeruginosa,* and *S. aureus.* Definitive diagnosis depends on the recovery of the organism from the lesion; therefore examination of smears or culture of pus for the presence of *A. lignieresii* is advisable. Isolation of the pathogen has been reported to be difficult from chronic lesions particularly when antimicrobials have been used. Full-thickness incision biopsies used for histopathological examination can be of value in diagnosis and show multiple pyelogranulomas in the deep dermis with distinct eosinophilic club rosettes surrounding gram-negative bacterial rods.

NECROPSY FINDINGS

Necropsy examination is not usually performed in cattle affected by the disease. In sheep, lymphangitis and abscesses containing thick, tenacious, yellow-green pus occur around the local lesion. Typical club colonies are visible on staining sections of affected tissue. Culture of material from lesions usually detects the presence of *A. lignieresii.*

DIFFERENTIAL DIAGNOSIS

- Foreign bodies in the mouth
- Rabies
- Esophageal obstruction
- Tuberculosis
- Cutaneous lymphosarcoma

TREATMENT

Iodides are still a standard treatment for both actinomycosis and actinobacillosis. In the former, the results are relatively inefficient, but in actinobacillosis, response is usually dramatic and permanent. Laboratory studies suggest that iodides have little bactericidal effect against *A. lignieresii.* It is probable that iodides exert their effect by reducing the severity of the fibrous tissue reaction.

Oral or intravenous dosing of iodides may be used. Potassium iodide, 6 to 10 g/day for 7 to 10 days, given orally to cattle, is effective. Treatment must be discontinued when symptoms of iodism develop. Lacrimation, anorexia, coughing, and the appearance of dandruff indicate that maximum systemic levels of iodine have been reached. Sodium iodide (70 mg/kg) can be given intravenously as a 10% or 20 % solution in one dose to both cattle and sheep. One course of potassium iodide or one injection of sodium iodide is usually sufficient for soft-tissue lesions, with the acute signs in actinobacillosis disappearing in 24 to 48 hours after treatment. At least one or preferably two further treatments at 10- to 14-day intervals are required for bony lesions.

Occasionally animals show distress, including restlessness, dyspnea, tachycardia, and staggering during injections of sodium iodide. Abortion occasionally occurs following the treatment of heavily pregnant cows with sodium iodide. This has not been reproduced in an experimental study; however, although uncommon, it is wise to advise the owner of this risk. Subcutaneous injections of sodium iodide cause severe irritation and local swelling immediately. The irritation

disappears within an hour or two but the swelling persists for some days. Subcutaneous injection is the standard route of administration for sheep, with the dose rate of sodium iodide being 20 mL of a 10% solution weekly for 4 to 5 weeks.

Sulfonamides, penicillin, streptomycin, and broad-spectrum antibiotics are also used. Streptomycin, given by intramuscular injection and repeated if necessary, has given good results in actinomycosis in cattle when combined with iodides and local surgical treatment. Isoniazid has been used as a treatment for actinomycotic infections in humans, and it has been reported on favorably as an adjunct to antibiotic or iodide therapy in cattle. The daily dose rate recommended is 10 mg/kg BW orally or intramuscularly, continued for 3 to 4 weeks.

Cutaneous actinobacillosis may require an extended course of treatment with streptomycin and/or dihydrostreptomycin for 2 to 4 weeks to achieve resolution.

TREATMENT AND CONTROL

Treatment

Sodium-iodide (as 10% or 20% solution) (70 mg/kg IV, may be repeated after 7–10 days) (R-2)

Potassium-iodide (6–10 g per animal orally every 24 h for 10 days) (R-2)

Isoniazid (2.5–5 mg/kg orally every 24 h for 30 days) (R-2)

Procaine penicillin (44,000 IU/kg IM every 24 h for 7 days) (R-2)

Florfenicol (20 mg/kg every 48 h IM) (R-2)

Oxytetracycline (10 mg/kg IM every 24 h for at least 7 days (R-2)

Oxytetracycline long-acting formulation (20 mg/kg every 72 h IM) (R-2)

Dihydrostreptomycin 10 mg/kg IM for at least 7 days) (R-2)

Control

Isolation or disposal of animals with discharging lesions (R-1)

IM, intramuscularly; IV, intravenously.

CONTROL

Restriction of the spread of disease is best implemented by quick treatment of affected animals and the prevention of contamination of pasture and feed troughs. Isolation or disposal of animals with discharging lesions is essential, although the disease does not spread readily unless predisposing environmental factors cause a high incidence of oral or skin lacerations.

REFERENCES

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2. DeKruif A, et al. *Vet Rec*. 1992;131:414.
3. Milne MH, et al. *Vet Rec*. 2001;148:273.
4. Muhammad G, et al. *Acta Vet Brno*. 2006;75:247.

ORAL AND LARYNGEAL NECROBACILLOSIS

SYNOPSIS

Etiology *Fusobacterium necrophorum*.

Epidemiology Oral infection of calves less than 3 months old; laryngeal involvement in older animals up to 18 months of age.

Clinical findings

Necrotic stomatitis: Fetid breath and necrotic ulceration of mucosa of cheek.

Laryngeal necrobacillosis: Fetid breath. Inspiratory dyspnea and stridor, necrotic lesions on arytenoid cartilages.

Lesions: Necrosis at site of lesion.

Treatment Antimicrobials. Surgical debridement of necrotic lesions and arytenoidectomy in unresponsive cases. Tracheostomy may be required to allow breathing with necrotic laryngitis.

Control None specific.

The term "necrobacillosis" commonly refers to infections associated with necrotizing lesions caused by *F. necrophorum*.[1] Although oral necrobacillosis refers to an inflammatory process affecting tissue of the oral cavity of calves, laryngeal necrobacillosis refers to an infection of the more caudal pharyngeal and laryngeal region. **Calf diphtheria** is a common synonym for necrobacillosis of the pharynx and larynx, and **necrotic stomatitis** is a synonym for the oral form. They are considered together because the essential lesion and infection are the same in both instances.

ETIOLOGY

F. necrophorum is a gram-negative, non–spore-forming, rod-shaped anaerobic but aerotolerant organism. It is a normal inhabitant of the ruminant oral cavity and upper digestive and respiratory tract and an opportunistic pathogen generally associated with abscesses and various necrotic infections.[2] Along with the oral/laryngeal necrobacillosis, *F. necrophorum* is also the causative agent of digital necrobacillosis (foot rot) and liver necrobacillosis (liver abscesses) in cattle.

Historically a subdivision of *F. necrophorum* divided into four different biotypes (A, B, AB, and C) was used. Biotypes A and B, which are considered to be most relevant in the etiology of *F. necrophorum*–associated diseases in cattle have been renamed as *F. necrophorum* subsp. *necrophorum* (formerly biotype A) and *F. necrophorum* subsp. *funduliforme* (formerly type B). The subspecies necrophorum is the more prevalent subspecies in necrobacillotic processes in animals. *F. necrophorum* subsp. *funduliforme* tends to occur more frequently in mixed infections.[2] Both *F. necrophorum* subsp. *necrophorum* and *F. necrophorum* subsp. *funduliforme* are associated with the disease.

F. necrophorum possesses a number of virulence factors such as endotoxic LPS, leukotoxin (LT) hemolysin, and hemagglutinin as well as others that are considered to be of critical importance for the anaerobic pathogen to penetrate, colonize, and proliferate in nonsuperficial tissue.[2] The pathogen is considered incapable of penetrating the intact mucosa or skin. Therefore other factors causing a primary tissue trauma, and thereby a portal of entry, are probably required. In the case of laryngeal disease, the point of entry is thought to be contact ulcers in the mucosa caused by repeated closure of the larynx.

EPIDEMIOLOGY

Occurrence

The disease has no geographic limitations but is more common in countries in which animals are housed in winter or maintained in feedlots. In the United States infections involving the pharynx and larynx appear to be more prevalent in the western states than in other sections of the country. It is a common disease in feedlots in yearling cattle, often in company with papillomatosis of the larynx. Laryngeal necrobacillosis is one of the most common infectious upper airway diseases associated with severe respiratory distress in calves observed in Belgium, the Netherlands, and parts of France. The condition in this region primarily affects **double-muscled Belgian Blue calves**, which are considered to be genetically predisposed to the condition.[3]

The disease is seen incidentally in sheep and goats. Laryngeal chondritis has been described in Texel sheep, which may be predisposed to the disease because of anatomic factors, namely the short head of the breed. This may affect the shape of the larynx or its relationship to adjacent tissues.

Transmission

Oral/laryngeal necrobacillosis is an infectious but noncontagious disease. The causative bacterium is a common inhabitant of the environment and upper digestive tract of cattle. It has been proposed that the infection may be spread through dirty milk pails and feeding troughs. Entry through the mucosa is probably affected through abrasions caused by rough feed and erupting teeth. The difficulty of reproducing the disease and the irregularity of its occurrence, even when *F. necrophorum* is known to be present, suggests the possibility of etiologic factors presently unknown.

Risk Factors

Host Risk Factors

Animals suffering from intercurrent disease or nutritional deficiency are most susceptible, but there is also an obvious **age predisposition** to the condition. Necrotic stomatitis is predominantly seen in weaned and unweaned calves 2 weeks to 3 months of age. Laryngeal infections commonly affect older calves up to 1 year of age and rarely occur in older animals up to 3 years of age.

An unusually high disease incidence has been observed in double-muscled Belgian Blue calves, a breed that is common in Belgium, the Netherlands, and some parts of France.[3]

Pathogen Risk Factors

A number of pathogen risk factors have been identified for *F. necrophorum,* of which LT and LPS are considered most important for the pathogenesis of necrobacillotic infections. Several investigations have reported that subtypes and strains of the bacterium vary in the amount of LT produced, which may contribute to the virulence of a specific strain. A correlation between LT production and the ability to induce abscesses has been reported in laboratory rats.[1]

Environmental Risk Factors

Necrobacillosis is highest in groups kept in confined quarters. Cases in pastured animals have been reported but are rare. Unsanitary conditions have been incriminated in facilitating the spread of the condition through contaminated nipples or pails.

PATHOGENESIS

F. necrophorum is a normal inhabitant of the oral cavity and causes inflammation and necrosis once it is able to penetrate tissue, e.g., through an injury of the mucosa of the oral cavity, pharynx, and larynx. Edema and inflammation of the mucosa of the larynx results in varying degrees of closure of the rima glottidis and inspiratory dyspnea and stridor. The presence of the lesion causes discomfort, painful swallowing, and toxemia. Extension of the lesion to the arytenoid cartilages will result in laryngeal chondritis. Involvement of the cartilage will usually result in delayed healing or failure to recover completely.

CLINICAL FINDINGS

In describing the clinical findings, a distinction must be made between calf diphtheria, which is characterized by the involvement of the larynx and necrotic stomatitis. In the former, a moist painful cough accompanied by severe inspiratory dyspnea that cause a roaring inspiratory sound ("honker calf" or "hard breather"), salivation, painful swallowing movements, complete anorexia, and severe depression are the characteristic signs. The temperature is high at 41°C (106°F), the pharyngeal region may be swollen and painful on external palpation, and there is salivation and nasal discharge. The breath has a foul rancid smell.

In cases of laryngeal necrobacillosis examination of the pharynx and larynx by visual inspection through the oral cavity with the aid of a speculum positioned over the base of the tongue will often reveal the lesions. The larynx can be viewed directly and illuminated with a strong source of light. A flexible endoscope is also useful when available and is necessary for examination of the larynx and cranial part of the trachea. The mucosa of the larynx and glottis are usually edematous and inflamed and a necrotic lesion is usually present and visible on one or both arytenoid cartilages. The opening of the larynx is often reduced because of the edema and inflammation. Careful visual inspection of the larynx during inspiration may reveal that the lesion extends into one or both vocal cords. The examination usually causes considerable discomfort, anxiety, and the production of purulent or bloodstained saliva.

Death is likely to occur from toxemia or obstruction to the respiratory passages on days 2 to 7. Most affected calves die without treatment, but only a small proportion of calves in a group are usually affected. Spread to the lungs may cause a severe, suppurative aspiration bronchopneumonia.

In calves affected with necrotic stomatitis, there is usually a moderate increase in temperature (39.5°C–40°C; 103°F–104°F), depression, and anorexia. The breath is foul and saliva, often mixed with straw, hangs from the mouth. A characteristic swelling of the cheeks may be observed posterior to the lip commissures, which, on opening the mouth this, is found to be caused by a deep ulcer in the mucosa of the cheek. The ulcer is usually filled with a mixture of necrotic material and food particles. An ulcer may also be present on the adjacent side of the tongue and cause severe swelling and protrusion of the tongue. In severe cases the lesions may spread to the tissues of the face and throat and into the orbital cavity. Similar lesions may be present on the vulva and around the coronets, and a spread to the lungs may cause fatal pneumonia. In other cases death appears to be caused by toxemia.

CLINICAL PATHOLOGY

Bacteriologic examination of swabs from lesions may assist in confirming the diagnosis.

NECROPSY FINDINGS

Severe swelling, caused by edema and inflammation of the tissues surrounding the ulcer, is accompanied by the presence of large masses of caseous material. Occasionally, lesions similar to those in the mouth, pharynx, and larynx may be found in the lungs and in the abomasum. Microscopically, areas of coagulation necrosis are bordered by large numbers of neutrophils and filamentous bacteria.

Samples for Confirmation of Diagnosis

- **Bacteriology:** anaerobic culture swab from deep within lesion (ANAEROBIC CULT)
- **Histology:** formalin-fixed sample of interface between ulcer site and normal tissue (light microscopy).

DIFFERENTIAL DIAGNOSIS

Necrotic laryngitis is characterized by inspiratory dyspnea and stridor, toxemia, fever, edema, (inflammation), and necrotic lesions of the laryngeal mucosa.

- **Neoplasms of the larynx** Occur only rarely, usually in mature cattle, and cause chronic inspiratory dyspnea.
- **Traumatic pharyngitis** May resemble laryngitis, but the lesions are obvious on visual inspection of the pharynx. In chronic cases of traumatic pharyngitis there may be periesophageal cavities containing rumen contents
- **Foreign bodies** Pieces of wire and small wooden sticks, for example, may become lodged in the mucosa of the arytenoid cartilages and cause clinical signs similar to necrotic laryngitis.

TREATMENT

The lesions of necrotic stomatitis will usually heal in a few days following debridement of the ulcers, application of a solution of tincture of iodine, and oral administration of sulfamethazine at a dose of 150 mg/kg BW daily for 3 to 5 days as labeled for use in food animals, or parenteral penicillin or broad-spectrum antimicrobials. Therapy should be at least for 5 days, and therapy for up to 3 weeks may be necessary.

Successful treatment of necrotic laryngitis is dependent on early recognition and prompt therapy with antimicrobials daily for several days. A broad range of antimicrobials have been proposed for the treatment of oral/laryngeal necrobacillosis. *F. necrophorum* is susceptible in vitro to β-lactam antibiotics, tetracyclines, macrolides, and lincomycins but is resistant to aminoglycosides and ionophore antibiotics.[2] The apparent sensitivity of this gram-negative pathogen to penicillins and cephalosporins is peculiar even based on its cell wall structure.[2] Although third- and fourth-generation cephalosporins (e.g., ceftiofur and cefquinome) are highly effective against *F. necrophorum,* these antimicrobials that have been classified as critically important for human and veterinary medicine are only indicated as second choice for cases that have poorly responded to other antimicrobials. Corticosteroids may be a beneficial adjunctive therapy, especially to reduce the edema. Tracheostomy may be necessary in some cases to relieve dyspnea. Failure to respond is usually associated with chronic suppurative chondritis, which requires subtotal arytenoidectomy.

TREATMENT AND CONTROL

Treatment

Procaine penicillin (22,000 IU/kg IM every 12 h or 44,000 IU/kg IM every 24 h for at least 7 days) (R-2)

Continued

Oxytetracycline (10 mg/kg IM every 24h for at least 7 days or long-acting formulation 20 mg/kg every 72 h) (R-2)

Ampicillin trihydrate (10 mg/kg SC or IM every 24 h for at least 7 days) (R-2)

Ceftiofur hydrochloride (2.2 mg/kg SC or IM every 24 h for at least 7 days) (R-2)

Dexamethasone (0.2–0.5 mg/kg IV or IM as a single dose) (R-2)

IM, intramuscularly; IV, intravenously; SC, subcutaneously.

CONTROL

Proper hygienic precautions in calf pens or feeding and drinking places together with avoidance of rough feed should prevent the spread of the disease. When the incidence is high, prophylactic antibiotic feeding may keep the disease in check.

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ENTEROHEMORRHAGIC *ESCHERICHIA COLI* IN FARM ANIMALS AND ZOONOTIC IMPLICATIONS

Enterohemorrhagic *Escherichia coli* (EHEC), particularly *E. coli* serogroup O157:H7, have been recognized as food-borne pathogens causing potentially fatal human illness since the 1980s and have since then become a worldwide public health concern of increasing relevance. First outbreaks of human EHEC infections were recorded in 1982 in Oregon and Michigan and have been associated to the consumption of undercooked hamburger patties.[1] Since then, *E. coli* O157:H7, and in more recent years also a number of other serotypes, have caused major human illness outbreaks worldwide with considerable morbidity and mortality. Clinical signs can range from mild diarrhea, bloody diarrhea, and hemorrhagic colitis to the hemolytic uremic syndrome (HUS).[1]

Ruminants, and cattle in particular, are the main reservoir of EHEC but do not typically develop clinical disease. Human infection is acquired through consumption of contaminated food or water, direct contact with EHEC-carrying animals, or via person-to-person transmission.[1]

ETIOLOGY

EHEC comprise a subgroup of so-called Shiga-toxin–producing serotypes of *E. coli* that have been implicated in severe human disease. Shiga-toxin–producing *E. coli* strains produce toxins similar to the one produced by *Shigella dysenteriae,* the so-called Shiga toxins (Stx), and are therefore also denoted as **Shiga-toxin–producing *E. coli* (STEC)**. The presence of Shiga-toxin is determined by the Vero cell toxicity test, so STEC are also called **verotoxin** or **verocytotoxin-producing *E.coli* (VTEC)**.[2] The only consistent difference between pathogenic STEC serovars and apathogenic *E. coli* strains is indeed the possession of Stx genes.[3]

The large majority of outbreaks and sporadic cases of severe disease in humans are associated with a very limited number of EHEC serotypes.[1] By far the most prevalent single serotype of *E. coli* associated with human illness is ***E. coli* O157:H7**. However, in recent years a number of other EHEC serotypes have been linked to human illness and are on the rise worldwide; EHEC strains have therefore been classified into *E. coli* O157:H7 and non-O157:H7 *E.coli* recently. The six most prevalent non-O157 STEC serogroups associated with clinical disease in humans are in descending order: O26, O111, O103, O121, O45, and O145.[4]

EPIDEMIOLOGY

The predominant carriers and shedders of EHEC are healthy domesticated ruminants, cattle in particular, and to a lesser extent sheep and possibly goats.[5] EHEC strains associated with clinical disease in humans constitute only a minor fraction of the STEC isolates that are routinely recovered from healthy cattle, whereas the large majority of bovine STEC isolates either do not occur at all or are greatly underrepresented in people.[5] Although the majority of STEC isolates carried by healthy ruminants are not transmitted to humans there is no doubt that cattle are the main source of human EHEC infection.[5] The highest recovery rates of STEC among non-ruminant farm animal species was reported for turkeys, whereas other species such as pigs or chicken are only incidental carriers.[5] Rodents, domestic animals, and flies have been identified as incidental carriers.

It is estimated that between 20% and 50% of human EHEC infections are attributable to non-O157 *E. coli* strains, but estimates vary greatly from country to country and within one country from region to region.[4] In North America, Japan, and the UK *E. coli* O157:H7 is the serotype most commonly associated with clinical disease in people, whereas in Europe, Australia, Argentina, or South Africa infections with non-O157 serotypes have been estimated to be at least as prevalent as infections with the O157:H7 serotype in people.[4] Human cases of HUS are in most cases associated with infections from the serotype O157:H7. Estimates of sporadic cases of HUS in people associated with non-O157 STEC are less than 10% in North America and between 10% and 30% in Germany, Italy, and Great Britain.[4]

Occurrence and Prevalence of Infection

Cattle

Ruminants, and cattle in particular, are the most important nonclinical natural reservoirs of STEC. Generally, cattle remain asymptomatic because intestinal mucosal cells lack the Stx-specific globotriaosylceramide receptor.[4]

Estimates of the prevalence of STEC fecal carriage among populations of cattle vary considerably, and data from different surveys are difficult to compare because of inconsistent experimental approaches, differences in sampling strategies, and applied analytical methods.[2] In many studies the analytical approaches used specifically aim at the detection of serotype O157:H7, whereas fewer surveys used laboratory methods suitable to detect all or at least selected non-O157 STEC serogroups.[2] Generally, the reported prevalence rates for non-O157 STEC strains in cattle are much higher than the prevalence rates of O157:H7 in cattle. Between 2007 and 2009 the fecal prevalence of STEC in cattle determined at the level of the European Union (EU) was between 2.2% and 6.8%. Strain O157 was isolated in between 0.5% and 2.9% of these samples.[2] The prevalence rates reported by different member states varied between 0% and 48.5%, which is at least in part due to the different sampling strategies and laboratory methods used in the different countries. Tested specimens included feces, ear, and hide samples.[2] The most sensitive sampling method, at least for STEC O157:H7, was found to be the rectal swab, which has been explained by the fact that STEC tend to specifically colonize the rectoanal junction of the intestinal mucosa that is directly sampled with the swab approach.[3] There is a correlation between the prevalence of *E. coli* O157:H7 in the feces, hides, and carcasses of beef cattle during slaughter. Overall, the prevalence of *E. coli* O157:H7 in feces and on hides was 28% and 11%, respectively.

A recent study investigating the prevalence of STEC O157 in Belgium found that the viable O157 strain was present in 37.8% of 180 participating farms. In this study dairy farms had the highest herd prevalence rate (61.2%) followed by beef (22.7%) and veal calf operations (9.1%).[6] Prevalence rates of STEC in dairy cattle in the United States range between 0.17% and 8.4% in cows, 1.7% and 9.5% in heifers, and 0.2% and 40% in calves.[7] Prevalence estimates in North American beef cattle range from 10% to 28% with a herd prevalence approaching 100%.[1]

Generally, prevalence rates are higher in calves and heifers than in adult cattle, which is an effect that has been attributed to the greater susceptibility to colonization of calves and heifers than for cows.[7] The prevalence of fecal STEC shedding is influenced by numerous variables, including the season, the scope, frequency and timing of sampling, and the conditions of sampling and storage. The organism can be found widely distributed in samples from several types of cattle including beef calves, stocker cattle, feedlot cattle, adult beef cows, dairy calves, water sources, and wildlife.

Shedding of STEC has been proposed to vary between individuals. Although most animals may only shed the bacteria transiently following exposure, some individuals shed the pathogen for prolonged periods and at much higher rates due to colonization of the terminal rectum of the gastrointestinal tract.[1] Cattle shedding STEC at much higher concentrations and for prolonged periods of time are so-called **supershedders**. The percentage of STEC-shedding cattle that are considered supershedders has been estimated at 3.9% for serovar O157 and 10% for non-O157 serovars.[3] Although supershedders constitute a small proportion of cattle in an infected herd, they are thought to substantially impact the on-farm epidemiology. It has been estimated that serotype O157:H7 supershedders may be responsible for over 95% of the bacteria shed.[1]

Prevalence of Infection in Cattle, Sheep, and Pigs at Slaughter

Abattoir surveys conducted in the UK determined prevalence rates of fecal carriage of STEC O157 between 4.7% and 15.7% in cattle and between 0.7% and 2.2% in sheep in the UK. STEC O157 was only isolated form 0.4% of slaughtered pigs.[5] In a Dutch abattoir study STEC O157 strains were isolated from 10.6% of slaughtered cattle and 4.0% of slaughtered sheep. An overall prevalence of *E. coli* O157:H7 fecal shedding by New York cull dairy cattle of 1.3% was found in specimens just before processing the packing plant. In a survey of downer cattle submitted to two slaughter facilities in Wisconsin, the prevalence of *E. coli* O157:H7 in the feces and/or tissues of downer dairy cattle was 4.9% compared with 1.5% in healthy cattle.

In an abattoir study the polymerase chain reaction (PCR) was used to detect virulence genes and molecular epidemiology of *E. coli* O157:H7 isolates. Samples included swabs of tools, knives, and saws; fecal samples; carcass samples; and ears removed after slaughter. From 1432 samples, 143 *E. coli* O157:H7 strains were isolated. These results indicate the increase in contamination frequencies during transportation to the abattoir and the lairage period before slaughter as a result of cross-infection caused by mixing of animals from different sources. The presence of supershedding animals at the abattoir increases the potential risk of beef contamination during the slaughtering process and stresses the need for correct hazard analysis and critical control points procedures. Carcass samples were taken at three points during processing: preevisceration, postevisceration before antimicrobial intervention, and postprocessing after carcasses entered the cooler. The prevalence of *E. coli* O157:H7 in the three postprocessing samples was 43%, 18%, and 2%, respectively. Antimicrobial intervention included steam pasteurization, hot water washes, organic acid washes, or combinations of these treatments. The reduction in carcass prevalence from preevisceration to postprocessing suggests that sanitary procedures can be effective within processing plants. Fecal and hide prevalence were significantly correlated with carcass contamination, indicating a role for control of *E. coli* O157:H7 in live cattle.

Sheep and Goats

Sheep and goats can be naturally infected with *E. coli* O157:H7, and sheep have been used as a model of ruminant infection. Sheep may harbor *E. coli* O157:H7 and non-O157:H7 STEC at rates similar to or higher than in cattle. Prevalence rates of 67% and 45% have been reported in Germany and Australia, respectively. Worldwide, sheep have been shown to shed several non-O157 strains in their feces. Several of these STEC serotypes have been associated with sporadic cases or major outbreaks of human illnesses. Thus lamb, mutton, and their products share a food safety risk factor similar to that of beef. Non-O157:H7 STEC have been found in sheep grazing irrigated pasture or arid rangeland forage in Nevada. In Brazil, STEC occurred in the feces of 51% of healthy sheep grazing on pasture.

Wildlife

Based on fecal samples of deer submitted by hunters, *E. coli* O157:H7 have been found in the feces of free-ranging white-tailed deer in Nebraska at a rate of 0.25%. The prevalence of infection of *E. coli* O157:H7 in white-tailed deer sharing rangeland with cattle was 2.4%. Deer experimentally inoculated with *E. coli* O157:H7 shed the pathogen for over 26 days to naive penmates. Fermented deer sausage was identified as a vehicle for *E. coli* O157:H7 transmission in Missouri.[8] The low overall prevalence of *E. coli* O157:H7 and the identification of only one site with positive deer suggest that wild deer are not a major reservoir of *E. coli* O157:H7.

High prevalence of fecal carriage of STEC O157 of 3.3% was reported in wild boars in Spain, and one strain was identical to a strain associated with clinical disease in people.[9]

Pigs

E. coli O157:H7 has been found in fecal samples of finished pigs at the time of slaughter, but the prevalence was very low at 0.08%. In experimentally infected pigs, *E. coli* O157:H7 can persist for more than 2 months. In a longitudinal study conducted in four U.S. swine farms STEC O157:H7 was isolated from 8.9% of rectal swabs; however, shedding was not associated with clinical disease, and isolated strains could have been nonvirulent.[10] Potentially pathogenic O157:H7 strains have, however, been isolated from 2% of slaughter pigs in one study as well as in feral swine in California.[5] Pigs may have the potential to be reservoirs hosts for *E. coli* O157:H7, but the magnitude of the risk needs to be determined.

Risk Factors

Animal Risk Factors

Cattle that are infected with *E. coli* O157:H7 remain free of disease because of the lack of specific vascular receptors for Stx and are tolerant of *E. coli* O157:H7 for their entire lives.

Although most exposed cattle shed STEC at less than 100 colony forming units (CFU)/g feces, a small subset of cows are predisposed to shed exceptionally high numbers of bacteria (>10^4 CFU/g feces) for prolonged periods of time. These individuals, also called **supershedders,** are estimated to be responsible for over 95% of the bacterial shedding within a herd.[11]

Age has been identified as an animal risk factor for STEC infection in cattle. Although preweaned calves were found to rarely carry STEC, postweaning calves and heifers have a higher fecal carriage prevalence of STEC and shed larger numbers of bacteria than older cows.[7] Studies conducted in colostrum-deprived calves suggest that the low prevalence of O157 carriage in unweaned calves is at least partly because of the protective effect of colostral antibodies during the first weeks of life.[5]

Higher prevalence rates of fecal STEC carriage in heifers than in young bulls suggest a gender effect that may be caused by hormonal effects around pregnancy and lactation.[5]

Environmental and Management Risk Factors

Although a larger number of STEC serotypes may be isolated from some farms, typically a herd only harbors a small number of isolates that tend to persist on the farm for over 2 years independently of animal carriers. This underscores the importance of environmental contamination and the circulation of the pathogen between animals and environment for the on-farm epidemiology of STEC infection.[5] Water tanks in feedlots, for example, were found to be frequently contaminated with STEC. *E. coli* O157:H7 was isolated from 13% of the water tanks in U.S. feedlots, with at least one water tank positive on 60% of the feedlots. Water tanks were five times more likely to be contaminated with *E. coli* O157:H7 if a pen was positive for bacteria, but the direction of the spread was not determined.[5] Similarly *E. coli* O157:H7 was isolated from 14.9% of the feed samples obtained from the feed bunks. Factors positively associated with *E. coli* O157:H7 in the feed were higher heat index at the time of sampling, the presence of cottonseed meal in the ration, and the feedlot location.

A seasonal effect is well established in temperate climates with peaks in the prevalence of fecal STEC carriage between late spring and early fall. For example, fecal samples and rope swabs from dairy herds collected over a period of 1 year in Alberta, Canada, revealed a 15-fold increase in

prevalence of positive samples between June and September compared with the rest of the year.[12] Several abattoir surveys conducted in different European countries revealed similar peaks in the fecal carriage prevalence during the summer months.[5] The specific factors contributing to this seasonal effect are not well understood.

Housing and Management Practices

Environmental dissemination of an inoculated strain of *E. coli* O157:H7 given to dairy calves spreads more quickly when calves are housed in groups compared with calves housed in individual pens from 7 to 110 days of age. The use of segregated penning systems rather than group housing of weaning calves may reduce the prevalence of these potential pathogens within the calf unit. If this results in a reduction in the general herd or farm STEC prevalence, then such changes in calf-rearing practice may offer a control point.

Pathogen Risk Factors

Virulence Attributes and Mechanisms

The primary feature of STEC isolates is their ability to produce potent cytotoxins encoded by *stx1* and *stx2* genes. They also have the ability to adhere to the intestinal mucosa in an intimate manner through the attachment and effacement protein intimin, encoded by the *eaeA* gene, and most produce a plasmid-encoded enterohemolysin, encoded by the *elixA* gene. STEC isolates that cause disease in humans usually have one or both of these virulence-associated factors and have been referred to as complex Shiga-toxin–producing *E. coli* (cSTEC). The most often reported STEC serotype causing diseases in humans worldwide is *E. coli* O157:H7, but non-O157 serotypes such as O8:H19, O8:H21, O22:H8, O113:H21, and Orough:NM (nonmotile) are commonly found to cause diseases such as HUS.[3] There are over 160 STEC serotypes that have been isolated from human patients around the world.

Acid Resistance

E. coli O157:H7 is extremely acid resistant, which contributes to the low infectious dose for humans; this has been estimated to be fewer than 100 CFU and possibly even as low as 10. Certain strains of *E. coli* O157:H7 have been considered to be more acid tolerant than some commensal *E. coli*. In addition, *E. coli* O157:H7 strains may become acid habituated by exposure to weak acids in the rumen. Consequently, *E. coli* O157:H7 may survive passage through the acid barrier in the abomasum, colonizing and replicating in the ruminant colon.

The acid-resistance characteristics of *E. coli* O157:H7 led to the hypothesis that feeding grain to cattle created an ideal environment in the gastrointestinal tract to promote the growth and persistence of the organism. The research data on the effects of grain versus forage feeding to cattle and its effects on fecal *E. coli* O157:H7 are limited and conflicting. Some early research indicated that grain feeding increased the dissemination of acid-resistant *E. coli* by cattle and that feeding hay for a brief period immediately before slaughter would decrease the shedding of *E. coli* O157:H7. The numbers, persistence, and acid resistance of generic coliforms and *E. coli* O157:H7 from various gastrointestinal tract sites of cattle fed grain or hay were compared. Grain feeding or hay feeding did not affect survival of *E. coli* O157:H7 in the rumen or its passage through the abomasum (pH 2.0) to the duodenum.

Recent studies on the effect of forage or grain diets have shown that cattle fed forage diets had ruminal persistence of fecal *E. coli* O157:H7 at quantifiable concentrations for twice as long as cattle fed grain diets. Diets high in grain generate high volatile fatty acid concentrations and low pH, creating a less conducive environment for *E. coli* O157:H7, whereas lower volatile fatty acid concentrations and higher pH in forage-fed cattle may be more conducive to the growth and survival of the organism. Monensin supplementation decreased the duration of shedding with forage diet, and the cecum and colon were culture positive for *E. coli* O157:H7 more often than the rumen of cattle.

Antimicrobial Resistance

Although antimicrobial therapy in cases of EHEC infection is considered to be contraindicated, numerous studies evaluating antimicrobial-resistance patterns of *E. coli* O157:H7 have been conducted. Antimicrobial resistance is common in O157:H7 and other STEC strains and include multiple drug resistance to streptomycin, tetracycline, and sulfisoxazole.[1] The prevalence of antimicrobial resistance among isolates of *E. coli* O157:H7 recovered from clinical cases in humans, pigs, cattle, and food over a 15-year period (1985–2000) in the United States has been described. There was a high prevalence of resistance to tetracycline, sulfamethoxazole, cephalothin, and ampicillin. The highest prevalence occurred among isolates from pigs, in which more than 50% of all isolates were resistant to sulfamethoxazole, cephalothin, or tetracycline and more than 20% were resistant to ampicillin or gentamicin.

Methods of Transmission

Sources of Organism

Ruminants as Reservoirs

E. coli O157:H7 is a transient inhabitant of the gastrointestinal tract of normal healthy ruminants. Cattle and sheep feces serve as sources for contamination of feed and water sources. Fecal shedding is transient in cattle, often lasting 1 to 3 months or less, but the organism can persist on individual farms for up to 2 years. Longitudinal surveys have shown that maintenance of *E. coli* O157:H7 and other STEC strains in cattle herds relies on continual reinoculation of individual cattle. Repeated isolations of *E. coli* O157:H7 from healthy beef and dairy cattle demonstrate that cattle are asymptomatic carriers of the organism. Short periods of relatively high prevalence of excretion are separated by longer periods of reduced or undetectable shedding. This has contributed to the variance in prevalence data reported in the literature.

Fecal shedding is more prevalent from spring to early fall than during the cold season of the year. Fecal shedding also varies among different classes of animal. Weaned heifers between 3 months of age and breeding age are more likely to shed STEC in feces than adult cattle or younger calves.

Contaminated water troughs, particularly those that are allowed to develop sediments, provide an environment for survival, proliferation, and horizontal spread of *E. coli* O157:H7 and other STEC serotypes. The organism can also proliferate to very high levels in moist silage.

The pattern of fecal carriage of *E. coli* O157:H7 in cattle finished under modern intensive feedlot management conditions has been examined. *E. coli* O157:H7 was isolated from 13% of fecal samples, with the highest prevalence values of the organism in pens supplied with chlorinated drinking water compared with nonchlorinated water pens. Over a period of 7 months from April to September, certain specific clonal types of *E. coli* O157:H7 persisted and predominated despite massive cattle population turnover. This suggests that the farm environment, and not necessarily the incoming cattle, is an important potential source of *E. coli* O157:H7 on farms.

Other Species

E. coli O157:H7 subtypes indistinguishable from those detected in cattle have been found in turkeys, pigeons, geese, horses, dogs, opossums, and flies. *E. coli* O157:H7 also has been isolated from insects in cattle environments, but their role in dissemination is uncertain.

Wild Birds

E. coli O157:H7 has been found in the feces of wild birds, which may contribute to the spread of the organism within and between farms. The presence of wild geese was a significant risk factor in the shedding of *E. coli* O157:H7 by beef suckler cows in Scotland.

Flies

The increased presence of flies around cattle during the summer months represents a potential mechanism for the spread of *E. coli* O157:H7 among farm animals. *E. coli* O157:H7 has been isolated from the crop of houseflies (*Musca domestica*) immediately after feeding on a bacterial preparation.

Environmental Sources

There are many possible sources of STEC in the farm environment, including manure piles, ponds, dams and wells, barns, calf hutches, straw and other bedding, feed and feed troughs, water and water troughs, farm equipment, ground surface and pasture, and watercourses. Once in the environment, the organism can be transferred to other sites by rainwater, wind, and removal and spreading of manure, including animals and humans.

Water Supplies for Livestock

Drinking water offered to cattle is often of poor microbiological quality, and the daily exposure of animals to various STEC strains from this source can be substantial. The degree of *E. coli* exposure is positively associated with proximity of water troughs to the feed bunk, protection of the trough from sunlight, and warmer weather. Cattle water troughs can serve as environmental reservoirs for STEC and as a long-term source infection for cattle.

The experimental inoculation of *E. coli* O157:H7 with 1 L of water into dairy calves in a confined environment resulted in shedding of the organism by the calves within 24 hours after administration. The duration of shedding varied from 18 to more than 43 days, and the number of doses necessary to initiate shedding varied among calves.

STEC is present in as many as 10% of water troughs, and water is more likely to be positive when *E. coli* O157:H7 was detected in the sediment. Chlorination of input water in feedlots was unable to reduce the prevalence of *E. coli* O157:H7-contaminated water troughs.

Water trough sediments with feces from cattle excreting STEC may serve as a long-term reservoir of the organism on farms and a source of infection for cattle. The accumulation of large amounts of organic matter would be expected to rapidly inactivate the biocidal activity of chlorine and provide an ideal niche for the survival of the organism. *E. coli* O157:H7 can survive in farm water under field and shed conditions at temperatures less than 15°C for up to 24 days. The addition of feces to water outdoors resulted in survival for 24 days.

E. coli O157:H7 has been isolated from surface waters collected from a Canadian watershed. Systematic sampling of surface water within the Oldman River basin in southern Alberta reveals that it is often contaminated with *E. coli* O157:H7 and *Salmonella* spp. The prevalence of *E. coli* O157:H7 and *Salmonella* spp. in water samples was 0.9% and 6.2%, respectively. The region surveyed is noted for high cattle density as well as for one of the highest incidences of gastroenteritis in Canada, resulting from infection by *Salmonella* spp. and *E. coli* O157:H7. Although the data indicated a relationship between high livestock density and high pathogen levels in southern Alberta, analysis of the point source data indicates that the predicted manure output from cattle, pig, and poultry feeding operations was not directly associated with the prevalence of either *Salmonella* spp. or *E. coli* O157:H7. Variations in time, amount, and frequency of manure applications onto agricultural lands may have influenced levels of surface-water contamination with these bacterial pathogens.

Feed Supplies

The prevalence of *E. coli* O157:H7 in cattle feeds in feedlots was 14.9%, which was higher than previously reported, and may be because of more sensitive detection methods. Feed may be a vehicle for dissemination and colonization; however, the source of the STEC contamination in cattle feed is uncertain. Possible sources include saliva and fecal contamination by cattle or other species, or by wildlife, including birds, rodents, and insects. Another possible source is contaminated feed components mixed into the feed. Pulse-field gel electrophoresis (PFGE) profiles of *E. coli* O157:H7 isolated from a component feed sample closely resembled that isolated later from the same farm, suggesting that cattle feed may be an important vector for the transmission of *E. coli* O157:H7.

Manure

Survival of STEC in manure and manure slurry has been observed under various experimental and environmental conditions. The use of manure as fertilizer could explain food-borne outbreaks of *E. coli* O157:H7 and other strains associated with unpasteurized apple cider, potatoes, and other vegetables. Because STEC can survive for extended periods of time, proper manure management is of major importance in preventing the spread of this organism to the environment. Composting is an effective method for eliminating pathogens such as *E. coli* O157:H7 from manure.

Soil

E. coli O157:H7 inoculated into loam and clay soils can survive for 25 weeks and in sandy soil for 8 weeks. The organism was detectable for up to 7 days after inoculation into the uppermost 2.5 cm of the soil and for up to 7 days on grass plots inoculated with a fecal slurry from dairy cattle at an application rate of *E. coli* O157:H7 of 660 CFU/m^2.

Animal-Holding Facilities

The organism can be cultured from rope devices in a feedlot pen that cattle rub or chew, and there is a correlation with the prevalence of cattle shedding the organism in the feces from within the same pen. This pen-test strategy may be useful for identifying pens of cattle posing a higher risk to food safety.

Immune Mechanisms

The Esp and Tir proteins secreted by some STEC strains play critical roles in the development of the attaching and effacing lesions and are recognized serologically in human patients with HUS. Antibodies to intimin, Esp, and Tir proteins have been detected in HUS patients following infections with EHEC.

In contrast, little is known about the immune responses of cattle to STEC infection. *E. coli* O157:H7 and other STEC serotypes are shed sporadically by cattle, and it appears that natural exposure to these organisms does not confer protection on the host. Calves 13 to 30 days of age developed anti-O157 IgG responses following experimental oral inoculation with *E. coli* O157:H7. Mature cows did not develop a significant increase in their serum anti-O157 IgG levels following oral inoculation. These observations suggest that local immunity to *E. coli* O157:H7 may not develop to any degree in the intestine and that immunization to reduce fecal shedding of *E. coli* O157:H7 may not be effective.

Vaccination of cattle with antigenic bacterial proteins involved in colonization can significantly reduce fecal shedding and prevalence of *E. coli* O157:H7 in cattle. Vaccination of cattle with *E. coli* O157:H7 type III secreted proteins can reduce the numbers of *E. coli* O157:H7 shed in the feces, the duration of shedding in experimentally challenged cattle, and in feedlot cattle under field conditions. Vaccination of pregnant gilts with intimin from *E. coli* O157:H7 induced high intimin-specific immune responses in the serum and colostrum, and suckling neonatal piglets had reduced bacterial colonization and intestinal lesions following experimental challenge. These results suggest that vaccination may be a useful preharvest strategy for reducing the prevalence of *E. coli* O157:H7 infection in cattle.

Zoonotic Implications

Enterohemorrhagic strains of *E. coli,* especially serotype *E. coli* O157:H7, have been linked in humans with hemorrhagic colitis, HUS, and thrombocytopenic purpura from eating contaminated foods such as beef and dairy products, vegetables, and apple cider, and from contaminated drinking water or from contact with infected animals or contaminated environments. As few as 100 *E. coli* O157:H7 bacteria can cause illness in humans.

In the United States the Centers for Disease Control and Prevention estimate that around 265,000 human STEC infections occur every year, of which approximately 36% are attributed to *E. coli* O157:H7 and the remainder to non-O157 serotypes.[13]

Between 2005 and 2009 a total of 16,263 confirmed cases of STEC infection in people have been reported from the 24 member states of the EU. For 2009 the notification rate of STEC infection within the EU was 0.75 per 100,000 population with between two and six deaths per year.[14] The highest

notification rate was recorded for the age group 0 to 4 years (7.2 per 100,000 population) followed by children aged 5 to 14 years (1.8 per 100,000 population).[14] Although outbreaks of EHEC infection in people are recorded regularly, public health surveillance data indicate that sporadic cases of infection greatly outnumber outbreak cases.[15]

The number of patients infected with EHEC that develop HUS, particularly children, has been estimated to be approximately 10%.[15] In 2009 a total of 242 cases of HUS were reported within the EU; the serogroup O157 was isolated in 47% of cases affecting children (0–4 years old) and the serogroup O26 in 15%.[14]

Most cases of STEC illness are attributable to food-borne infection, and in particular to the consumption of undercooked ground beef; however, acquisition of disease by direct contact with animals and manure at petting zoos and dairy farms are of increasing concern. Consumption of pink hamburgers at home or in restaurants is a risk factor for EHEC infection. Microbiological testing of ground beef patties from a large outbreak that occurred in the Pacific northwest between November 1992 and February 1993 suggested that the infectious dose for *E. coli* O157:H7 is fewer than 700 organisms. This represents a strong argument for enforcing zero tolerance for this organism in processed food and for markedly decreasing contamination of raw ground beef. In 2009 overall 9285 beef samples have been tested for the presence of EHEC in the EU; 2.3% were found positive for EHEC and 0.7% contained EHEC serogroup O157.[14] Argentina is the country with the highest recorded incidence of HUS in the world with around 400 cases per year. It also has the highest per capita consumption of beef of any country in the world.

A major source of the bacteria in ground beef is bovine feces, which contaminates carcasses before evisceration; the organism is thought to be spread from contaminated hides to the surfaces of carcasses at slaughter. In addition to feces and hides, STEC has been isolated from the oral cavities of cattle.

In May 2000, *E. coli* O157:H7 and *Campylobacter jejuni* contaminated the drinking water supply in Walkerton, Ontario, Canada. As a result, seven people died and over 2000 became ill. The pathogens causing the outbreak were attributed to contamination of the town's water well arising from cattle manure from a nearby cattle farm following a period of heavy spring rainfall. Failure to adequately chlorinate the water supply resulted in the contaminated water being consumed by the people in the town.

Visits to farms for recreational or educational purposes have become an important part of the tourism and leisure industries in some countries. The emergence of STEC, with its very low infectious dose and associated risks of serious human illness, has greatly increased the potential for zoonotic disease acquired from livestock, including those on open farms. The livestock of these farms may include sheep, goats, mature cattle and calves, pigs, donkeys, ponies, rabbits, guinea pigs, chipmunks, laying hens, bantams, ducks, geese, and a variety of waterfowl. Outbreaks of *E. coli* O157:H7 infection have occurred in people visiting these farms, and the *E. coli* O157:H7 has been isolated primarily from the calves and goats.

In a large outbreak of *E. coli* O157:H7 infections among visitors to a dairy farm (predominantly children), high rates of carriage of *E. coli* O157:H7 among calves and young cattle most probably resulted in contamination of both the hides of the animals and the environment. Contact with calves and their environment was associated with an increased risk of infection, whereas hand washing was protective. Thirteen percent of the cattle were colonized with *E. coli* O157:H7, which had the same distinct pattern on PFGE found in isolates from the patients. The organism was also recovered from surfaces that were accessible to the public.

Transmission of EHEC occurs by three major routes: food items such as undercooked meat, unpasteurized milk or cheese made from raw milk, person-to-person spread, and direct or indirect contact with animals. Infections have been associated with visits to cattle farms and farms open to the public, with consumption of farm products, and with camping on a cattle-grazing site. Infections have also been described in farm family members and other farm dwellers.

Economic Importance

The economic consequences of beef contaminated with *E. coli* O157:H7 are enormous. Since 1994 in the United States, millions of kilograms of ground beef have been recalled from retail outlets because of contamination with *E. coli* O157:H7. Such beef products must be destroyed and not used for animal or human food. Human illness associated with the most common food-borne pathogens alone cost the U.S. economy more than $7 billion each year. Some of these human outbreaks have been linked to the consumption of meat-based products or to contact with animals and their wastes.

PATHOGENESIS

EHEC are characterized by the presence of Stx genes, **locus for enterocyte effacement (LEE),** and a high molecular weight plasmid that encodes for a hemolysin. These three virulence factors are present in most *E. coli* associated with bloody diarrhea and HUS in humans.

The LEE is a large cluster of genes that are collectively responsible for the intimate attachment of the bacterium to the apical membrane of the enterocyte and subsequent destruction or effacement of the microvilli. The intimate attachment of the bacterial cell to the epithelium is attributed to the adhesin **intimin** and Tir, a bacterial protein, which is inserted into the host membrane and serves as the response for intimin. Both factors are part of the LEE in enteropathogenic *E. coli (EPEC)* and EHEC. Intimin appears to be an essential component in initiating attachment, colonization, and the subsequent pathologic changes that follow infection with EPEC and EHEC.

E. coli O157:H7 also possesses a high molecular weight plasmid that contains several putative virulence genes, including a pore-forming hemolysin. Virulence plasmids are common features of pathogenic *E. coli,* encoding toxins, adhesins, and other factors necessary for colonization, survival, and ability to cause disease in its animal host.

In ruminants, STEC persists and proliferates in the lower gastrointestinal tract and does not remain for long periods in the ruminant stomachs or duodenum. *E. coli* O157:H7 exhibits a tropism for the terminal rectum in cattle. In calves experimentally infected with *E. coli* O157:H7, in almost all persistently colonized animals, the majority of tissue-associated bacteria identified are in a region within 3 to 5 cm proximal to the rectoanal junction. This region contains a high density of lymphoid follicles, and microcolonies of the bacterium are readily detectable on the epithelium of this region by immunofluorescence microscopy. As a consequence of this specific distribution, *E. coli* O157:H7 are present predominantly on the surface of the fecal mass. Sampling the feces and terminal rectum or swabbing the rectal mucosa of cattle immediately after slaughter found higher numbers of *E. coli* O157:H7 at the site closer to the rectoanal junction, and low-level and high-level carriers (so-called supershedders) were identified. Carriage on the mucosal surface of the terminal rectum was associated with high-level fecal excretion.

Experimental Reproduction

Experimentally, *E. coli* O157:H7 causes fatal ileocolitis in newborn calves under 36 hours of age. Affected calves developed diarrhea and enterocolitis with attaching and effacing lesions in both the large and small intestines by 18 hours after inoculation.

Natural and experimental infection of calves from 13 to 30 days of age and mature cows with *E. coli* O157:H7 do not result in any clinical signs of disease, and no lesions were present at necropsy. A serologic response occurred in the calves but not in the cows.

Attaching and effacing intestinal lesions can be produced by experimental inoculation of 6-day-old conventionally reared lambs with *E. coli* O157:H7. All animals remain normal clinically, but attaching and effacing lesions occur in the cecum at 12 and 36 hours postinoculation and in the terminal

colon and rectum at 84 hours. This indicates that the well-characterized mechanisms for intimate attachment encoded by the LEE of *E. coli* O157:H7 may contribute to the initial events of colonization. Similar lesions can be produced in ligated intestine loops of 6-month-old sheep using *E. coli* O157:H7.

CLINICAL PATHOLOGY

STEC comprises over 400 different serotypes with diverse biochemical and physiologic characteristics and accordingly a large variety of detection methods are used. With the exception of the serotype O157:H7 for which an International Organization for Standardization (ISO) protocol for the detection in food and animal feedstuff is available, there are currently no internationally standardized procedures for the detection of non-O157 STEC.[15] For *E. coli* O157:H7, the most prevalent single STEC serotype associated with human illness, genetic detection assays, and the use of culture and enrichment media are well developed and widely used as routine diagnostic procedures. Non-O157 serotypes have been recognized as potential human pathogens with increasing occurrence worldwide; accordingly, detection methods and culture and enrichment broths to isolate the most prevalent pathogenic serotypes have been developed in recent years but have not yet been standardized.

Food, feedstuff, or feces samples may be directly plated onto selective media and/or differential agars, which are reliable in detecting STEC O157 at densities above 100 CFU/g.[5] Food samples, however, often contain few colony-forming units that still may suffice to cause clinical disease in people. Direct plating may fail to identify bacteria stressed or injured by manufacturing processes, transport, or storage. Furthermore, STEC cells may enter a dormancy state in which they are viable but not culturable, which can lead to an underestimation of the number of bacteria contained in the sample or even failure to isolate STEC.[16] Regardless of the culture protocol used, recovery of *E. coli* O157:H7 is more likely from fresh fecal samples than from frozen samples.

Enrichment before plating facilitates the recovery of injured bacteria and can decrease the detection limit to below 5 CFU/g. Tryptone soya broth and *E. coli* broth incubated at 35°C to 37°C for 18 to 24 hours are commonly used for nonselective enrichment. Selective enrichment media are supplemented with selective agents or antimicrobials to inhibit growth of competing microflora.[15] Several studies have reported incidental susceptibility of STEC O157 to various selective components in the enrichment medium, which may hamper the growth not only of apathogenic microflora but also of some potentially pathogenic STEC strains. The use of nonselective enrichment media such as buffered peptone water is therefore preferred over the use of selective enrichment media.[15]

Enrichment may be followed by immunomagnetic separation (IMS) with beads coated with O157-specific antibody before plating onto agar. Immunomagnetic separation is part of the official standard procedure for the detection of STEC O157:H7.

CONTROL

Studying STEC during the entire cattle-production process is problematic because of the complexity of the system and the complexity of the ecology of the organism. The development of economically feasible intervention strategies that are effective in reducing food-borne pathogens is a priority for both the beef and dairy industries.

The effective control of *E. coli* O157:H7 and other STEC serotypes will require the implementation of several different infectious disease control strategies and management procedures extending from the farm environment to the meat processing plant, the retail handling and processing of meat products, and the handling and cooking of beef products in the home.

The features of the ecology of *E. coli* O157:H7 that are important to consider in a control program include the following:

- Lack of a host specificity such that indistinguishable isolates can be found in a variety of species.
- Near ubiquitous distribution on cattle farms.
- Transient residence in the gastrointestinal tract of individual animals that is not associated with disease.
- A higher prevalence in animals with gastrointestinal flora disturbances such as those associated with transit, feed changes, or antimicrobial dosing.
- A markedly higher prevalence during warm months.
- Molecular subtyping indicates that specific subtypes can persist on a farm for years.
- Commercial feeds are sometimes contaminated with STEC and it seems likely that feeds represent an important route of dissemination.
- Mixed feeds collected from feeding troughs are commonly positive for STEC, as are water troughs, and feed and water probably represent the most common means of infection.
- Environmental replication in feeds and in the sediments of water troughs occurs and may account for the higher level of fecal shedding in the summer months.
- Because *E. coli* O157:H7 has been found to persist in and remain infective for at least 6 months in water trough sediments, this may be an important environmental in which the organism survives during periods when it cannot be detected, especially during cold months.
- Traditional means of controlling infectious diseases, such as eradication or test and removal of carrier animals, do not appear to be feasible.
- It is virtually impossible to exclude *E. coli* O157:H7 from beef-processing plants and carcasses.
- Cross-contamination of whole carcasses with fecal-derived bacteria occurs as a result of airborne transmission (during removal of the hide). Contaminated equipment and cross-contamination is inevitable during boning-out and grinding (where portions of carcasses from a large number of animals are commingled or make contact with a common piece of equipment).
- The very small numbers of STEC predicted to contaminate carcasses under highly effective control could be spread to a large volume of beef product during processing and multiply if the product experienced temperature abuse. Because the dose of STEC to cause human illness is very low, this dispersion of the organism throughout a high volume of product may constitute the greatest risk to public health.

The control of STEC will depend on implementation of management procedures that extend from the farm (preharvest), slaughtering process (postharvest), and retail handling and processing, to ultimately the consumer.

Preharvest beef safety production programs consist of policies, strategies, and procedures that are performed on food-producing animal farms with the objective of producing a safe and wholesome product free of antibiotic or chemical residues and with a minimum of pathogens that could be transferred through meat to humans. Some examples follow here.

Specific Strategies for Control of *Escherichia coli* O157:H7 at Preharvest Level

A stochastic simulation model was used to assess the benefit of measures implemented in the preslaughter period that are aimed at reducing the contamination of beef carcasses with STEC O157:H7. Control measures were based on either reducing the herd prevalence of infection; reducing the opportunity for cross-contamination in the processing plant by reordering of the slaughter procedures; reducing the concentration of *E. coli* O157:H7 in fresh feces or reducing the amount of feces, mud, and bedding ("tag") transferred from the hide to the carcass. Simulations suggested that the greatest potential is associated with vaccination and with an agent that reduces shedding of *E. coli* O157:H7 in feces. An industrywide reduction in the amount of tag attached to hides and addition of a source of cattle having a prolonged average fasting time were not predicted to have a large impact on the mean amount

of carcass contamination with *E. coli* O157:H7.

Animal Management Strategies

Water Systems and Runoff

Interventions at the water trough level offer significant potential to reduce STEC contamination and cross-contamination. Suggested potential strategies to reduce STEC survival in the water supply include chlorination, ozonization, frequent cleaning, and screens that reduce organic solids in water troughs. However, field studies found that chlorination of water troughs did not alter the prevalence of *E. coli* O157:H7 in the troughs or in the feces of cattle in those pens.

Environmental Control of STEC

The survival of STEC for extended periods of time (weeks to months) in livestock production environments may enable transfer of the organism back to cattle through contaminated feed or water. This creates a cycle of infection allowing STEC to be maintained in cattle herds. Effective control of STEC requires suppression at as many points in the cycle of infection as possible to reduce its spread. Minimizing contamination of water troughs and feed bunks together with adequate manure management should contribute to a significant reduction in the spread of STEC in cattle, crops, and water sources.

The fecal prevalence of STEC among mature dairy cattle is associated with the choice of bedding material used on a farm. The use of sawdust for bedding material for lactating dairy cows, as opposed to sand, was associated with a significantly higher fecal prevalence of *E. coli* O157:H7. The overall average herd prevalence was 3.1% and 1.4%, respectively, for cows on sawdust and on sand. The total number of days on which herds were positive for *E. coli* O157:H7 was higher for sawdust-bedded herds than for sand-bedded herds; 22 versus 14, respectively. These results provide evidence that specific farm management practices can influence the prevalence of *E. coli* O157:H7 on the farm.

Diet Changes

Feedlot and high-producing dairy cattle are fed rations with a high percentage of grain. When the starches that escape the ruminal microbial degradation move on to the large intestine, EHEC ferment the sugars and the populations of *E. coli* increase. Cattle fed grain rations shed larger numbers of *E. coli,* especially *E. coli* O157:H7 in barley-fed cattle. When cattle are abruptly switched from a high-grain ration to a forage diet, generic *E. coli* populations decline by 1000-fold within 5 days. Cattle naturally infected with *E. coli* O157:H7 shed smaller numbers of the organism when the ration is changed to a forage-based diet compared with cattle fed continuously on a high-grain diet. However, the magnitude of reduction is highly variable between studies and thus is not currently recommended. Fasting for 48 hours and type of diet before fasting has no effect on fecal shedding of *E. coli* O157:H7 in cattle. Thus feed withdrawal before slaughter should not increase the risk of STEC entering the food chain. However, refeeding 100% forage following a 48-hour fast results in a significant increase in the number of animals shedding *E. coli* O157:H7. This may occur when feeder cattle are moved from one farm to another through a sale barn and may be one of the reasons for the higher incidence of *E. coli* O157:H7 shedding by cattle when they first enter the feedlot.

Proposals aimed at dietary modifications must be balanced with the practical applications of commercial livestock feeding operations.

Direct Antipathogen Strategies

Several strategies have been examined that specifically target and directly kill pathogenic bacteria. These include the use of antibiotics, antimicrobial proteins produced by bacteria, bacteriophages, compounds that specifically target the physiology of pathogenic bacteria, and vaccination.

Vaccination Against Escherichia coli O157:H7

There is evidence that virulence factors secreted by the type III system can be used as effective vaccine components for the reduction of colonization of cattle by *E. coli* O157:H7. Vaccination of cattle with proteins secreted by *E. coli* O157:H7, three times at 3-week intervals, significantly reduced the numbers of bacteria shed in feces, the numbers of animals that shed, and the duration of shedding in an experimental model. Vaccination of cattle also significantly reduced the prevalence of *E. coli* O157:H7 in a clinical trial conducted in a typical feedlot. The pretreatment prevalence of animals shedding *E. coli* O157:H7 averaged 30%. The average proportion of cattle shedding the organism in vaccine-treated pens was 8.8%, and in nonvaccinated pens 21.3%. Because the type III-secreted antigens are relatively conserved among non-O157 EHEC serotypes, the vaccine formulation might be broadly cross-protective.

Using the pig as an experimental model, pregnant dams were vaccinated with *E. coli* O157:H7 adhesin ($intimin_{O157}$) at 2 and 4 weeks before farrowing. *E. coli* O157:H7 adhesin ($intimin_{O157}$)-specific antibody titers in colostrum and serum of dams were increased after parenteral vaccination. Neonatal piglets were allowed to suck vaccinated dams for up to 8 hours before being inoculated with a Shiga-toxin–negative strain of *E. coli* O157:H7. Piglets that had ingested colostrum containing *E. coli* O157:H7 adhesin ($intimin_{O157}$)-specific antibodies from vaccinated dams, but not those nursing sham-vaccinated dams, were protected from *E. coli* O157:H7 colonization and intestinal lesions. This supports the hypothesis that $intimin_{O157}$ is a potential antigen for an *E. coli* O157:H7 antitransmission vaccine.

A vaccination field trial evaluated the efficacy of *E. coli* O157:H7 vaccine in a sample of feedlots in Alberta and Saskatchewan. Pens of cattle were vaccinated once on arrival processing and again at reimplanting. The *E. coli* O157:H7 vaccine included 50 µg of type III-secreted proteins. Fecal samples were collected from 30 fresh fecal droppings within each feedlot pen at arrival, at revaccination, and within 2 weeks of slaughter. The mean pen prevalence of *E. coli* O157:H7 in feces was 5.0%, ranging from 0% to 90%. There was no significant association between vaccination and pen prevalence of fecal *E. coli* O157:H7 following initial vaccination at reimplanting or before slaughter.

Competitive Enhancement Strategies

The use of native or introduced microflora to reduce pathogenic bacteria in the intestine is termed a "probiotic" or competitive enhancement strategy. The principle is to promote growth of groups of beneficial bacteria that are competitive with, or antagonistic to, pathogens.

Probiotics

Probiotic bacteria are effective in reducing the duration of ruminal carriage of *E. coli* O157:H7 in cattle. Probiotics are live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance. The principle is that these beneficial organisms will combat the effects of stress and prevent undesirable microorganisms from becoming established in the gastrointestinal tract. Dietary supplementation of cattle with *Lactobacillus*-based and *Propionibacterium*-based direct-fed microbials reduced the prevalence of *E. coli* O157:H7 in both fecal and hide samples.

Sodium Chlorate Supplementation

Chlorate supplementation has been investigated as a preharvest strategy to reduce populations of *E. coli* O157:H7 and *Salmonella* spp. in food animals. Certain bacteria can respire anaerobically by reducing nitrate to nitrite via the intracellular enzyme nitrate reductase. This same enzyme also reduces chlorate to chlorite, a cytotoxic end product. Chlorate significantly reduced *E. coli* O157:H7 populations in ruminal fluid incubations, wild-type *E. coli,* inoculated *E. coli* O157:H7 and total coliforms in cattle, and inoculated *E. coli* O157:H7 in sheep. The administration of sodium chlorate in the feed of cattle preharvest for 24 hours reduced the population of *E. coli* O157:H7 strains approximately by two logs (10^4–10^2) in the rumen and three logs (10^6–10^3) in the feces.

Control of *Escherichia coli* O157:H7 During Slaughtering and Postharvest Stage

Meat Inspection Service and Surveillance

As a result of public concern about *E. coli* O157:H7, the meat inspection service in many countries has been reorganized to deal with control of the organism in the processing of beef. In the United States, the presence of *E. coli* O157:H7 in ground beef was declared an **adulterant**. Surveillance systems have also been established in many countries to obtain more information about the presence of the organism and to report outbreaks, and considerable research has emerged.

Elaborate *E. coli* O157:H7 detection systems are now in place in abattoirs in many countries as part of the **Hazard Analysis of Critical Points System (HACCP)** to ensure that contamination of beef carcasses with *E. coli* O157:H7 is below certain legislated levels. Although laboratory testing focuses on this serotype in many countries, screening has been extended to other pathogenic serogroups associated with illness in humans, such as O26, O103, O91, O145, and O111 in several countries.[2]

Major progress has been made in the last decades in the processing of beef carcasses following slaughter to reduce the microbial contamination of beef using the HACCP.

HACCP is a process control system designed to identify and prevent microbial and other hazards in food production. It includes steps designed to prevent problems before they occur and to correct deviations as soon as they are detected. Such preventive control systems with documentation and verification are widely recognized by scientific authorities and international organizations as the most effective approach available for producing safe food.

In the United States, as of 1996, the U.S. Department of Agriculture (USDA) adopted the Pathogen Reduction HACCP system, which includes four major elements:

- Every plant must adopt and carry out its own HACCP plan, which systematically addresses all significant hazards associated with its products.
- Mandatory *E. coli* testing in slaughter plants: Every plant must regularly test carcasses for *E. coli* to verify the effectiveness of the plant's procedures for preventing and reducing fecal contamination.
- Pathogen reduction performance standards for *Salmonella*: All plants and plants producing raw ground products must ensure that their *Salmonella* contamination is below the current national baseline prevalence.
- Sanitation standard operating procedures: Every plant must adopt and carry out a written plan for meeting its sanitation responsibilities. Effective sanitation in slaughter and processing plants is essential to prevent adulteration of meat and poultry products.

HACCP is endorsed by such scientific and food safety authorities as the National Academy of Sciences and the National Advisory Committee on Microbiological Criteria for Foods, and by such international organizations as the Codex Alimentarius Commission and the International Commission on Microbiological Specifications for Foods.

Postharvest Decontamination Techniques

Meat carcasses may become contaminated from fecal material, the stomach contents, and the hide. Additional sources of cross-contamination exist in the slaughter process, such as processing tools and equipment, structural components of the facility, human contact, and carcass-to-carcass contact.

Decontamination techniques for carcasses are targeted at reducing or eliminating bacteria that may be human pathogens as well as those that may cause meat spoilage. The pathogenic bacteria of most concern include *E. coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes, Campylobacter* spp., *C. botulinum, C. perfringens, Staphylococcus aureus, Aeromonas hydrophila,* and *Bacillus cereus.*

Meat processors strive to produce raw products that have low levels of bacteria on the surface and no pathogenic bacteria. However, the process is not done in a sterile environment and contamination is unavoidable, and occasionally pathogenic microorganisms may come into contact with the surface of the meat carcass. Routine slaughter practices have evolved over the years to reduce the likelihood of inadvertent microbial contamination. This evolution has led to the adoption of the **hurdle technology** approach to microbial carcass interventions.

The principles of hurdle technology state that, if the initial microbial load is substantially reduced as a result of carcass decontamination procedures, fewer microorganisms are present, which are then more easily inhibited in subsequent processing steps. The effectiveness of hurdle technology has been demonstrated experimentally for beef decontamination technologies under controlled conditions. The concept of hurdle technology for beef carcass decontamination has also been validated to be effective in field studies in beef-processing facilities.

The following are some of the more widely used and researched intervention strategies:

- **Hot water rinse**. There is substantial scientific evidence that hot water (>74°C) will produce a sanitizing effect on beef carcasses, and this is widely practiced in the industry.
- **Steam pasteurization**. The commercialization of the steam pasteurization system has been successful and it is in use in many large beef slaughter facilities in North America. Hot water/steam vacuum systems are designed to remove visible spots of contamination from small areas on the carcass and are used to augment the traditional knife trimming. Steam pasteurization is a process in which beef carcasses are placed in a slightly pressurized, closed chamber at room temperature and sprayed with steam that blankets and condenses over the entire carcass. This raises the surface temperature to 90°C (195°F) or 93°C (200°F) and kills nearly all pathogens. Carcasses then are sprayed with cold water.
- **Steam vacuum**. Steam or hot water is sprayed on a beef carcass followed by vacuuming, which has the combined effect of removing and/or inactivating surface contamination. The handheld device includes a vacuum wand with a hot water spray nozzle, which delivers water at approximately 82°C to 88°C (180°F–190°F) to the carcass surface, as well as the vacuum unit. Steam vacuuming is approved for use by the USDA-Food Safety and Inspection Service (FSIS) as a substitute for knife trimming for removing fecal and ingesta contamination when such contamination is less than 2.54 cm at its greatest dimension.
- **Chemical rinses**. Organic acids are typically applied as a rinse to the entire surface of the carcass. The USDA-FSIS approved the use of organic acid solutions such as acetic, lactic, and citric acids at concentrations of 1.5% to 2.5%. Acetic and lactic acids have been most widely accepted as carcass decontamination rinses. The effectiveness of organic acids is best achieved shortly after hide removal, when the carcass is still warm.

Progress Made With Decontamination Processes

The multiple decontamination processes, as applied in actual plant settings, have resulted in significant improvements in the microbiological quality of beef. There is considerable evidence to support the effectiveness of in-plant application of multiple decontamination technologies (hurdle technology). Reductions were achieved from 43% of lots sampled preevisceration as positive for *E. coli* O157:H7 to 1.9% remaining positive post-processing after multiple decontamination methods on the slaughter floor.

In February 2005, the beef industry welcomed news from the USDA-FSIS showing a significant drop in *E. coli* O157:H7 prevalence in 2004, compared with 2003. The FSIS data showed that the percentage of *E. coli* O157:H7–positive ground beef samples collected in 2004 fell by 43.3% compared with

the previous year. The data showed that, between 2000 and 2004, the percentage of positive samples of *E. coli* O157:H7 had declined by more than 80%. FSIS also reported that there were six recalls related to *E. coli* O157:H7 in 2004 compared with 12 in 2003 and 21 in 2002.

Irradiation

Irradiation of beef in the postharvest stage is a process that could be used to inactivate pathogens. At the present time, the percentage of beef being irradiated is very small. Constraints include reluctant consumer acceptance of radiation-treated food, increased price of production, and the irradiation's negative effect on odor and flavor.

Consumer Education on Handling and Cooking Meat

To prevent infection with STEC, consumers must be encouraged to follow four simple steps: chill promptly; clean hand and kitchen surfaces; separate, do not cross-contaminate; and cook thoroughly.

Visitors to Animal Farms

Farm animals and the farm environment present a variety of possible sources of infection with STEC. Farm visits are popular among city families for holidays and family gatherings, and schools in urban areas frequently promote educational farm visits for their students. The consumption of unpasteurized milk by visiting children and close physical contact with animals have been documented as most likely sources of infection in some outbreaks of *E. coli* O157:H7 infection. Farm animals and the farm environment present a variety of possible sources of infection. Visitors to animal farms, especially groups such as schoolchildren, must avoid petting animals whose hair coats and skin may harbor *E. coli* O157:H7. STEC of bovine origin can infect humans in the farm environment. Many dairy-farm residents regularly consume unpasteurized milk, which is a potential source of STEC.

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BRAXY (BRADSOT)

Braxy is an acute infectious disease of sheep in Britain characterized by inflammation of the abomasal wall, toxemia, and a high mortality rate. The disease was common in the early twentieth century but now is extremely rare.

SYNOPSIS

Etiology *Clostridium septicum* and ingestion of frosted feedstuffs

Epidemiology Weaners and yearling sheep in winter

Clinical findings Rapid death

Clinical pathology Death too rapid

Necropsy findings Pathognomonic lesion in abomasum

Diagnostic confirmation Typical abomasal lesion and positive fluorescent antibody staining of organism in lesion

Treatment None

Control Annual vaccination preceding the period of risk

ETIOLOGY

C. septicum is a common cause of malignant edema in animals.

EPIDEMIOLOGY

Braxy occurs only in midwinter when there are heavy frosts and snow, and usually only in weaner and yearling sheep. It has occurred in experimental sheep receiving infusions of acetic acid into the abomasum, and these were thought to cause abomasitis. Adult animals in an enzootic area appear to have acquired immunity.

C. septicum is a soil-borne organism and in many areas can be considered as a normal inhabitant of the ovine intestinal tract.

The disease occurs in the UK and various parts of Europe and has been reported in the southern part of Australia but appears to be rare in North America. It is now not of major importance because of its low prevalence, although it once was sufficiently common to be an important cause of loss in some countries. In affected sheep the case–fatality rate is usually about 50%, and in enzootic areas an annual loss of 8% has been reported.

PATHOGENESIS

Presumably a primary abomasitis, associated with the ingestion of frozen grass or other feed, permits invasion by *C. septicum,* resulting in a fatal toxemia.

CLINICAL FINDINGS

There is a sudden onset of illness with segregation from the group, complete anorexia, depression, and high fever 42°C (107°F) or more). The abdomen may be distended with gas, and there may be signs of abdominal pain. The sheep becomes recumbent, comatose, and dies within a few hours of first becoming ill.

CLINICAL PATHOLOGY

Antemortem laboratory examinations are of little value in establishing a diagnosis.

NECROPSY FINDINGS

There are localized areas of edema, congestion, necrosis, and ulceration of the abomasal wall. Congestion of the mucosa of the small intestine may also be present, and there may be a few subepicardial petechiae. *C. septicum* can be isolated by smear from the cut surface of the abomasal wall or by culture from the heart, blood, and other organs of fresh carcasses. Bacteriologic examinations of tissues must be performed within an hour of death if the diagnosis is to be confirmed.

Mortality in calves with braxy-like lesions in the abomasum is also recorded.

Samples for Confirmation of Diagnosis

- Bacteriology: frozen abomasum, in air-tight container; four air-dried impression smears from freshly cut surface of abomasal mucosa (anaerobic culture, fluorescent antibody test)
- Histology: fixed abomasum

DIFFERENTIAL DIAGNOSIS

Clinical diagnosis of braxy is difficult. At necropsy the lesions of abomasitis are characteristic, especially if the disease occurs under conditions of severe cold. Overeating on grain may cause local patches of rumenitis and reticulitis, but there are no lesions in the abomasum. Braxy may resemble infectious necrotic hepatitis, but there are no liver lesions in braxy. The final diagnosis depends on isolation of *C. septicum* from typical alimentary tract lesions.

TREATMENT

No treatment has been found to be of any value.

CONTROL

Management of the flock is important. The sheep should be yarded at night and fed hay before being let out to the frosted pasture each morning. Vaccination with a formalin-killed whole culture of *C. septicum,* preferably two injections 2 weeks apart, is also an effective preventive.

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ENTERIC DISEASE ASSOCIATED WITH *CLOSTRIDIUM PERFRINGENS*

C. perfringens resides in the intestinal tract of domestic animals and can produce a number of toxins that result in enteric and histotoxic disease. *C. perfringens* isolates are classified into one of five types, types A–E, depending on their ability to produce the four major lethal toxins: α-toxin, β-toxin, ε-toxin, and ι-toxin. The activities of these major lethal toxins are the basis of the pathogenesis of the classical enterotoxemias attributed to this organism and described later. More recently, it has been recognized that *C. perfringens* produces other toxins that are probably important in animal disease. These include an enterotoxin and a cytotoxic β-2 toxin, the latter encoded by the *cbp2* gene.[1,2] Regulation of the expression of the genes responsible for α-, β-, β-2-, and NetB-toxin production, the latter involved in the pathogenesis of necrotic enteritis in chickens, is performed by the proteins VirR and VirS, whereas the regulation of ε- and ι-toxins is not yet fully understood.[1,3]

The amino acid sequence of the β-2 toxin has little homology with that of the major β-toxin, and they are only weakly related immunologically, but the biological activity of the two toxins is similar and both are cytotoxic and cause hemorrhagic necrosis of the intestinal wall. The importance of enterotoxin and the β-2-toxin to animal disease is still uncertain. Both appear important in the cause and pathogenesis of enteric disease in pigs. The β-2-toxin may be important in enterocolitis in foals and adult horses as Cbp2-positive *C. perfringens* type A has been isolated from diarrheic foals and adult horses; however, the significance of these isolations to the disease is still not fully determined. *C. perfringens* normally resides in the intestine, but plasmids encoding virulence genes can be transferred to resident strains from environmental ones converting these into enteropathogens.[4] Surplus dietary carbohydrate or protein that exceeds the capacity of intestine to absorb it are used by *C. perfringens* for growth and toxin production, so it is a risk factor for *C. perfringens*-associated disease.[1] A multiplex PCR has been described for the rapid toxin typing of *C. perfringens* isolates.[5]

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ENTEROTOXEMIA ASSOCIATED WITH *CLOSTRIDIUM PERFRINGENS* TYPE A

The role of *C. perfringens* type A in the pathogenesis of diseases of animals is uncertain because the organism forms part of the bacterial flora of the alimentary tract in many normal animals. However, there are an increasing number of reports that attribute disease and mortality to this organism. The validity of these attributions remains to be fully determined, but they are listed next.

Enterocolitis in Horses

Enterocolitis in foals and adult horses is an etiologically poorly defined syndrome. Enterocolitis associated with *C. difficile* is one cause and is covered under that heading elsewhere in this text. In addition, enterocolitis associated with *C. perfringens* type C is covered under that heading. There remains a syndrome of enterocolitis that is manifested with enteritis, diarrhea, and colic, and a high case fatality, and one that is diagnosed most often at postmortem. It appears to occur worldwide and although occurrence is sporadic, there is the perception that there is an increasing prevalence of this disease. A study of risk factors in the western United States found that stock horse breeds were more at risk and that the presence of other livestock on the farm, and housing in a stall or drylot for the first 3 days of life, was associated with increased risk. Other studies have implicated barn hygiene as a factor that should be considered in preventive procedures.

There have been a number of clostridial species that have historically been associated with the syndrome besides *C. perfringens* type A. In some cases, this association has been by identification of type-specific toxin in the intestine of the affected horse but in others it has been made by the presence of large numbers of the incriminated organism in affected animals in comparison to occurrence and numbers in normal horses. This association has risk because the number of clostridia in the intestine can be influenced by diet, clostridia can multiply in the intestine following death, and they can exist in different forms that may be variably isolated with different cultural techniques.

Equine Intestinal Clostridiosis

A syndrome historically named equine intestinal clostridiosis has been attributed to intestinal infection with *C. perfringens* type A in adult horses. The syndrome was characterized by an acute profuse watery diarrhea with high mortality in adult horses and the demonstration of large numbers of *C. perfringens* type A in the intestine at postmortem. It was described as occurring in horses with hemorrhagic cecitis and colitis similar to colitis X, in horses collapsing and dying following exercise, and in other circumstances. Diarrhea and death were reproduced with massive (biologically implausible) oral challenge with broth cultures of these organisms, and colic and hemorrhagic gastroenteritis were produced by intravenous injection of ponies with *C. perfringens* type A enterotoxin. The evidence of an association of *C. perfringens* type A with disease in these early studies was equivocal, but *C. perfringens* type A can be isolated from both foals and adults with enterocolitis. However, isolation from this disease and causal association remain to be determined. *C. perfringens* is common in the environment of foals, and one study in over 128 healthy foals found that *C. perfringens* type A could be isolated from the feces of the majority of foals at 3 days of age. *C. perfringens* with the gene for β-2-toxin expression were found in the feces of 28 foals and with the enterotoxin gene in five foals. Consequently, the isolation of *C. perfringens* type A expressing the gene for β-2-toxin does not constitute a causal diagnosis. There is, however, a suggestion that *C. perfringens* type A that expresses the gene for β-2-toxin may be the particular subset of this isolate that is responsible for this disease.

Enteritis in Piglets

C. perfringens type A is associated with diarrheic food poisoning in humans, and a similar diarrhea in pigs may be produced by infection with this organism. The disease is manifested with a watery yellow diarrhea occurring in piglets under 5 days of age, usually in the first 3 days of age, and a high morbidity but low case fatality. At postmortem, there is a mild enterocolitis and villous atrophy. It is controlled with sanitation procedures or with the type of prophylactic procedures used with enterotoxemia associated with *C. perfringens* type C. Simultaneous infection with *Isospora suis* and *C. perfringens* may cause more severe disease.[1,2]

Hemorrhagic Enterotoxemia and Hemolytic Disease in Cattle, Sheep, and Goats

There are reports of a highly fatal hemolytic disease in cattle, sheep, and lambs (yellow lamb disease), of an acute hemorrhagic enteritis in calves and adult cattle, and of an acute hemolytic enterotoxemia in foals and goats, associated with the presence of large numbers of *C. perfringens* type A in the intestine. These reports have some credibility because of the activity of the primary toxin of *C. perfringens* type A, α-toxin, which possesses

phospholipase C and sphingomyelinase activity and consequently hemolytic action. The presence of β-2-toxin in these strains may also contribute to the pathogenicity.

Some credibility is also engendered by reports, albeit occasional, of similar syndromes in different geographic areas and by different institutes.

In the hemolytic disease there is an acute onset of severe depression, collapse, mucosal pallor, jaundice, hemoglobinuria, dyspnea, and the presence of severe abdominal pain. Temperatures range from normal to 41°C (106°F). The disease is highly fatal, most affected animals dying within 12 hours of the onset of illness, although occasional animals survive for several days. Large numbers of *C. perfringens* and the presence of the specific toxin in feces is used to make a presumptive diagnosis. At necropsy the cardinal features are pallor, jaundice, and hemoglobinuria. The kidneys are swollen, dark brown in color, and may contain infarcts; the liver is pale and swollen and there may be hydropericardium and pulmonary edema. There is extensive necrosis of the small intestine. Clostridia dominate the bacterial population of the small intestine, as indicated by smears made from the contents, and α-toxin is present in large quantities. The toxin is present in large quantities in the intestine, which is indicative of the existence of the disease.

The syndrome is very similar to that associated with chronic copper poisoning and leptospirosis in calves.

In the hemorrhagic enteritis of calves, foals, and adult cattle the syndrome observed is indistinguishable from that associated with *C. perfringens* types B and C. The disease in adult cattle is most common in the period shortly after calving. The experimental disease in lambs and calves produced by the intravenous injection of toxin is characterized by transitory diarrhea and hyperemia of the intestinal mucosa. Type A antiserum has been effective in prevention of the disease in calves, and a formalinized vaccine has shown some immunizing capacity in sheep.

Abomasal Ulcer

C. perfringens type A has been suspected in the etiology of abomasal ulcers in suckling beef calves in western North America and is less common elsewhere.[3,4] A clonal population of *C. perfringens* type A was isolated from ulcers in a 3-month-old calf, but its role in the causation of this syndrome is still unclear. A study of the prevalence and bacterial colonization of fundic ulcers in veal calves, which are more associated with welfare and nutritional factors than pyloric ulcers, recovered less *C. perfringens* from affected compared with healthy abomasa.[5]

Jejunal Hemorrhage Syndrome in Cattle

C. perfringens type A has been proposed as a cause of this disease, largely based on the isolation of this organism from the intestine of affected animals.[6] This organism is present in the intestinal tract of normal animals and, although it is possible that a subset of Cbp2-positive, β-2-toxin–producing *C. perfringens* type A organisms are responsible for this disease, the current evidence is equivocal.[7] Animals are often found dead, but clinical signs include going off-feed, restlessness, incoordination and staggering, tachycardia, weak ruminal contractions, and abdominal colic.[8] Adult cattle are more typically affected, but cases in 9-month-old calves have been described.[8] Despite the pathogenesis of this disease being imperfectly understood,[9] a commercial toxoid and autogenous vaccines against *C. perfringens* type A are available in North America.

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ENTEROTOXEMIA ASSOCIATED WITH *CLOSTRIDIUM PERFRINGENS* TYPES B, C, AND E

SYNOPSIS

Etiology β-Toxin, a trypsin-sensitive toxin produced by *C. perfringens* types B and C, produces hemorrhagic enteritis and ulceration of the intestinal mucosa resulting in diarrhea and dysentery in young lambs, goats, calves, pigs, and foals. β-2 toxin also contributes to these diseases. A number of diseases associated with these clostridia occur in different parts of the world and are given specific names.

Epidemiology Young animals, with the exception of struck in sheep, often occurring as outbreaks with a high case–fatality rate in which there is buildup of infection.

Clinical findings Rapid course with hemorrhagic diarrhea, abdominal pain, and toxemia.

Necropsy findings Focal (type B) or extensive (type C) areas of necrosis in the small intestine.

Diagnostic confirmation Clinical signs, gross and microscopic pathology from sacrificed or freshly dead animals, and direct or cultural examination for clostridia.

Treatment Antibiotics, specific antitoxin, supportive therapy.

Control Vaccination.

ETIOLOGY

The general etiology of these diseases is given in this section but, because of differing circumstances of occurrence, the description of the epidemiology of these diseases is given separately according to animal species.

The causative clostridia are commonly found in soil, the animals' housing environment and the alimentary tract of healthy animals, and management factors precipitate disease. The diseases produced by these organisms occur in animals in the first few days of life, with the exception of the disease struck in sheep. Their predominance in very young animals may be caused by the immaturity of their alimentary tracts, the β-toxin being readily inactivated by trypsin, and because of the ready colonization of the gut by *C. perfringens* in the absence of a mature intestinal flora. It is probable that many animals become subclinically challenged but do not show clinical illness, because antitoxin has been detected in clinically normal animals.

The bacteria are capable of forming spores that survive for long periods. Generally, rapidly growing, well-nourished animals are most susceptible. The toxins produced are alpha, beta, and epsilon in type B, and alpha and beta in type C.

β-2 toxin is also produced by some of these organisms and appears important to their pathogenicity in pigs and horses. There are subtypes of these organisms with differing toxin production abilities. α- and ι-toxin are produced by type E, which is a far less common cause of enterotoxemia in calves, kids, and lambs but has been associated with an outbreak of enterotoxemia of adult cattle in Argentina.[1]

The diseases that are produced by these organisms in the different animal species, and the organisms' names, are as follows:

- **Lamb dysentery** associated with *C. perfringens* type B. An enterotoxemia of young lambs is also associated with *C. perfringens* type C.
- **Goat enterotoxemia** associated with *C. perfringens* type C and rarely by type B.
- **Necrotic enteritis of pigs, pig enterotoxemia** associated with *C. perfringens* types C and less commonly by type B.
- **Foal enterotoxemia** associated with *C. perfringens* types C and B.

- **Calf enterotoxemia** associated with *C. perfringens* types B and C (and rarely E).
- **Struck**, associated with *C. perfringens* type C, affects adult sheep, particularly when feed is abundant.

EPIDEMIOLOGY

Lamb Dysentery and Type C Enterotoxemia

Occurrence

Lamb dysentery associated with type B occurs in Great Britain, Europe, and South Africa and is an important disease in these countries. In contrast, this disease is rare or absent in Australia, New Zealand, North America, and Japan, in which type C infections are more important. The geographic variation may be caused by variation in the occurrence of the types of *C. perfringens*. Lamb dysentery does not occur in New Zealand, and *C. perfringens* type B has not been found in sheep or soil samples in that country.

Type C enterotoxemia in lambs and goats occurs particularly with shed lambing in North America and where there is close stocking of ewes and lambs at lambing. It is also recorded in Australasia.

Animal and Environmental Risk Factors

In lamb dysentery, the incidence of clinical disease in groups of lambs may reach as high as 20% to 30%. In an outbreak, the disease initially affects 1- to 4-day-old lambs and the clinical course is very short. A characteristic of the disease is the tendency for an increase in incidence rate as lambing progresses and for the involvement of older lambs, up to 2 to 3 weeks of age, which survive for longer periods. The case–fatality rate approaches 100%.

In Great Britain lamb dysentery occurs primarily in the hill breeds of sheep, breeds that have a small litter size but good milk production, and the appearance of the disease in a particular year appears to be related to weather conditions that allow sufficient pasture growth to produce profuse lactation in the ewes. The time of onset of the disease in a lambing season is related to the weather conditions that predispose to its occurrence.

Type C enterotoxemia in lambs and goats is prevalent in cold weather and on farms in which ewes are kept closely confined in small yards or fields for lambing and kidding. Gross contamination of the surroundings with the causative bacteria is likely to occur in these circumstances. The disease can occur as an outbreak with an attack rate of 15% to 20% and a case fatality that approaches 100%. Type C enterotoxemia is more common in single-born lambs and is largely restricted to lambs 12 hours to 4 days of age. Sporadic disease occurs in orphan lambs reared on milk replacer, which appear particularly at risk and may develop the disease at up to 2 weeks of age.

Necrotic Enteritis of Pigs

Occurrence

Necrotic enteritis is an important disease of piglets, particularly in intensive pig units. It occurs in most countries but is most common in certain areas in the United States, Europe, and the UK.

Animal and Environmental Risk Factors

The organisms are recoverable from the skin of sows and the feces of affected piglets, and infection probably occurs during suckling. The number of piglets affected varies between herds and between litters. The disease may occur sporadically in a piggery but commonly occurs as an outbreak affecting several litters within a given time period. Pigs up to 7 days of age are most commonly affected, and susceptibility to disease and its severity decreases with age. Peracute disease with rapid death occurs in piglets affected at 1 to 2 days of age, whereas piglets affected at 1 to 2 weeks show a more protracted clinical course. The case fatality is high, and in severe outbreaks 80% of piglets at risk may die. The disease tends to become endemic in pig units and to recur on the same premises in succeeding years.

Insufficient cleaning and disinfection of farrowing pens, the housing of pigs on concrete, and the routine use of antibiotics to which *C. perfringens* is resistant, such as the aminoglycosides, have been proposed as risk factors for buildup of infection in swine units.

Enterotoxemia in Foals

Enterotoxemia in foals has been associated with both *C. perfringens* type B and type C. Type C predominates in reports from North America. Cases are usually sporadic, with most in single animals under 7 to 14 days of age, although *C. perfringens* type C and β-toxin has been demonstrated in cases of necrotic enteritis in adult horses.[2] The factors that predispose to disease in foals are poorly defined. Isolates of *C. perfringens* from 55 Canadian horses with clinical colitis, including 12 foals, were less cytotoxic than a β-toxin–producing control, and none were positive for enterotoxin, NetB, or large cytotoxin gene.[3]

Enterotoxemia in Calves

Enterotoxemia caused by *C. perfringens* type B or C is uncommon in calves. The disease usually occurs as outbreaks of severe dysentery with some deaths in calves 7 to 10 days old, although calves up to 10 weeks of age may be affected.

Struck in Sheep

Struck in adult sheep on good pasture in spring is limited in its occurrence to certain localities in Britain and is rarely reported.

PATHOGENESIS

The organism is ingested from soil and fecal contamination on the surface of the dam's udder. It proliferates and attaches to the surface of the epithelial cells of the intestinal villus, but toxin production and mucosal damage may precede attachment. Information on the rate of carriage in normal animals is scant and the factors that allow proliferation and attachment are poorly understood.[4] Toxigenic strains of *C. perfringens* types B and C produce both α- and β-toxins.

The α-toxin is a lethal toxin that is produced in varying amounts by isolates of both types. It is a phospholipase, and hydrolysis of membrane phospholipids in erythrocytes, platelets, leukocytes, and endothelial cells results in cell lysis or other forms of cytotoxicity. The β-toxin causes increased capillary permeability and may facilitate its uptake from the intestine. β-Toxin is a necrotizing toxin and initially produces damage to the microvilli with degeneration of mitochondria, with eventual destruction and desquamation of the intestinal epithelial cells and the production of a hemorrhagic enteritis and ulceration of the intestinal mucosa.[5]

The age incidence of these diseases may be partially explained by the observation that β-toxin is highly sensitive to inactivation by trypsin, which is a component of normal pancreatic proteases. Colostrum contains a trypsin inhibitor, and trypsin is decreased or absent in affected pigs. Experimentally administered soybean flour used as a protease inhibitor converts experimentally induced clostridial enteritis from a nonfatal to a fatal disease.

CLINICAL FINDINGS

Lamb dysentery can be manifested by sudden death without premonitory signs in peracute cases. In the more common acute form, there is loss of sucking drive and severe abdominal pain manifested by bleating, stretching, and looking at the abdomen. Lambs pass brown, fluid feces sometimes containing blood, and defecation is often accompanied by painful straining. Death usually occurs after a period of recumbency and coma and within 24 hours of the onset of illness. On farms in which the disease has become established, cases may occur in older lambs up to 3 weeks of age and occasional cases may survive for several days. A chronic form of the disease in older lambs is called "pine," and manifests with chronic abdominal pain and reluctance to suck but no diarrhea, and is recognized and responds to treatment with specific antiserum.

Necrotic enteritis in piglets is also manifested with rapid death in young animals and more prolonged disease in slightly older piglets. Affected pigs become dull and depressed and exhibit diarrhea, dysentery, and gross reddening of the anus. The feces of piglets affected within 2 to 3 days of life is watery and initially yellow but in a

proportion of pigs will become hemorrhagic and red-brown in color and contain necrotic debris. The clinical course in piglets affected at this age is usually less than 24 hours; they rapidly become dehydrated, hypoglycemic, hypothermic, and comatose. Piglets affected at an older age have a fluid, yellow-colored diarrhea and blood may not be evident. Frequently the majority of litters born during an outbreak will be affected, although affected litters may include some normal pigs. Occasionally weaned pigs are affected. Acute outbreaks in herds may be followed by the occurrence of chronic necrotizing enteritis.

Foals with enterotoxemia associated with *C. perfringens* type B or C show evidence of severe depression, pronounced toxemia, and marked abdominal pain. Affected foals are a few days old and have an acute attack of collapse with bloody feces, subnormal temperature, fast pulse and respiratory rate, and death within a few hours. Colic may be evident, and a major differential diagnosis is an acute intestinal accident. The clinical course is very short and diarrhea does not occur in many cases. There are limited descriptions of the clinical disease in foals because of its sporadic occurrence and rapid course.

In calves, signs include diarrhea, dysentery, and acute abdominal pain accompanied by violent bellowing and aimless running. There may be additional nervous signs, including tetany and opisthotonus. In very acute cases, death occurs in a few hours, sometimes without diarrhea being evident. In less severe cases, the illness lasts for about 4 days and recovery is slow, usually requiring 10 to 14 days.

Struck in adult sheep is manifested only by sudden death with no clinical signs observed beforehand. Occasionally death is preceded by abdominal pain and convulsions.

CLINICAL PATHOLOGY

The disease in all species is so acute and so highly fatal that the diagnosis is usually made on necropsy material.[6] Antemortem laboratory examinations are not widely used in diagnosis and there is no database, but the predominance of clostridia in a fecal smear may suggest a diagnosis of hemorrhagic enterotoxemia. Specific antitoxins are detectable in the sera of recovered animals. A severe hypoglycemia has been observed in baby pigs dying of the disease, but this is not specific in this infection.

NECROPSY FINDINGS

The major lesion in all species is hemorrhagic enteritis, with ulceration of the mucosa in some cases. With type B infections the lesions occur as localized areas of necrosis, usually most evident in the ileum. The intestinal mucosa is dark red and the ulcers are large (up to 2.5 cm in diameter) and almost transmural. Intestinal contents are bloodstained and may contain fibrin clots, and there is often excess serosanguineous fluid in the abdominal cavity.

With type C infection the areas of necrosis are more extensive, involving entire segments of small intestine and often inducing a peritonitis.[7] Subendocardial and subepicardial hemorrhages are common in ruminants dying of enterotoxemia. If the necropsy of adult sheep is delayed for several hours, the fascial tissues may develop the appearance of malignant edema. Carcasses of 7- to 10-day-old pigs may lack the severe hemorrhagic enteritis typical of the disease in newborn pigs. The less acute disease course in this older age group often results in a yellow, fibrinous deposit on the intestinal mucosa, accompanied by large quantities of watery, lightly bloodstained ingesta in the lumen.

Generally, the histologic features of gut segments affected by these types of enterotoxemia include mucosal hemorrhage, necrosis, fibrin exudation, and a neutrophilic infiltrate. Large numbers of bacterial rods line the luminal surface of these lesions. Unfortunately, postmortem autolysis frequently eliminates the possibility of identifying some of the features.

Smears of intestinal contents can be stained and examined for large numbers of clostridium-like organisms, and filtrates of the contents may be tested for toxin content. Definitive typing of the clostridia has traditionally been via in vivo assays, but these are undesirable on humanitarian grounds and are being replaced by immunoassays, such as enzyme-linked immunosorbent assay (ELISA) and passive latex agglutination. A rapid passive latex agglutination test permits confirmation of the presence of α-toxin but does not permit distinction between the various types of *C. perfringens* capable of producing the toxin. A multiplex PCR test enables characterization of *C. perfringens* isolates based on their genotypic potential for toxin production. PCR techniques detect the genes encoding the major toxins and are promoted for replacing in vivo tests for toxin.[8] The PCR test can differentiate toxigenic clostridial isolates recovered from diseased animals and nontoxigenic isolates recovered from normal animals.

Samples for Confirmation of Diagnosis

- Bacteriology: 20 to 30 mL of intestinal content, frozen in a glass or plastic leak-proof container (latex agglutination, anaerobic CULT, bioassay, PCR); air-dried smears of mucosal surface from several levels of small intestine (cyto: Gram stain)
- Histology: fixed ileum, jejunum (several segments of each)

TREATMENT

In individual cases the disease is often too acute for effective therapy but fluid and supportive therapy are indicated. Hyperimmune serum is the specific therapy and the major therapy of value. Oral and parenteral administration of penicillin may prevent further proliferation of organisms and production of toxins.

CONTROL

Vaccination, preferably with type-specific toxoid or bacterin, is the specific preventive measure. Recombinant vaccines appear to evoke a similar or better antibody response and may be more cost-effective to produce.[9]

Outbreaks

In outbreaks, because of the need for rapid action, it is usually necessary to proceed with vaccination before typing of the organism can be performed. Cross-protection occurs between *C. perfringens* types B and C because the β-toxin is produced by both strains and is central to the disease produced by both strains. Lamb dysentery antiserum will protect against type C infections. Type C toxoid and antiserum are also available.

DIFFERENTIAL DIAGNOSIS

The rapid course and typical necropsy findings suggest the diagnosis, but the major differential is with other causes of diarrhea in young animals.

All species

- Enteritis associated with *Clostridium perfringens* type A
- Salmonellosis
- Enteric colibacillosis
- Cryptosporidiosis

Foals

Enteritis associated with:

- *Strongyloides westeri*
- *Clostridium difficile*
- *Actinobacillus equuli*

Piglets

- *Isospora suis*
- Transmissible gastroenteritis

Struck in sheep is strictly regional in distribution and in affected areas can usually be diagnosed on the basis of necropsy lesions.

When an outbreak occurs all pregnant animals can be vaccinated to provide some colostral immunity to their progeny. However, vaccination of the dam requires a period of at least 2 weeks before there is sufficient protective antibody in colostrum. As a result, there will be a period of time between vaccination and protection of the newborn, and animals born during this period need to be provided with protection by the administration of specific antiserum. Antiserum will protect susceptible animals and can be administered immediately after birth. An alternate, and sometimes more

cost-effective procedure, is to administer benzathine or benethamine penicillin G or depot amoxicillin at birth and to repeat as required during the period of susceptibility.

Long-Term Control

Long-term control is by vaccination of the dams. To initiate the program two injections of vaccine are necessary 1 month apart, the second injection being given 2 to 3 weeks before parturition. For the prevention of lamb dysentery the two vaccinations of ewes may be spaced 2 to 5 weeks apart and the second injection can be given as early as 2 months before lambing, thus avoiding handling of heavily pregnant ewes. In subsequent years, ewes require only one booster injection immediately before parturition.

For the protection of piglets the sow is vaccinated 5 and 3 weeks before farrowing, but vaccination at mating, repeated 2 to 3 weeks before farrowing, is adequate.

Attention should be given to the unitage of the antigen or antitoxin present in clostridial toxoids and antisera. These vary widely and the manufacturer's instructions should be followed closely. Anaphylaxis may occur with some antisera of equine origin, and treated animals should be kept under close observation for 24 hours and treated quickly if signs of dyspnea and muscle shivering occur.

In the face of an outbreak the lambing area should be moved, or with piglets the farrowing rooms vigorously cleaned and disinfected. The feeding of bacitracin (300 mg/kg of feed) or salinomycin (60 mg/kg feed) to the sow for 1 to 2 weeks before farrowing has been shown to reduce disease incidence, possibly by decreasing the level of excretion of *C. perfringens* by the sow.

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JEJUNAL HEMORRHAGE SYNDROME (HEMORRHAGIC BOWEL SYNDROME, HEMORRHAGIC JEJUNITIS, OR JEJUNAL HEMATOMA) IN CATTLE

Jejunal hemorrhage syndrome, also known as hemorrhagic bowel syndrome, hemorrhagic jejunitis, or jejunal hematoma, is a recently recognized disease of cattle characterized clinically by a syndrome similar to obstruction of the small intestine causing abdominal distension, dehydration, and shock caused by necrohemorrhagic enteritis affecting primarily the small intestine. At necropsy there is segmental necrohemorrhagic enteritis of the small intestine and large intraluminal blood clots. In spite of intensive medical and surgical therapy, the prognosis is unsatisfactory and the case–fatality rate approaches 100%, unless surgical intervention is early.

The first case series reports were of five affected Holstein Friesian cows from Idaho in the United States in 1991[1] and two cows from Pennsylvania in 1992,[2] although the first report of jejunal hemorrhage syndrome appears to be in a 1990 paper from Ohio documenting the condition in a Holstein Friesian cow.[3]

ETIOLOGY

The etiology is uncertain. *C. perfringens* type A has been frequently isolated from the intestines of naturally occurring cases in dairy cattle, but its significance is uncertain. This is because *C. perfringens* type A is a normal inhabitant of the intestinal tracts of healthy cattle and is able to proliferate quickly after death. *C. perfringens* type A isolates that contain the β-2-toxin gene (*cpb2*) were initially thought to play an important role in the disease.[4] A subsequent study of five cases of jejunal hemorrhage syndrome failed to identify the presence of any known or possible virulence-associated genes, and the authors concluded that a *C. perfringens* type A "virulence signature" did not exist.[5] Studies in beef cattle suggest that mycotoxins and STEC are part of the disease complex for jejunal hemorrhage syndrome and that *C. perfringens* type A or mycotoxigenic fungi did not play a role in the disease.[6] The latter findings suggest that *C. perfringens* type A plays a secondary role in the disease. It is important to note that all attempts to reproduce the disease using *C. perfringens* type A isolates have been unsuccessful.

The fungus *A. fumigatus* has also been implicated as a causative agent of jejunal hemorrhage syndrome, but there is minimal enthusiasm for this being a primary agent.

EPIDEMIOLOGY

Although the first reports of jejunal hemorrhage syndrome were from the United States, the disease has now been identified in many countries in Europe and the Middle east, as well as multiple cases from Canada. The disease occurs sporadically, primarily in mature lactating dairy cows at peak dry matter intake and milk production. Cases occur throughout the year with slightly more reported in winter in the United States. Among dairy breeds, Brown Swiss cattle appear overrepresented in published case series.[4,7] Individual cases have also occurred in beef cows. In Germany, the disease occurs in Simmental cattle. The morbidity is low but the case–fatality rate is very high, even with surgical intervention.

Investigations of herds with cases have failed to identify any reliable possible risk factors. Most cases occur in lactating dairy cows in the first 3 months of lactation. In a single dairy herd, 22 cases occurred in a period of 4 years. Affected cows ranged from 2 to 8 years of age and the time since parturition ranged from 9 to 319 days.

As part of the National Animal Health Monitoring System's Dairy 2002, information was collected about jejunal hemorrhage syndrome in dairy cattle in the United States. The disease was observed in 9% of herds within the previous 5 years and in 5% of herds during the preceding 12 months. Risk factors found to be associated with the disease during the preceding 12 months were large herd size, administration of bovine somatotropin, and routine use of milk urea nitrogen concentration to determine ration composition. Use of pasture as part of the lactating cow ration during the growing season was associated with decreased odds of the disease in herds with a rolling herd average milk production of 9000 kg (20,000 lb) or less, whereas in herds with higher milk production, use of pasture was not associated with the occurrence of the disease. For individual cows with signs consistent with the disease, the third lactation was the median of the parity distribution and the medial time between parturition and the onset of clinical signs was 104 days. In summary, management practices implemented to achieve high milk production may increase the risk of developing the disease in dairy cattle. Increased consumption of a high-energy diet seems to be the most plausible common pathway of all the risk factors that have been described.

Feeding rations high in soluble carbohydrates has been suggested as a possible risk factor by providing the intestinal environment for *C. perfringens* type A to proliferate and produce enterotoxins, similar to the situation that may cause hemorrhagic enteritis, abomasitis, and abomasal ulceration in calves.

PATHOGENESIS

The primary lesion is an acute localized necrotizing hemorrhagic enteritis of the jejunum leading to the development of an

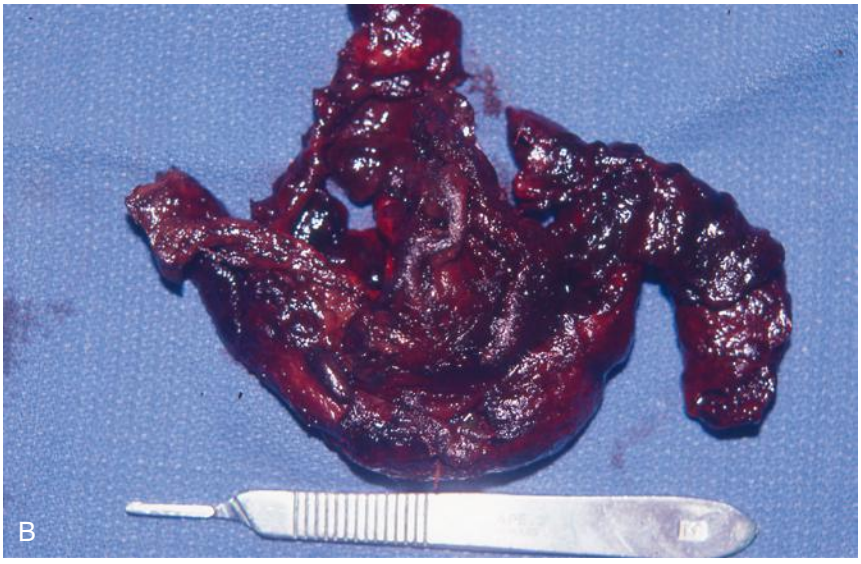

Fig. 8-26 Holstein Friesian cow with jejunal hemorrhage syndrome. **A,** Indicates the typical lesion in the jejunum as visible through a right flank laparotomy with the cow standing. The cow's head is to the right. **B,** Organized blood clot that was removed from the intestinal lumen from an enterotomy distal to the lesion.

intraluminal blood clot, which causes a physical obstruction of the intestine and ischemia and devitalization of the wall of the affected segment of the intestine (Fig. 8-26). The lesion is similar to hemorrhagic enterotoxemia associated with *C. perfringens* in young rapidly growing calves, lambs, or piglets. Puzzling and unexplained factors of the disease are that it is a focal lesion in the midjejunum. A satisfactory reason for both factors has yet to be developed.

Recent detailed histologic examination of 21 cases showed that 6 cases identified the presence of an **intramural hematoma** that split the muscularis mucosa at its margins and dilatation of villus lacteals.[8,9] These observations led to the suggestion that the initial disturbance was a decrease in blood or lymphatic flow leading to leakage into the lamina propria followed by development of an intramural hematoma. *C. perfringens* type A then proliferates in the presence of ischemic tissue and extravascular blood, and in this scenario *C. perfringens* type A acts as a secondary and not a primary agent.

There is gastrointestinal stasis with accumulation of intestinal gas and fluids proximal to the obstructed intestine, resulting in distended loops of intestine, hypochloremia, hypokalemia, dehydration, and varying degrees of anemia. The serum biochemistry changes are those of an obstruction of the upper small intestine and sequestration of abomasal secretions, with resultant hypochloremia, hypokalemia, and strong ion (metabolic) alkalosis. The hemorrhagic enteritis is progressive, with the ischemia and necrosis extending through the intestinal wall, and within 24 to 48 hours there is marked fibrinous peritonitis, dehydration, continued electrolyte imbalance, marked toxemia, and death.

CLINICAL FINDINGS

Common historical findings include sudden anorexia and depression, marked reduction in milk production, abdominal distension, weakness progressing to recumbency, bloody to dark-red feces or dry scant feces, dehydration, and abdominal pain, including bruxism, vocalization, treading, and kicking at the abdomen. Sudden death without prior clinical findings has been reported.

On clinical examination there is depression, dehydration, and the body temperature may be normal to slightly elevated; the heart rate is increased to 90 to 120 beats/min; the mucous membranes are pale; and the respiratory rate is increased. The abdomen is usually distended moderately over the right side. The rumen is usually hypomotile but distended.[10] Fluid-splashing sounds are commonly audible by succussion over the right abdomen. In some cases, a ping can be elicited over the right abdomen.

On rectal examination, the feces are black-red, jelly-like, and sticky, and smell like digested blood. On deep palpation of the right abdomen, distended loops of intestine may be palpable, some of which are firm (those loops containing the blood clot), whereas others may be resilient, representing loops of intestine proximal to the blood clot obstruction that contain excessive fluid and gas and in which the intestine is in a state of ileus.[10] The disease is difficult to differentiate from jejunal intussusception from the results of the physical examination.

The course of the disease in most cases is 2 to 4 days. Even with intensive fluid and electrolyte therapy, affected animals continue to worsen progressively, become weak, recumbent, and die, or euthanasia is chosen.

Ultrasonographic examination of the abdomen from the right flank using a 5-MHz linear transducer was very helpful in

identifying the presence of distended loops of intestine (diameter 4.3–12.0 cm, mean 6.8 cm) and reduced or absent intestinal motility. In 19% of cases, the jejunum was observed to contain localized hyperechoic material consistent with blood clots, confirming a diagnosis of jejunal hemorrhage syndrome.[11] The presence of fluid between intestinal loops and fibrin was observed in some cases; this usually indicates more advanced disease and could be used to identify poor surgical candidates.

On laparotomy, the abomasum is commonly distended with fluid. Up to 60 to 100 cm of small intestine may be distended and firm to touch, with a markedly dark red to purplish hemorrhagic serosal surface covered with fibrin tags. The mesenteric band may be too tense to allow exteriorization of the affected intestine. Manipulation of the affected intestine may lead to its rupture because of its thin and fragile intestinal wall caused by ischemia and devitalization. The small intestine proximal to the affected segment is usually distended with fluid and gas and compressible; that distal to the affected segment is usually relatively empty.[12]

CLINICAL PATHOLOGY

Hematology

The hemogram is variable and not diagnostic. Leukocytosis and mature neutrophilia with increased band neutrophils and increased fibrinogen concentrations are common, but neutropenia with a left shift may also occur. The PCV and plasma protein concentrations are variable.

Serum Biochemistry

Metabolic alkalosis with compensatory respiratory acidosis, hypokalemia, and hypochloremia are common, which is consistent with abomasal outflow obstruction due to the obstruction caused by the clotted blood or ileus.

NECROPSY FINDINGS

The abdomen is moderately distended as a result of marked dilatation of the small intestine, which is dark red, hemorrhagic, and commonly covered by fibrinous exudate. The affected segment of intestine, especially the jejunum and ileum, may be 1 m or more in length and contains a firm blood clot, adherent to the mucosa, which is necrotic and hemorrhagic over the entire length of the affected portion.

Histologically, there is multifocal submucosal edema and neutrophil infiltration, segmental necrosis, ulceration, and mucosal and transmural hemorrhage (hematoma) of the jejunum. Frequently, the epithelium is completely sloughed and, in the area of attachment of the blood clot, the mucosa is absent. Extensive fibrin and neutrophil infiltration occur on the serosal surface and fibrinous peritonitis is common.

C. perfringens type A has been isolated from the intestinal contents of typical cases, but its significance is unknown.

TREATMENT

No specific medical treatment is available, and surgical confirmation and correction is recommended. For valuable animals, intensive fluid and electrolyte therapy is indicated. Because of the possibility of clostridial infection, penicillin is indicated if treatment is attempted. Recent histologic examination of the lesion suggests that the primary hemorrhagic area is intramural and not intraluminal.[8,9] Based on this information, it would appear that right flank laparotomy and resection of the affected segment of the intestine and anastomosis is the preferred surgical approach; support for routine resection of the lesion is provided by the results following surgery in one case series,[10] whereas another case series reported good success treating less extensive (and presumably earlier) lesions by massaging the intraluminal blood clot to break up the intraluminal obstruction;[7] presumably in these cases the intramural damage was milder and recoverable without resection. The overall success rate is usually poor because of the advanced nature of the lesion.

DIFFERENTIAL DIAGNOSIS

The disease must be differentiated from other causes of acute physical or functional obstruction of the small intestine causing distended loops of intestine, fluid-splashing sounds on ballottement of the abdomen, and dehydration and electrolyte imbalances. These include intussusception, volvulus of the jejunal flange, incarceration of small intestine through an omental rent, ileal impaction, and diffuse peritonitis (causing ileus). In ileal impaction in mature cows, distended loops of intestine are palpable on rectal examination but on laparotomy the abnormalities consist of ileal impaction and distended loops of intestine, which are amenable to treatment.

Diseases causing melena and dysentery include bleeding abomasal ulcers, acute salmonellosis, and acute bovine viral diarrhea.

Transabdominal ultrasonography (Fig. 8-27) can be used to detect ileus of the small intestine and distension of loops of small intestine with homogeneous echogenic intraluminal material compatible with intraluminal hemorrhage and clot formation.

CONTROL

Solid control or prevention strategies have not been identified because the cause of jejunal hemorrhage syndrome has not been confirmed. However, because of the association between the incidence of jejunal hemorrhage syndrome and nutritional factors, the following strategies are suggested:

- Increase fiber in the diet, prevent cattle from sorting feed at the bunk, and decrease the amount of rapidly fermentable carbohydrates in the diet.
- Keep feed pushed up, maintain constant feed intake to minimize bolus ingestion

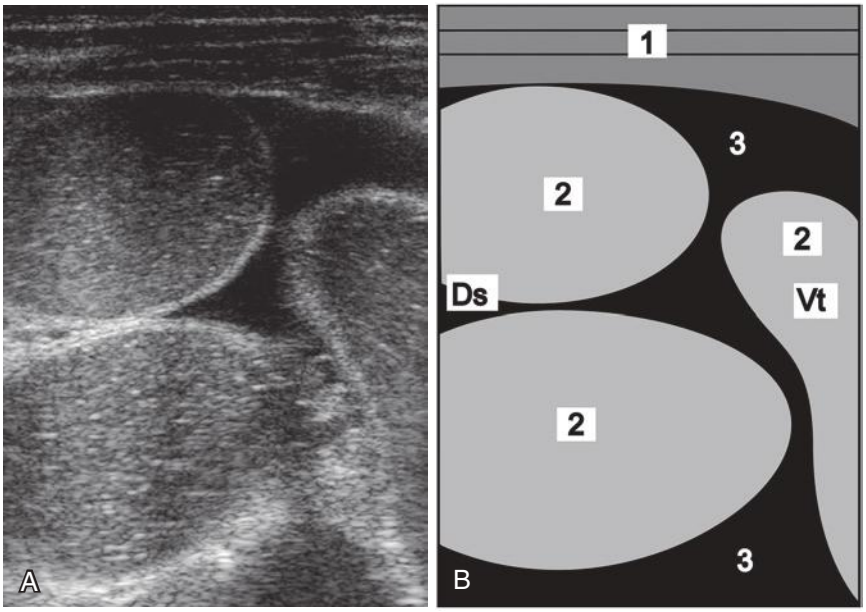

Fig. 8-27 A and B, Ultrasonogram and schematic of the abdomen in a cow with ileus caused by obstruction of the jejunum with coagulated blood (hemorrhagic bowel syndrome). The jejunal loops are dilated and there is anechoic fluid (transudate) between the dilated loops. The ultrasonogram was obtained from the right abdominal wall caudal to the last rib using a 5.0-MHz linear scanner. *1,* Lateral abdominal wall; *2,* dilated jejunal loops; *3,* anechoic fluid between the jejunal loops; *Ds,* dorsal; *Vt,* ventral. (Reproduced with kind permission of U. Braun.)

of rapidly fermentable carbohydrates, and avoid sudden ration changes.
- Test forage for presence of *C. perfringens* and *A. fumigatus*.

Consider administering autogenous vaccines containing *C. perfringens* type A from an affected case on the farm; commercially available vaccines targeting *C. perfringens* types C and D are very unlikely to be efficacious based on current knowledge. There is no published randomized clinical trial demonstrating vaccine efficacy, and the role of *C. perfringens* type A as a primary agent is questioned.

FURTHER READING

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PARATUBERCULOSIS (JOHNE'S DISEASE): CATTLE

SYNOPSIS

Etiology *Mycobacterium avium subspecies paratuberculosis* (MAP).

Epidemiology Occurs in cattle, sheep, goats, and camelids. High prevalence of infection in cattle population and among herds. Ten percent to 15% of infected animals develop clinical disease. Primarily transmitted by fecal–oral route. Intrauterine infection occurs. Highest susceptibility to infection in the first months of life. Long incubation period.

Clinical signs Chronic or recurrent progressive intractable diarrhea with concomitant weight loss and decreased milk production in adult cattle while appetite often remains unaffected. Subcutaneous edema may develop between mandibles. Disease progresses over several weeks and months leading to progressing emaciation and eventual death.

Clinical pathology Culture or polymerase chain reaction on fecal material, and serologic tests (ELISA, AGID, CF). Low serum protein.

Lesions Chronic granulomatous enteritis, regional lymphangitis, and lymphadenitis.

Diagnostic confirmation Presence of intestinal lesion and identification of organism. Positive serologic test.

Treatment No specific treatment of significant value.

Control Identify and eliminate clinically and subclinically infected animals from the herd. Prevent introduction of infected animals into the herd. Prevent exposure of calves and young stock to MAP through contact with fecal material of infected animals. Improve management and hygiene to minimize spread of infection in herd with emphasis on avoiding infection of newborn calves.

Differential diagnosis list

Diarrhea in adult cattle
- Intestinal parasitism (ostertagiasis)
- Salmonellosis
- Secondary copper deficiency

Emaciation in adult cattle
- Chronic traumatic reticuloperitonitis
- Malnutrition
- Pyelonephritis
- Lymphosarcoma
- Amyloidosis

AGID, agar gel immunodiffusion; CF, complement fixation; ELISA, enzyme-linked immunosorbent assay; MAP, Mycobacterium avium subspecies paratuberculosis.

ETIOLOGY

The causative agent of paratuberculosis in ruminants is *Mycobacterium avium subspecies paratuberculosis* (MAP), a slow growing acid-fast aerobic microorganism forming part of the *M. avium complex*. Although MAP is an obligate intracellular pathogen requiring a host for replication, its tenacity allows it to survive for longer than 1 year in the environment. MAP has been subdivided into two main lineages designated as the slow growing type I (or S for sheep) and the faster growing type II (or C for cattle) according to the species from which these lineages were first isolated. Type I strains indeed appear to have a strong host preference for sheep and are more virulent for this species, whereas type II strains are more commonly isolated from cattle and a broad range of other species. Notwithstanding this association of each lineage with either cattle or sheep, it is not exclusive as strains of each lineage can cause disease in all type of ruminants.[1] Whereas S strains are predominantly isolated from sheep in Australia and New Zealand, in Europe C strains are more commonly isolated from sheep, cattle, and other species.

Molecular epidemiology studies of MAP have identified a high degree of genetic similarity within the bovine isolates, regardless of geographic origin, indicating that only a few closely related clones of MAP may be responsible for widespread infection in cattle, other ruminants, and possibly wildlife. In contrast a higher degree of genetic heterogeneity among MAP isolates recovered from human and ovine sources has been reported. Extensive analyses of the IS900 restriction fragment length polymorphism (RFLP) patterns have identified that Johne's disease in cattle and other species such as goats and rabbits is associated with indistinguishable strains. Bovine strains infect cattle, goats, and deer, and rarely sheep.

EPIDEMIOLOGY

Occurrence

The disease occurs worldwide most commonly in cattle and to a lesser extent in sheep and goats. Paratuberculosis is widespread in cattle in Europe and has been spread to many countries by the export of infected clinically normal purebred stock. The incidence is greatest in animals kept intensively under climatic and husbandry conditions as is common in dairy herds. Only a few countries in the world have no record of diagnosed paratuberculosis in ruminants, making this condition a globally endemic disease in livestock. During the last century MAP spread globally from Western Europe. Increasing incidences in eastern European countries over the past decades were attributed to intensifying life animal trade after the fall of the "Iron Curtain" and eastward extension of the EU.

Paratuberculosis was first confirmed in Australia in 1980 and is now considered endemic in Victoria and in the dairy population of New South Wales. Western Australia in contrast is considered free of paratuberculosis. Similarly, paratuberculosis is now endemic in dairy cattle in New Zealand. Data over the prevalence of this condition in Africa are scant, but paratuberculosis was diagnosed at least incidentally in most African countries.[2] Clinical and subclinical cases have also been reported from Mexico, Brazil, and Argentina.

Morbidity and Case Fatality

With only 10% to 15% of infected animals expected to develop clinical disease, the incidence of clinical disease in an infected herd rarely exceeds 5% of mature animals. The population mortality rate is less than 1% per year but under exceptional circumstances can reach 5% to 10%. For every clinical case of Johne's disease in a herd, it is estimated that there are 15 to 25 additional infected animals in various stages of clinical disease; 4 to 8 cases of subclinical disease and carrier adults; and 10 to 14 with silent infection in calves, young cattle, and adults.

Wildlife and Exotic Species

MAP has a very broad host range. Infection may also occur in many different wildlife and exotic species. Water buffalo and captive and free-living wild ruminants including deer, bighorn sheep, Rocky Mountain goats,

aoudads, mouflon sheep, camels, mountains goats, reindeer, antelopes, New World camelids, and yaks are all susceptible. Outbreaks of the disease have occurred in farmed red deer and the incidence is increasing in some regions.

There is evidence that wildlife in Scotland are naturally infected with MAP and that the host range is much wider than previously thought. The organism has been found in fecal cultures from foxes, stoats, crows, weasels, jackdaws, hares, badgers, rooks, rats, and wood mice. Such environmental contamination with the organism can pose a risk to grazing livestock and farms adjoining paratuberculosis-infected properties. Paratuberculosis has been found in wild rabbits (*Oryctolagus cuniculus*) in Scotland. Analysis indicates a significant relationship between a past or current problem of paratuberculosis in cattle and in the wild rabbit population on infected farms. On infected farms, rabbits potentially input millions of viable MAP organisms per hectare per day onto pasture grazed by livestock through fecal contamination. Also, grazing cattle do not avoid rabbit fecal contaminated pasture, which is the only recorded example of a herbivore species not avoiding their own feces or the feces from sympatric wildlife species. The greatest overlap between habitat use by rabbits and livestock grazing occurred in rough grazing and gorse scrub habitats, particularly in autumn. Therefore a reduction in potential transmission risk could be achieved by reducing contact between livestock and rabbits in these habitats, especially reducing access to these habitats by young livestock because they are more susceptible to infection.

In the Czech Republic, paratuberculosis has been diagnosed in all four of the most common wild ruminant species including red deer, roe deer, fallow deer, and mouflon. The highest incidence of clinical disease in wild ruminants was in farmed deer. Using RFLP, transmission from domestic infected ruminants to wild animals could be confirmed, whereas the transmission from wild animals to domestic ruminants was uncertain. Nonvertebrates, wild ruminants, or nonruminant wildlife can be vectors and potentially become a risk factor in the spread of MAP.

The epidemiologic implications of cattle and wildlife comingling on the same pasture are unknown. The rate of infection can be the same in both species and it seems that both share a common source, which might well be a common herd of deer and cattle. Pigs mixed with infected cattle may develop enlargement of the mesenteric lymph nodes suggestive of tuberculosis and from which the causative organism can be isolated. Pigs and horses infected experimentally develop granulomatous enteritis and lymphadenitis. Mice and hamsters are also susceptible and are used in experimental work.

Prevalence of Infection

The prevalence of infection in a region is difficult to estimate because of the difficulty in diagnosing subclinical infection and the failure to report diagnosed cases unless a specific survey or control program is undertaken. Numerous studies reporting herd and animal prevalences in different regions have been published. Nonetheless, results are difficult to compare because different diagnostic approaches (e.g., fecal cultures, PCR or serology in blood or milk) with markedly different sensitivities have been used and several studies suffer from an important sample selection bias.[3]

In a recent meta-analysis the estimated animal prevalence of paratuberculosis in the European cattle population was estimated to be over 20% with lowest reported animal prevalences of at least 3% to 5% in some countries. The herd prevalence of paratuberculosis in European cattle herds was estimated to be above 50%.[3] The UK reported a herd prevalence for paratuberculosis of almost 35%. In dairy herds in the United States, the overall animal seroprevalence ranged from 5% to 17%.[4] The most recent survey conducted in the top 17 U.S. dairy states using environmental fecal cultures reported a herd prevalence of approximately 68% in U.S. dairy herds.[5] In this study over 95% of dairy operations with over 500 cows were found to be infected, whereas only 63% of smaller dairy operations with fewer than 100 cows were infected with MAP.[5] In dairy herds in Alberta, Canada, the herd prevalence determined by ELISA was 26.8%. The herd prevalence as determined by fecal culture ranged from 27% to 57%. In Australia the herd prevalence in dairy herds of the infected southeast part of the country is about 15%, whereas the western part of Australia is considered free of MAP.

Although much fewer reports of the prevalence of MAP infection in beef cattle are available reported herd-level and animal-level prevalences are consistently lower than in dairy cattle. In the United States the prevalence of MAP infection in beef herds of 23 states was determined based on serology and revealed a herd prevalence of 7.9% and an animal prevalence of 0.4% in the studied population.[6] Smaller scale studies conducted in Louisiana, Florida, and Missouri estimated the animal seroprevalence in beef cattle between 4% and 8% and herd prevalence in beef herds between 30 and 40%. Serologic surveys conducted in Canada revealed between 0.8% and 1.7% of seropositive animals in 3% to 11% of beef herds in Western Canada.[7]

Methods of Transmission

The main route of transmission of paratuberculosis is widely accepted to be through oral uptake of MAP by susceptible animals via ingestion of contaminated milk, water, and other feed products or uptake from the environment. With newborn calves being the most susceptible age group for MAP infection, contaminated colostrum and milk are considered a primary source of infection. MAP is introduced into milk and colostrum either via contaminated teats or direct shedding of the organism into the colostrum/milk. Infected cows and other species excrete MAP directly into the milk during at least the late disseminated stage of the infection. Up to 45% of clinically affected cows may excrete the organism in milk, which was isolated from 36% of colostrum samples from heavy shedders and 9% of samples from light shedders (nearly three times as often as it is found in milk).

MAP was isolated from dust and bioaerosols collected in barns housing MAP-infected cows, suggesting that inhalation or ingestion of these bioaerosols has the potential to function as an alternative route of infection.[8]

Vertical transmission of infection in utero is well established in cattle, and intrauterine MAP infection was identified as significant risk in dairy herds.[9] Data suggest that up to 9% of fetuses from subclinically infected and 39% of fetuses from clinically infected dams contract infection with MAP.[9] Transmission of the organism from moderate shedders via the trophoblast is unlikely before the stage of development of cotyledons. Transfer of embryos from infected to uninfected dams is thus unlikely to present a risk of disease transmission. It is hypothesized that the epitheliochorial placenta is impermeable to the organism from 42 to 49 days postinsemination but that this could change after 60 days. Isolation of MAP from the semen of bulls and rams is unusual and represented by single case reports.

Because of the normally long incubation period, infected animals may excrete organisms in the feces for 15 to 18 months before clinical signs appear. Also, animals reared in a contaminated environment may excrete MAP in feces without being infected, becoming so-called "**pass-through shedders**."[10] Spread of the organism from farm to farm is usually caused by trading of livestock, which are unknown infected carriers and shedders of the organism, but lateral spread of feces across boundary fences also occurs.

Field studies have shown that the nymphs of the Oriental cockroach (*Blatta orientalis*) may serve as a passive vector of MAP. Also, earthworms and adult Diptera may be vectors of the organism on cattle farms with paratuberculosis. Ovine trichostrongylid larvae (*Haemonchus contortus, Ostertagia circumcincta,* and *Trichostrongylus colubriformis*) may become contaminated with MAP and may play a role in the transmission of the organism.

The survival of MAP in amitraz-based dip fluid for at least 2 weeks suggests that dips could play some role in the transmission of Johne's disease in cattle. The main risk is to calves suckled by cows that have just been dipped and whose udders are covered in dip fluid.

Risk Factors

Animal Risk Factors

Age of Animal

A distinguishing characteristic of paratuberculosis is that resistance to infection increases with age. Experimental and field studies showed that infection becomes more difficult when calves are 4 months or older, and susceptibility to infection from 1 year of age on appears to be similar to that of adult animals.[11] Although the mechanism rendering young calves more susceptible to infection is not entirely understood, considerable importance is attributed to the permeability of the intestinal mucosa for large molecules in early life. Immaturity of the innate immune response in neonatal calves may contribute to higher susceptibility to MAP infection in calves. Increased resistance of adult cattle to MAP infection is thought to result primarily from effective containment or even elimination of infection rather than from impaired penetration of the intestinal mucosa.[11] The age-related resistance to MAP may be overwhelmed when very high doses of MAP are ingested in a heavily contaminated environment

Because of a long incubation period of over 2 years, in most cases clinical disease does not occur until 2 to 5 years of age. Notwithstanding this age limit should not be used as a reliable diagnostic criterion; in extreme circumstances the magnitude of the ingested *MAP* dose will affect the course of the disease. Clinical disease incidentally was reported to occur at 12 to 18 months of age.

Breed Incidence and Genetic Susceptibility

Breed differences have been suggested based on the different prevalences of MAP infection between beef and dairy cattle or differences in prevalence in different geographic regions in which different cattle breeds predominate. However, these differences cannot lead to reliable conclusions concerning breed effects given the confounding effects of animal husbandry. Studies conducted in Texas reported that *Bos indicus* purebred and crosses had odds ratios 17- and 3.5-fold greater than *Bos taurus* breeds for positive serologic results. Although these results could also suggest differences in response to MAP infection (i.e., seroconversion) rather than susceptibility to MAP infection they provide evidence for a breed or subspecies effect.[12] Evidence for a certain degree of genetic variation in host susceptibility to MAP infection has accumulated in recent years.[13,14] Heritability of host susceptibility to MAP infection has been studied at sire level using the phenotype of daughters as a key parameter. Heritability estimates in dairy cattle range between 1% and 18% with the majority of estimates between 9% and 12%.[10] Although these heritability estimates are modest, these data suggest that genetic selection of bulls with the objective of breeding more resistant animals may be a potentially useful tool contributing to the control of paratuberculosis in the future. Whole Genome Association Studies identified SNPs in multiple genes like TLR-2 or NOD-2, indicating that susceptibility or resistance to MAP is likely caused by multiple genes.[14]

Other Diseases and Stressors

Factors that affect susceptibility to infection include size of infective dose, level of dietary iron intake, age, stress, and immunosuppressive agents such as BVD virus. These factors may affect the probability of development of clinical disease, but they have not been well documented. Field observations indicate that stress, including parturition, transportation, and nutritional deficiencies or excesses may influence the development of clinical disease. Housed animals are subjected to a high risk of infection because of the heavy contamination by feces and the long survival of the bacteria in protected sites.

The possible cross-protection between tuberculosis and paratuberculosis suggests that eradication of tuberculosis may make the cattle population generally more susceptible to paratuberculosis, but this has not been borne out by field experience in North America.

Herd Characteristics

A computer simulation model of paratuberculosis in dairy cattle has been used to examine the course of the disease in a herd. Seven variables were specified at the initial stage of the model:

- Herd size
- Annual herd birth rate
- Annual herd replacement rate
- Number of infected cows at time zero
- Number of herd replacements purchased each year
- Risk of purchasing an infected heifer
- Number of effective cow–calf contacts per year

All variables affect the course of paratuberculosis spread in herds, but the model is most sensitive to the effective contact rate. This is consistent with the findings of other infectious disease models and with recommendations on Johne's disease control, namely **minimize cow–calf contact** to prevent transmission of infection.

The prevalence of infection in purchased cattle directly affects the risk of buying infected cattle and the rate at which herds become infected in the model. Purchase of a large percentage of replacement heifers from populations with modest infection rates annually will quickly result in infection of a herd. Age-specific culling rates are also important in the development of the model. Accurate prediction of the rate at which infected cattle leave a herd was a major determinant of the course of the epidemic because each year an infectious cow remained in the herd, the cow contributed in an exponential manner to the generation of infected calves and thus the number of infected herd replacements. Over the range of realistic values for all variables in the model, the prevalence of the disease in infected herds continued to increase until a plateau was reached. True prevalence rates in the model generally plateaued at 40% to 60% of the herd. These data results suggest infection is spreading quickly in dairy cattle.

Environmental and Management Risk Factors

Management factors that were identified as important in influencing the prevalence of infection include the following:

- Newborn calf care
- Bred heifer management
- Environmental conditions
- Handling of manure
- Care and management of growing calves

These are not cause-and-effect relationships but hypotheses based on observations in dairy herds.

Care of the Newborn Calf

The fecal–oral route is widely accepted as the major route of transmission for MAP. Because of the increased permeability of the intestines in the first hours after birth, the first hours and days of life are deemed to bear the highest risk of infection for a calf. Exposure of the newborn calf to MAP generally occurs by feeding colostrum contaminated with feces, by directly nursing from teats contaminated with feces, by ingesting colostrum from MAP-infected dams that are at increased risk of shedding MAP through the mammary gland, or through direct contact with feces from infected dams. Accordingly control strategies to prevent infection of neonatal calves must focus on providing excellent sanitary conditions in the maternity area, avoiding contamination of colostrum with manure by thoroughly cleaning teats before milking or the calf nursing the dam, separating calves as soon as possible from adult animals to minimize contact with manure, avoiding the presence of infected dams in the maternity area, and avoiding the use of colostrum from MAP-infected dams. Additional measures recommended to reduce to risk of transmission of MAP to neonatal calves include avoiding the use of pooled colostrum, the use of heat-treated colostrum, and feeding milk replacer or pasteurized milk.[10]

Calf Rearing

Although risk of infection is highest in the first days of life experimental studies showed that increased susceptibility to infection persists at least for the first 4 months of life.[11] Feeding whole milk or feed contaminated with MAP or allowing contact of calves with manure from MAP-infected cows present the highest risks of infection. Transmission of MAP may also occur horizontally from an infected calf to its herdmates. Although

model studies suggest that calf-to-calf transmission does not constitute a major route of MAP transmission, experimental studies showed that calves and young stock are capable of excreting detectable quantities of MAP in feces.[15,16] More recently the presence of MAP in dust and bioaerosols in barns housing MAP-infected cows was documented. Although the transmission of MAP infection through bioaerosols needs further investigation, it was suggested that growing calves housed in the same barn with infected cows are more likely to be exposed to MAP than calves housed separately from adult-infected cows.[8] Recommendations for calf rearing to reduce the risk of MAP infection include raising young stock well separated from adult cattle; feeding unweaned calves milk replacer or pasteurized milk; preventing contamination of feed, water, and pens of young stock with manure from adult cows; and avoiding to feed leftovers from adult cows to young stock.[10]

A survey of farms in Scotland found the factors that increased the likelihood of a farm having Johne's disease included large numbers of rabbits, access of wildlife to feed supplies, the application of manure to grazing pasture, the type of water supplies, and the number of crows.

Soil Characteristics and Manure Handling

An association between high prevalence of MAP infection in ruminants and soil type has been recognized. The evidence strongly implicates regional soil acidification, excesses of iron and molybdenum, and marginal deficiencies in copper and selenium in the progressive expression of Johne's disease. Survival of the organism may be enhanced by silt or sand content in loamy soils.

The organism can persist without multiplication in pasture for up to 1 year. MAP is relatively susceptible to sunlight and drying, to high calcium content, and high pH of the soil. Continuous contact with urine and feces reduces the longevity of the bacteria. The alkalinity of the soil may also influence the severity of the clinical signs. Herds raised on alkaline soils, particularly in limestone areas, may have a high incidence of infection but little clinical disease.

Environmental conditions and manure handling are correlated with prevalence and are reflected in overall cleanliness of the farm and the amount of contamination resulting from faulty design, maintenance, location of housing facilities, and frequency of cleaning by the farm operator.

The distribution of MAP in the environment surrounding dairy farms and its relationship to fecal pool prevalence in herds known to be infected and uninfected was described and compared. Environmental samples were culture positive in 78% of infected herds. Environmental samples were cultured positive in cow alleyways (77% of herds), manure storage (68%), calving areas (21%), sick cow pen (18%), water runoff (6%), and postweaned calves areas (3%). Herds with both areas cultured negative were estimated to have 0.3% to 4% fecal pool prevalence. Herds with both areas having a heavy load of bacteria were estimated to have 53% to 73% fecal pool prevalence. These findings support the concept that targeted sampling of cow alleyways and manure storage areas may be a suitable alternative strategy for herd screening and MAP infection status assessment and for estimating herd fecal prevalence.

Pathogen Risk Factors

MAP is an obligate pathogen and parasite of animals and in theory can be eradicated by removal of all infected animals. However, the organism can survive for long periods in the environment, enabling it to persist and spread in the grassland environment and to withstand a periodic lack of suitable hosts.

Survival and Dormancy of Organism in the Environment

Bovine strains of MAP can be extremely persistent in nature, with survival for more than 1 year. Studies of the survival of the organism on Australian farms on which paratuberculosis is prevalent indicate that when the organism in feces becomes mixed with soil, there is a reduction of 90% to 99% in the apparent viable count of the organism. This is thought to be caused by binding of bacteria to soil particles, which are excluded from culture by sedimentation during sample preparation. Survival of the organism in fecal material applied to soil was greatest in a fully shaded environment and was least where fecal material and soil were fully exposed to the weather and where vegetation was also removed. Significant degrees of pasture decontamination can be achieved in a relatively short period, which will be beneficial for disease reduction in a herd because of the beneficial effects lower doses of the organism would have on the incubation period and disease outcome. Pasture management, such as selective grazing with no susceptible hosts or mechanical slashing, may be used to maintain a relatively low level of shade at the soil surface to hasten decontamination.

Thermal Resistance of Organism

MAP was found to be more heat resistant than other mycobacteria. Studies investigating the effectiveness of different pasteurization protocols detected viable MAP after standard thermal treatments such as low-temperature holding at 63°C for 30 minutes or high temperature-short time (HTST) at 72°C for 15 seconds. MAP strains were reported to be able to survive HTST pasteurization with survival rates ranging from 3% to 5% in bovine tissue.[17] Extending holding time from 15 to 25 seconds as implemented in the UK in 1998 for commercial milk pasteurization in an effort to increase the effectiveness of the pasteurization process was found to be no more effective at killing MAP than conventional HTST pasteurization. These findings are corroborated by several independent retail-milk surveys reporting recovery of viable MAP from retail HTST pasteurized milk.[18,19] It is a general consensus that the presence of MAP in concentrations greater than 10^4 CFU/mL in milk may not be completely destroyed by HTST pasteurization. Pasteurization of colostrum with a temperature of 63°C for 60 minutes was recommended as suitable procedure under field conditions even when using large batches (30 L) to eliminate MAP from colostrum in most cases.[20] Pasteurization of colostrum resulted in a decrease in colostral IgG concentrations but not to a level that would preclude its use for transfer of passive immunity.

Economic Importance

The economic impact of MAP infection for the dairy industry is substantial and occurs across all herd sizes and regions. At the end of last century estimated costs ranged between US$40 and 227 per cow per year. Economic losses result from decreased milk production, decreased lifetime production caused by premature culling, decreased fertility, decreased slaughter value of the carcass, potentially delayed genetic improvement caused by involuntary culling of genetically valuable animals, replacement costs, and costs associated with MAP control programs. Some studies reported higher risk for mastitis, increased somatic cell counts, and decreased milk fat and milk protein production in MAP-infected dairy cows.

Infection of MAP was found to be associated with decreased milk production in dairy cows in several studies. Depending on the degree of shedding milk production of infected cows was found to be decreased between 2.1 and 6.0 kg/day.[21] The magnitude and direction of the association between subclinical MAP infection and milk production depends on the parity of the animal, stage of disease, and stage in lactation. In herds with an average parity of 2 or less, subclinical infection may have little impact on milk production. In herds maintaining an average herd parity of 2, many subclinical infected animals would be culled before experiencing any decline in milk production in which case the direct economic losses attributable to reduced milk production would be negligible.

Progressively decreasing feed efficiency in clinical and subclinical paratuberculosis results in loss of body condition despite unaffected feed intake. Decreased slaughter weight at culling has been documented for clinically and subclinically infected cows. Reduction of slaughter value was estimated to range between 5% in subclinical cases and 30% of clinical cases of paratuberculosis.

Associations between MAP infection in dairy cows and incidence of mastitis, somatic cell count, and milk constituents were studied with conflicting results. Although several studies documented a higher proportion of cows culled for mastitis in a group of animals with subclinical MAP infection compared with uninfected control cows, other studies failed to find an association between MAP infection status and mastitis or even reported lower rates of mastitis in MAP-infected cows. Studies reporting an association between subclinical MAP infection and increased somatic cell counts stand against reports failing to reveal a significant difference in somatic cell counts between infected and uninfected dairy cows or even documenting lower somatic cell counts in MAP-infected cows compared with uninfected control cows.[22-24] The effect of MAP infection on milk constituents such as protein and fat has been studied with similarly inconsistent results. Some authors reported that milk-fat and milk-protein production or the mature equivalent for milk fat and milk protein in subclinically MAP-infected cows were significantly lower than in uninfected herdmates, whereas other studies did not reproduce significant differences between infected and uninfected cows.[25]

Because the incidence of infertility was reported to be significantly higher in the cohort of MAP-infected cows than in uninfected control cows, reduced fertility is likely to contribute to the economic losses associated with MAP infection on a herd level.

A large fraction of economic losses associated with paratuberculosis are considered to result from loss of future income. Under normal conditions productivity and thus average income of a dairy cow increases with age. Culling seropositive or culture-positive animals before they reach their peak productivity therefore contributes to undetermined economic losses resulting from the lost production potential and potential breeding value.

Losses at National Dairy Industry Level

In 1996, averaged across all dairy herds in the United States, Johne's disease cost the dairy cattle industry, in reduced productivity, $22 to 27 per cow or $200 to 250 million annually. The economic impact of the disease in Australia and New Zealand and regions of the United States have been estimated, but their validity is questionable because of the accuracy of the diagnostic tests and the survey methodology. Some observers have indicated that paratuberculosis has emerged as one of the most prevalent and costly diseases of dairy cattle, but this is not well-documented. There is insufficient information available on the economic importance of paratuberculosis in the beef cattle industry.

Zoonotic Implications

Potentially, MAP is of great public health significance because it is speculated to be involved in **Crohn's disease** in humans. Crohn's disease is an inflammatory bowel disease of unknown etiology that can affect any portion of the gastrointestinal tract although the terminal ileum and colon are most commonly affected. It is characterized clinically by chronic weight loss, abdominal pain, diarrhea or constipation, vomiting, and generalized malaise. Surgical resection of the affected intestine is often necessary because of complications. Crohn's disease and paratuberculosis share many similarities in gross pathology, histopathology, clinical presentation, and epidemiology. Furthermore MAP has been recovered from tissue and blood samples of patients suffering from Crohn's disease, which led to concerns over the potential role of MAP in the development of Crohn's disease in humans.

A number of studies attempting to determine the prevalence of MAP in patients diagnosed with Crohn's disease have been conducted. A study using nested PCR and culture to detect the presence of MAP detected this pathogen in 26% of patients with noninflammatory bowel disease and 92% of those affected by Crohn's disease. More recently a study attempting to directly visualize mycobacteria in tissue of Crohn's disease patients found these microorganisms in just over 50% of samples of patients with Crohn's disease but rarely in control samples.[26] The organism has been cultured from the peripheral blood of a higher percentage of individuals with Crohn's disease than in controls, which does not prove that MAP is a cause of the disease, but suggests that a larger scale investigation is needed to ascertain the role of the organism in the illness.

Thus far there has been no definitive evidence for or against the theory assigning a causative role to MAP in the etiology of Crohn's disease in humans. There is no epidemiologic evidence at present to indicate that the incidence of Crohn's disease is associated with possible exposure to organisms such as might be expected in farmers, animal health care workers, or other individuals with direct contact with infected animals.

Although the role of MAP in the etiology of Crohn's disease is still under debate, it is rational to consider that in case MAP would be the causative agent or a contributing factor in the etiology of Crohn's disease this microorganism might be acquired through ingestion of foodstuffs. Also, the epidemiology of the disease, which includes rising incidence rates in Western societies concurrent with low rates in developing countries over the second half of the twentieth century and high rates among immigrants to Western societies, is consistent with the possibility that a critical infection may be acquired from cattle or other farm animals via milk or meat ingestion, staples of Western diets, and cause Crohn's disease in subjects with an appropriate genetic predisposition.

In Manitoba, Canada, the reported incidence of Crohn's disease at 15 patients per 100,000 people per year is among the highest in the world. Population-based case-control studies of the seroprevalence of MAP in patients with Crohn's disease and ulcerative colitis have concluded that a high seroprevalence in Manitoba raises the possibility that the high rates of Crohn's disease in Manitoba could be related to high exposure rates for MAP. However, MAP is not serologically specifically associated with Crohn's disease in a community with a relatively high prevalence of Crohn's disease.

If MAP does have a role in Crohn's disease, then milk and possibly meat from infected animals may be a potential vehicle of transmission of the organism from animal to man. MAP has been cultured from milk from cows with subclinical and clinical paratuberculosis. Laboratory studies investigating the thermal tolerance of MAP showed that standard pasteurization procedures such as HTST either for 15 or 25 seconds destroy large numbers of MAP in milk, although they may not kill 100% of MAP cells. Accordingly, several independent surveys conducted on HTST pasteurized retail milk reported that viable MAP was occasionally present in retail milk.[18,]

The organism can also survive in cheese made from raw milk. MAP is resilient and is able to withstand the acidic conditions in cheese. During the laboratory manufacture of soft cheese using raw milk spiked with MAP, the majority of MAP cells are concentrated into the cheese curd rather than lost with the whey. When the resulting soft cheese was stored at 4°C, MAP could still be cultured after 35 days.

Meat for human food consumption was found to be a potential source of MAP, most commonly originating from surface contamination of the carcass with fecal material during processing in the abattoir. Few studies investigated the prevalence of MAP contamination in U.S. packing plants suggesting that few MAP are present on the carcass even following decontamination.[27] In addition to the possibility of being present on the surface, MAP was also isolated from within muscle tissue and different organs such as liver, heart, spleen, and lymph nodes of infected animals used for human food. Ground beef represents the highest risk for containing MAP because it is in a large part produced from cull dairy cows, the subgroup of cattle having the highest animal prevalence for MAP infection. Ground beef not only consists of blended meat from different animals,

increasing the risk of containing MAP, but it also contains lymph nodes in which MAP concentrate.[28]

Because MAP is currently not recognized as a human pathogen by regulatory authorities in most countries, there are generally no restrictions on the slaughter of cattle identified as MAP infected and culling subclinical and even clinical cases of paratuberculosis is common practice.

Although the amount of MAP is likely to be greatly decreased by cooking meat to a well-done condition, consumption of undercooked meat could potentially harbor viable MAP.[28]

PATHOGENESIS

Following oral ingestion, the organism localizes in the mucosa of the small intestine, its associated lymph nodes, and, to a lesser extent, in the tonsils and suprapharyngeal lymph nodes. Although MAP can invade the organism through the tonsils and then spread either hematogenously or via lymph nodes, the primary portal of entry is the terminal part of the small intestine and the large intestine.

Susceptibility to Infection

It is widely assumed that calves contract MAP infection in their first month of life and are the most susceptible to infection in the first hours and days of life. The mechanism behind the age-dependent resistance to MAP infection is not entirely understood, but several hypotheses have been proposed. The "open gut" theory suggests that increased permeability of the neonate's intestinal mucosa facilitating immunoglobulin uptake from the intestinal lumen facilitates the penetration of MAP through the mucosal membrane. Other hypotheses suggest that immaturity of the innate and adaptive immune response in the newborn calf contributes to the higher susceptibility to MAP infection in early life.[11]

Susceptibility to MAP infection is likely not only driven by the age of the host but also by the degree of contamination with MAP of the environment. Higher doses of MAP may not only increase the risk of infection in early life but may also overwhelm age-dependent resistance, extending the period of susceptibility of infection.

The presence of MAP within the intestinal submucosa and mesenteric lymph nodes triggers an inflammatory response as well as the attraction of more macrophages and lymphocytes to the area. The result is a granuloma formation with multinucleated giant and immune cells infiltrating the intestinal submucosa, which results in decreased absorption, chronic diarrhea, and ensuing malabsorption. There is a reduction in protein absorption and leakage of protein into the lumen of the jejunum, termed protein losing enteropathy. The loss of protein results in muscle wasting, hypoproteinemia, and edema.

Immune Response and Spectrum

The differentiation of at least three different groups of animals, depending on the host–bacteria relationship that becomes established, has been proposed in the literature. In the first group, animals develop resistance quickly, control the infection, and do not become shedders (**infected resistant**). In the second group, the infection is not completely controlled; some animals will partially control the infection and will shed the organism intermittently, others will become **intermediate** cases that are incubating the disease and will be heavy shedders of the organism. In the third group, the organism persists in the intestinal mucosa, and it is from these animals that the **clinical** cases eventually develop. The different possibilities are summarized in Table 8.9.

The first line of defense against invading MAP in the ruminant intestine involves M cells (special epithelial cells associated with ileal Peyer's patches and lymphoid follicles that actively take up particulate matter from the intestinal contents; they are the portal of entry for bacteria and viruses) and phagocytic macrophages. In early stages of infection the organism is found in phagocytic macrophages in the intestine. Once inside the phagosome of an infected macrophage, the organism interferes with the normal course of phagosome maturation into phagolysosome, escaping the process of destruction. The infection of inactivated macrophages within the intestine is the first step in establishing persistent infection and in the subsequent development of disease. The host immune system begins a series of attacks against MAP-infected macrophages, including the rapid development of activated T helper 1 cells (Th1), CD4+ T cells, and cytolytic CD8+ cells. Activated Th1 cells are characterized by their production of interferon-γ (IFN-γ) and IgG2. Later in the course of infection a Th2 cell response becomes predominant over the Th1-mediated response. The Th2-type cell immune response is characterized by the production of cytokines such as interleukin (IL)-4 and IL-10 and is associated with an enhanced humoral immune response, whereas cell-mediated immunity wanes. Progression of cattle from subclinical to clinical Johne's disease is associated with a decreased ability of mononuclear cells to produce IFN-γ, both specifically and nonspecifically, at the site of infection and in the blood. The loss of putatively protective Th1 cell response leads to a lack of control of mycobacterial replication and, subsequently, to fecal shedding and the progressive granulomatous enteritis typical of bovine paratuberculosis. In contrast to the Th1-mediated cellular immune response, antibodies against MAP do not protect the organism from clinical disease. During the final stages of disease, lack of antigen-specific cell-mediated immune response or complete anergy may result, allowing for rapid dissemination of the infection throughout the host.

The immunologic response following infection is highly variable. Generally, infected animals initially develop a cell-mediated response, followed by a humoral response initiated by the release of bacteria from dying macrophages as the disease progresses. It has been speculated that the time to occurrence of seroconversion and fecal shedding in infected animals depends on the infective doses occurring with natural infection.[29]

There appears to be an immune spectrum, and no serologic or cellular immunity test will identify all animals in the spectrum. There are infected-resistant animals that control their infection but are unable to completely eliminate the organism. These animals do not react in antibody assays, only rarely or never shed organisms, and respond to the lymphocyte transformation test because their circulating lymphocytes are sensitized. In the intermediate stage, the animal fails to control the infection, antibodies appear in the serum, and organisms are shed in the feces. In the stage of clinical disease, the organisms are shed in the feces and the antibody responses and skin tests are variable.

The bacteria are carried by macrophages to other sites, particularly the uterus, the fetus, and the mammary gland, as well as the testes and semen of bulls. The postprimary dissemination of the lesions is more widespread in adult animals than in calves, and

Table 8-9 The relationship between the stages in the pathogenesis of Johne's disease, the presence of clinical disease and the results of diagnostic test

	Resistant animals	Intermediate (incubation period)	Advanced clinical disease
Clinical signs present	–	+	+++
Fecal shedding	+ (–)	++	+++
Antibody response	–	++	+++
Skin test	+ (–)	+ (–)	+ (–)
Lymphocyte transformation	+++	+++	+ (–)

the early lesions are more severe in the former but the organisms do not persist. Disseminated lesions consisting of microgranulomas in lymph nodes and other organs have been described in mature cattle. In calves, the organism proliferates slowly, particularly in the small intestinal site, which results in a massive cellular infiltration of the intestinal submucosa. In adult cows, infection may penetrate to the fetus and cause prenatal infection.

Important features of the natural history of the disease are the long incubation period of 2 years or more and the development of sensitization to johnin and to mammalian and avian tuberculin. This sensitivity develops in the preclinical stage but has disappeared in most cases by the time clinical signs are evident. On the other hand, complement-fixing antibodies appear late in the disease and generally increase with increasing severity of the lesions. This suggests that two independent antibodies are involved in the two reactions.

CLINICAL FINDINGS

Stages of Disease

Four stages of paratuberculosis in cattle have been described.

Stage One

Silent Infection. Calves, heifers, and young cattle up to 2 years of age are affected. There are no clinical signs and no effects on BW gain or body condition, but these animals may shed the organism. Clinicopathologic tests cannot detect the infection, but culture of the feces or demonstration of the organism in tissues may be possible.

Stage Two

Subclinical Disease. Carrier adults show no specific clinical signs but may be affected by other abnormalities such as mastitis or infertility. Most of these animals will be negative on fecal culture but 15% to 25% may be positive on fecal culture. These are also negative to most serologic tests.

Stage Three

Clinical Disease. Clinical disease is the tip of the iceberg in terms of the total number of infected animals in the herd. The "iceberg concept" states that for every animal with clinical signs born in the herd, another 15 to 20 animals are infected and less than half of whom will be detected by fecal culture. Clinical signs in most cases do not appear before 2 years of age and are most common in the 2- to 6-year-old age group. Cases occur only sporadically because of the slow rate of spread of the disease. A hallmark of the clinical stage is gradual loss of BW despite a normal appetite. During a period of several weeks, concurrent with the weight loss, diarrhea develops. Milk production declines but the temperature, heart rate, and respirations are within normal limits. The fall in milk yield is often apparent in the lactation before diarrhea commences. The feces are soft and thin, homogeneous, and without offensive odor. There is marked absence of blood, epithelial debris, and mucus. Diarrhea may be continuous or intermittent with a marked tendency to improve in late pregnancy only to reappear in a severe form soon after parturition. A temporary improvement may also occur when animals are taken off pasture and placed on dry feed.

Stage Four

Advanced Clinical Disease. As the disease worsens, emaciation is the most obvious abnormality and is usually accompanied by intermandibular edema, which has a tendency to disappear as diarrhea develops. The diarrhea is characterized by a fluid "waterhose" or "pipestream" passage of feces. The course of the disease varies from weeks to months but always terminates in severe dehydration, emaciation, and weakness with an ultimately fatal outcome.

CLINICAL PATHOLOGY

In an infected herd, animals may be divided into four categories:

- Animals with clinical disease and shedding the organism
- Subclinical infection and shedding the organism (intermediate and incubating)
- Infected, but not ill or shedding enough bacteria to be culturally detectable (infected resistant)
- Uninfected cattle

For successful eradication and control of the disease a diagnostic test is required that is able to identify the intermediate group. The primary hindrance to making a diagnosis in the live animal is the paradoxical immune response during various stages of the disease. Subclinical infection is characterized by a strong cell-mediated immune response that can be detected by such assays as lymphocyte proliferation to a T-cell-independent mitogen and delayed-type hypersensitivity reactions or skin tests. A negligible humoral response during subclinical infection reduces the usefulness of serologic diagnostic tests. In contrast, clinical disease is characterized by a strong humoral immune response and a weak cell-mediated response. During clinical disease, high numbers of MAP are shed in the feces, and one of the definitive tests is culture of the organism from feces.

Diagnosis of MAP Infection

To diagnose paratuberculosis in an individual animal several testing methods are available. Although in clinical cases the clinical presentation can be highly suggestive of paratuberculosis, confirmation of the diagnosis will require either directly identifying MAP in feces or tissue or identifying a humoral or cell-mediated immune response of the affected animal.

Direct identification of MAP in feces or tissue can be achieved by microscopy, culture, or the use of specific DNA probes in combination with PCR. Serologic tests for paratuberculosis in cattle identifying the presence of specific antibodies include the absorbed ELISA, complement fixation (CF), and agar gel immunodiffusion (AGID). The choice of the appropriate test must be based on the intended purpose of testing.

Culture or Detection of Organism

Bacteriologic Culture. Examination **of the feces** is a valuable diagnostic aid for detecting infection in clinically diseased animals and to some extent in apparently healthy cattle in known infected herds. **Fecal culture** is presently recognized as the **most reliable index of infection in live cattle.** Conventional MAP culture is preceded by specimen decontamination and concentration of the organisms before inoculating a growth medium. Culture of MAP can either be done on solid or liquid growth medium requiring an incubation period of 4 to 8 weeks for liquid culture media and 8 to 16 weeks for solid culture media. A technique of radiometric culture based on the release of radioactive CO_2 from bacterial metabolism that reduces the incubation period is available but requires the use of radioactive reagents.

Sensitivity of the fecal culture varies with the stage of infection. In clinical cases fecal culture sensitivities of 70% and higher were reported, whereas in clinically healthy but infected cows the sensitivity of fecal cultures was reported to range between 23% and 29%.[29] The specificity of fecal culture is estimated to be at least 98%. False-positive results may occur in a population in which the noninfected animals are subject to contamination from infected herdmates.[29] Cattle kept in a heavily MAP-contaminated environment are at increased risk of oral ingestion and consequent fecal excretion of MAP without being infected, yielding potentially false-positive results through the so-called "pass through" effect. It is therefore advised to cautiously interpret fecal samples yielding a low degree of MAP shedding in herds with a high prevalence of paratuberculosis.[10]

Counting the number of colony forming units on solid medium or measuring the time to detection on liquid medium in fecal cultures allows one to assess the degree of shedding and thus the risk of disease transmission presented by an individual.[10] Cultured isolates must be subjected to appropriate testing, such as PCR, to confirm that isolates are MAP.[29]

Fecal cultures can be performed either on feces collected from individual animals, from pooled fecal samples, or from environmental fecal samples.

Pooled Fecal Samples and Culture. The culture of pooled fecal samples from several animals in a herd has been evaluated as a

means of determining a herd's infection status. Pooling samples reduces the number of fecal cultures necessary to determine infection in low-prevalence herds, reducing the cost of a large-scale Johne's disease control or eradication program. Strategically pooled culture specimens (five animals of the same age per pool) compared with individual fecal specimens can yield a sensitivity and specificity of 86% and 96%, respectively.

Environmental fecal samples collected from cow alleyways and manure storage areas appear to be an alternative strategy for herd screening and MAP infection status assessment and for estimating herd prevalence.

Microscopic examination of Ziehl–Neelsen stained smears of feces for the presence of typical clumps of acid-fast bacteria has been an attractive alternative to fecal culture because the results are available within an hour. However, the sensitivity and specificity of the microscopic examination have always been in doubt. It may be difficult to distinguish MAP from other acid-fast organisms, which are frequent in feces. Also, it may be necessary to examine smears on several occasions to obtain a positive result. Clumps of acid-fast bacteria in epithelial cells are diagnostic and are more likely to be observed during a diarrheic phase, when epithelial cells are more likely to be shed, than in a period when feces are normal. Generally, the microscopic examination of fecal smears for the presence of acid-fast clumps is an unreliable method of detecting MAP in bovine feces. A pinch biopsy collected with the fingernails, or scrapings of rectal mucosa, are of no great advantage compared with fecal smears, because it is probably only in the late clinical stages that the rectal mucosa is invaded. If rectal scrapings or rectal pinch biopsy are used, a positive finding is clumps of acid-fast bacilli in epithelial cells or macrophages.

DNA Probes and Polymerase Chain Reaction. Using DNA probes and PCR to determine the presence of specific MAP DNA in a specimen greatly reduces turnaround time for the diagnosis of paratuberculosis compared with culture. The majority of commercial paratuberculosis PCR tests use the IS900 sequence. This DNA sequence has the advantage of being present with several copies in the MAP genome, increasing sensitivity. Because this sequence also forms part of the genome of a few other environmental mycobacteria, the diagnostic specificity of the IS900 DNA probe is impaired. The use of unique sequences in the MAP genome such as ISMap02, ISMav2, F57, or Hsp X result in higher specificity but are less sensitive because fewer copies of these sequences are present in the MAP genome compared with IS900. Molecular methods to detect MAP include single PCR, nested PCR, and real-time PCR and are preceded by concentration and separation steps. Real-time PCR monitors amplification of the specific DNA sequence after each replication cycle. Therefore, in contrast to other methods, it provides a quantitative result for estimating the amount of MAP DNA present in the specimen and thus the degree of fecal shedding.[10] Positive PCR results prove the presence of MAP DNA in the specimen but do not confirm the presence of viable MAP.

Culture of Milk and Blood. The organism can be cultured in the milk of subclinically infected cows, and the prevalence of infection of milk is highest in samples from cows with heavy fecal shedding and lowest with light shedding. A nested PCR test has been used to detect MAP in the blood and milk of cattle with clinical and subclinical infection. Between 8% and 22% of subclinically infected and about 35% of clinically affected cows harbor MAP in their udders.

Tests on Tissue Samples. Diagnosis of paratuberculosis may be attempted by surgically collecting a full-thickness biopsy of the ileum (>1 g) in combination with a biopsy of an ileum-associated lymph node (>1 g). Obtained specimens should be used for culture and histologic examination. Because acid-fast organisms are not necessarily encountered in all tissue specimens of infected animals negative results based on a single biopsy should be interpreted cautiously.

Serologic Tests

The serologic tests commonly used to identify humoral immune responses to MAP infection in cattle are the CF test, the ELISA, and the AGID. Cellular immune responses are commonly identified by means of the IFN-γ assay.

Complement Fixation Test. Historically the most widely used serologic test for the diagnosis of bovine paratuberculosis was the CF test. Although diagnostic sensitivities of approximately 90%, and specificities of approximately 70%, for the CF test have been reported in clinical cases, early cases and nonclinical carriers fail to give positive reactions. Moreover a number of nonspecific, transient, positive reactions caused by cross-reactions do occur, impairing the specificity of the CF test. Notwithstanding some countries require that cattle have a negative CF test before importation. Negative test results in apparently normal cattle should be interpreted with caution; positive test result can be regarded as a presumptive diagnosis of infection but should be confirmed by fecal culture.

Agar Gel Immunodiffusion. The sensitivity of the AGID test for the diagnosis of clinical paratuberculosis is 96% with a specificity of 94%. It is considered to be the most appropriate test available for the diagnosis of clinical disease. The test has one-third the diagnostic sensitivity of fecal culture in the diagnosis of subclinical infection. The test is inexpensive and the results are available within 48 hours. Because positive reactions are given by tuberculous animals, the test is limited to use in tuberculosis-free herds.

A fluorescent antibody test is available but is unable to distinguish between the antigens of *M. avium* and *M. paratuberculosis.* It does distinguish between *M. paratuberculosis* and *C. renale*, which are easily confused by the CF test. Combined with the CF test the fluorescent antibody test is used to detect early, subclinical cases, but the results are far from accurate. A refinement of the conventional fluorescent antibody test, which gives greater accuracy in identifying specific mycobacterial antigens, is the observation of the uptake by macrophages of fluorescein-coated insoluble spheres.

Enzyme-Linked Immunosorbent Assay. ELISA is considered the test for serum antibodies against MAP with the highest sensitivity and specificity available. Although the test accuracy in clinical cases is similar to the CF test, ELISA outperforms other serologic tests to identify subclinically infected carriers. Generally, the sensitivity of the ELISA is limited by the nature of the immune response to MAP infection in which antibodies are only produced in advanced stages of infection. The sensitivity of serum ELISA is considered to be medium to high in clinical cases of paratuberculosis. In contrast the sensitivity of ELISA used to detect infected but subclinical cases reported in the literature ranges between 7% and 39%.[30]

The ELISA response to MAP may also vary according to the characteristics of the cow and stage of lactation. The probability of being ELISA positive may be two to three times lower for cows in parity 1 compared with cows in later parities. In early lactation the probability of being positive was highest in the milk ELISA. In the serum ELISA the odds of being positive was highest at the end of lactation.

These results demonstrate the effect of stage of infection on serodiagnosis. The subclinical, light-shedding cattle are usually seronegative, whereas heavily infected animals are usually seropositive. In most cows in the early stages of infection when fecal shedding is low, the humoral antibody response is below the limit of detection, and currently available serologic tests are inadequate to detect those animals. As the infection progresses, the humoral response increases, and heavy fecal shedders and clinically affected animals are more readily detected.

It has been recommended to use quantitative results of the serum ELISA (i.e., Optical Density or S/P ratios) rather than simple dichotomous (positive/negative) results in the decision-making processes of a

control program. These quantitative values correspond well with the degree of shedding and thus the infectious risk of an individual animal.[10]

Using milk samples from dairy cows to detect antibodies to the organism facilitates the testing of large numbers of animals and has already been incorporated into routine milk testing programs in some countries. The sensitivity of different milk ELISAs has been studied on a herd level and on an individual animal level and were found to be similar to serum ELISA sensitivity.[23,30] The odds for a cow testing seropositive with a milk ELISA were higher for animals in the first 2 weeks of lactation and again after 45 weeks of lactation. This was explained with higher amounts of immunoglobulins lost into the udder at the onset of lactation. Decreasing transfer of immunoglobulins to the mammary gland and increasing milk production supposedly result in dilution of milk antibodies after the early postparturient period. Decreasing milk production toward the end of lactation is thought to be the main reason for increased odds to test positive with a milk ELISA in later stages of lactation.[23] Accordingly high-yielding dairy cows were found to be less likely to test positive with milk ELISA than low-producing cows. Although this observation could be explained by higher dilution of antibodies in dairy cows with higher milk production, decreased milk production in MAP-infected cows has been documented. It is therefore not clear if higher milk production of uninfected cows or higher antibody titers in milk in infected cows are the underlying mechanisms behind this observation. These results indicate that sensitivity of milk ELISA is improved when conducted in cows in either early or late lactation.

Immunity Tests. The in vivo tests of cell-mediated immunity included the skin and intravenous johnin tests, which were the original tests used. They are no longer used because of inadequate sensitivity and specificity. The IFN-γ assay is based on the release of this compound from sensitized lymphocytes during incubation with a specific antigen. The amount of released IFN-γ is quantified with a sandwich ELISA. Results from this assay are frequently difficult to interpret because neither the amount of antigen used nor the interpretation criteria have been standardized. Depending on the applied interpretation criteria, sensitivity for the IFN-γ assay in MAP-shedding cattle reported in the literature range between 13% and 85% and specificity between 67% and 94%.[29] Because of costs and variable performance of this diagnostic test it is currently not recommended.[10]

Summary of Diagnostic Testing

Apart from postmortem examination most diagnostic tests provide adequate specificity but only moderate to weak sensitivity to diagnose subclinical MAP infection. Fecal cultures provide the highest specificity but have a long turnaround time because of long incubation periods. Genetic probes and PCR yield results within days but suffer from inferior sensitivity compared with fecal culture specifically in low-shedding MAP carriers. ELISAs for serum or milk are the most commonly used diagnostic tests. The sensitivity of the ELISA test is highest in animals in the later stages of the disease, usually when clinical disease is developed. However, the absorbed ELISA sensitivity for stage 1 animals will be low at about 10%. Overall the absorbed ELISA detects approximately 35% of the animals found positive by concurrent fecal culture. Only repeated testing of cattle, especially young animals, from infected herds will provide the data to determine the true infection rates within infected herds.

Diagnostic Strategies for Different Situations

Eight specific testing purposes were considered[30]:

- **Herd classification** (infected/noninfected). In dairy herds bacterial culture of six environmental fecal samples is considered sufficiently sensitive and most cost-effective to determine the infection status of a dairy herd. Negative culture results on all six samples suggest that the herd is either not infected with MAP or has a low herd prevalence. For beef cow–calf operations fecal cultures or serum ELISA can be conducted on the whole herd. If case serology is chosen, positive ELISA results should be confirmed by fecal culture. Alternatively, target testing of a particular group of animals (e.g., thin animals over 30 months of age or all animals over 36 months of age) by fecal culture or serology as described previously can be conducted.
- **Precise estimation of within-herd prevalence.** This testing objective is expensive and is of limited value for the control of paratuberculosis under field conditions but may be appropriate for certain experimental conditions. For the precise estimation of the within-herd prevalence a large number of animals that must be determined by the use of standard epidemiologic equations have to be tested. For herds with up to 300 animals all animals must be tested. For herds with over 1000 animals a statistically determined and randomly selected subset of animals can be chosen. Diagnostic tests used include fecal cultures, PCR assay on feces, or ELISA. To be able to reliably follow the longitudinal development of the within-herd prevalence application of the same diagnostic procedure in subsequent tests is required.
- **Disease control in herds with known high infection prevalence (>10%) and clinical disease.** The main objective of a paratuberculosis control program is to reduce the economic impact of the infection rather than eradication of the disease. Because greatest economic losses are caused by animals in advanced stages of infection, in which seroconversion occurred in many cases, testing by ELISA is recommended as part of a control program. ELISA has a low cost and the sensitivity was estimated to approximate 85% in MAP-shedding cattle.[30] Effective control strategies require that highly positive animals on ELISA are removed from the herd. Although testing of beef cow–calf operations by ELISA also has been recommended, the economic impact of this control strategy has not accurately been documented. Because of the generally lower within-herd incidence of MAP infection in beef cow–calf operations the motivation of beef producers to invest in control programs is rather low.
- **Surveillance (estimation of biological burden).** Objective of MAP surveillance is to monitor the infectious pressure in herds in which paratuberculosis is controlled. Corrective measures will be implemented when surveillance testing indicates an increase of infectious pressure above a specified threshold value. Whereas in dairy herds surveillance presents a low-cost strategy to monitor the disease prevalence in herds in which MAP infection is controlled, in beef cow–calf operations MAP surveillance is not considered economically effective. Periodic target testing of thin cows, cultures of environmental fecal samples and identification of clinical cases either by fecal culture or ELISA are the most commonly used approaches.
- **Eradication (elimination of MAP from the herd).** Disease eradication is the logical step following effective disease control that led to a low within-herd prevalence. Although theoretically possible there is currently no convincing data available supporting the assumption that MAP eradication under field conditions is actually possible. For commercial herds disease eradication is unlikely to be a cost-effective option. Because of the presumed low disease prevalence in operations attempting to eradicate paratuberculosis, the diagnostic test with the highest sensitivity, which is fecal culture, is the best choice. Because there is limited loss in test sensitivity, the use of pooled fecal samples (five samples per pool) for fecal cultures is a valid testing alternative. Regular whole-herd testing is required

over several years, and positive animals must imperatively be removed from the herd.

- **Confirmation of clinical diagnosis in herds with no prior history of paratuberculosis.** In herds without history of paratuberculosis appropriate confirmation of a suspected case of paratuberculosis is essential. Postmortem examination, which includes identification of pathognomonic gross lesions as well as histology and bacteriology of ileal and mesenteric lymph node tissue, presents the most sensitive and definitive diagnosis. Suitable antemortem tests include fecal culture or PCR assay on a fecal sample.
- **Confirmation of clinical diagnosis in herds with prior history of paratuberculosis.** In a herd with previously confirmed cases of MAP infection confirmation of the diagnosis is a useful tool for any control or surveillance strategy. Fecal culture or *PCR* assay on a fecal sample as well as serum or milk ELISA are all acceptable antemortem diagnostic tests with high sensitivity and specificity in clinically affected animals.
- **Biosecurity (prepurchase testing).** The objective of prepurchase testing is to reduce the risk of introducing infected replacements animals into the herd. Evidently the most effective approach is to avoid or at least minimize the number of purchased animals introduced into the herd. When considering the acquisition of an animal, evaluation of the infection status of the herd of origin rather than the test result of the animal in question is critical. The objective should be to only purchase animals that are test negative and originate from herds that have a within-herd prevalence that is at least 50% below the within-herd prevalence of the buyer's herd.

NECROPSY FINDINGS

Lesions are confined to the posterior part of the alimentary tract and its associated lymph nodes. The terminal part of the small intestine, the cecum, and the first part of the colon are usually affected. In advanced cases the lesions may extend from the rectum to the duodenum. Typically, the intestinal wall is three or four times normal thickness, with a corrugated mucosa and prominent thickened serosal lymphatics. The ileocecal valve is always involved, with the lesion varying from reddening of the lips of the valve in the early stages to edema with gross thickening and corrugation later. A high incidence of arteriosclerosis has been observed in advanced cases of Johne's disease, with a distinct correlation between the vascular lesions and macroscopic changes in the intestine. The mesenteric and ileocecal lymph nodes are enlarged and edematous, but unlike tuberculosis, foci of necrosis and mineralization are rarely visible. The characteristic microscopic findings include large numbers of epithelioid macrophages and multinucleate giant cells within the lamina propria and submucosa of affected gut segments and within the paracortical areas of draining lymph nodes. A granulomatous lymphangitis is often visible.

DIFFERENTIAL DIAGNOSIS

The characteristic features of clinical paratuberculosis include chronic diarrhea, which does not respond to therapy; progressive weight loss; and emaciation in a single animal. The definitive etiologic diagnosis can be obtained by using a combination of serologic tests, fecal culture, polymerase chain reaction on fecal material; and histologic examination of ileal and mesenteric lymph node tissue.

The clinical disease must be differentiated from diseases that cause chronic diarrhea in adult cattle. The chronic nature of Johne's disease is usually sufficient to differentiate it from the other common enteritis of cattle. **Salmonellosis, coccidiosis**, and **gastrointestinal helminthiasis** are usually acute, and the latter two occur principally in younger animals and are distinguishable on fecal examination for oocysts and helminth eggs. **Secondary copper deficiency (chronic molybdenum poisoning)** is likely to be confused with Johne's disease in cattle, but is usually an area problem affecting large numbers of animals and responds well to the administration of copper. Other debilitating diseases in which diarrhea is not an important clinical finding are **malnutrition, chronic reticuloperitonitis, hepatic abscess, pyelonephritis, lymphosarcoma, and amyloidosis.**

Idiopathic eosinophilic enteritis in cattle is characterized clinically by chronic diarrhea and weight loss, and recovery may occur following treatment with dexamethasone.

TREATMENT

Currently there are no definitive cures for paratuberculosis and no therapeutic agents registered for the treatment of MAP infection. Because of this lack of efficacy and the failure of any of the antimicrobials to provide a bacteriologic cure, treatment is not recommended. If initiated, treatment that typically must be maintained for life is aimed at reducing clinical signs and possibly the degree of fecal MAP shedding.[10] Treatment attempts can potentially increase environmental contamination by extending the life of the treated animal and should therefore only be considered for exceptional circumstances such as the treatment of valuable sport or zoo animals.

The antimicrobials which have been used are summarized here:

- **Isoniazid** given to cattle at between 10 and 20 mg/kg BW orally daily has been used with varying degree of success. Isoniazid kills MAP only in the replication phase and thus only has a bacteriostatic effect creating a state of remission, whereas treatment is administered without eliminating MAP. Isoniazid is metabolized by the liver and has a narrow therapeutic range. Avoiding overdosage and periodic monitoring of liver function is therefore advisable
- **Rifampin** has been used extensively for the treatment of human tuberculosis and *Rhodococcus equi* infections in foals. For the treatment of paratuberculosis in rabbits rifampin (10 mg/kg once daily orally) has successfully been used in combination with streptomycin (10 mg/kg twice daily intramuscularly). Combinations with other drugs such as levamisole have also been proposed. Based on pharmacologic studies a dosage between 10 and 20 mg/kg administered orally has been recommended.
- **Clofazimine**, a phenazine derivate, was originally used for the treatment of sulfone-resistant leprosy and later also to treat paratuberculosis in experimentally and naturally infected small ruminants. Although complete cure was not achieved, clinical improvement and decreased fecal shedding was reported in clinical cases of paratuberculosis after oral treatment with an oral daily dose of 2 mg/kg. Dosage recommendations are 600 to 1000 mg orally per animal per day for the rest of the animal's life.
- **Monensin**, a carboxylic polyether ionophore, has been widely used in beef cattle as a growth enhancer as well as to control coccidiosis. In dairy cattle monensin is registered in different countries as a feed additive to improve energy metabolism. In Canada monensin is labeled for the indication of reducing fecal shedding of MAP in adult cattle in high-risk Johne's disease herds. Several studies demonstrated a decrease in number in colony forming units in different tissues of naturally and experimentally infected cattle treated with monensin. Monensin was also evaluated as an infection-prevention drug in calves experimentally challenged with MAP. Monensin-treated calves had fewer culture-positive tissue and fecal samples and fewer colony forming units compared with a control group. Dairy cows treated with monensin as a feed additive were found less likely to test positive for MAP infection by milk ELISA. Anecdotal reports of clinical improvement in advanced clinical cases after treatment

with monensin administered at the dose approved for other indications are available.[10] The use of monensin at the dosage approved for other indications may therefore be a suitable component of a MAP control program provided its use is legally permitted.[10]

- **Dietzia subspecies (C79793-74)**, a probiotic bacterium, was reported to hamper growth of MAP in vitro and treatment of infected animals with Dietzia with daily doses of 2 to 5×10^{11} CFU per cow per day was associated with longer survival times and lower MAP antibody titers.[31-33] Neonatal calves fed live Dietzia but not calves fed inactivated Dietzia at a dose of 1 to 2×10^{11} CFU per calf per day over 60 days were reported to be resistant to MAP infection. Because all publications come from one research group with commercial interest, further independent research is needed to substantiate the effectiveness of Dietzia for the treatment and control of paratuberculosis.

TREATMENT AND CONTROL

Treatment

Isoniazid (10 and 20 mg/kg BW every 24 h orally for life) (R-3)

Rifampin (10 and 20 mg/kg BW orally for life) (R-3)

Clofazimine (600–1000 mg per animal every 24 h orally for life) (R-3)

Monensin (185–660 mg per lactating animal every 24 h orally for life or 115–410 mg per nonlactating animal every 24 h orally for life) (R-2)

Dietzia subspecies C79793-74 ($2–5 \times 10^{11}$ CFU per animal every 24 h orally long term) (R-2)

Prevention in calves

Dietzia subspecies C79793-74 ($1–2 \times 10^{11}$ CFU per calf every 24 orally for the first 60 days of life) (R-2)

BW, body weight; CFU, colony-forming units.

CONTROL

The control of Johne's disease in ruminants is challenging because of the ubiquitous nature of the organism, the long incubation period, most cases are subclinical, and the laboratory tests available lack sufficient sensitivity to identify subclinically infected animals.

Because of the difficulty in diagnosing subclinical cases eradication strategies are usually not practical for economic reasons. Most current paratuberculosis control programs therefore are aimed at containing the disease at a low-prevalence level rather than entirely eliminating it. Before establishing a paratuberculosis control program at a herd level it is essential to educate the producer about risks and costs associated with the disease as well as proper hygiene and biosecurity measures.[10] A successful control program requires long-term commitment and strict compliance of the producer.

Principles of Control

Decreasing within-herd MAP prevalence involves 3 basic steps:

- Identify and eliminate MAP-infected animals from the herd
- Prevent introduction of infected animals into the herd
- Prevent exposure of susceptible animals to MAP

Identification and Elimination of Infected Animals

As a first step a producer might want to **determine the herd status and roughly estimate the herd prevalence** of a previously untested herd independently of an official control program. Collection of several environmental fecal samples obtained from cow congregation areas for culture or PCR is an appropriate and cost-effective approach for dairy herds for initial determination of herd infection status.[10] To determine the prevalence of infection within a herd, testing of individual animals over 36 months of age using either the ELISA (serum or milk) or individual fecal culture or PCR has been advised.[10] Ultimately the choice of the specific testing strategy will depend on factors such as herd size, costs, goals of the producer, and possible participation in official paratuberculosis control programs.

The first test to estimate the prevalence of infection will identify seropositive or MAP-shedding animals, which along with their offspring can be culled and sold only for slaughter. Calves from infected animals can be kept separate and grown and fed under feedlot conditions until ready for market. Because of the low sensitivity of standard diagnostic tests, repeated testing of the herd at 6- to 12-month intervals at least until two consecutive negative herd tests are achieved is required. This method has the advantage that many heavy fecal shedders are detected early before showing clinical signs, reducing the environmental contamination with MAP. Intermittent-MAP–shedding and low-MAP–shedding animals may escape detection.

An economic decision analysis model of paratuberculosis in a dairy herd indicates that a test-and-cull program should be profitable when the pretest prevalence of infection is greater than 5%. The model predicted that the best diagnostic test would be the one with the highest specificity and lowest cost, with test sensitivity of secondary importance. Given the costs of various types of diagnostic technology, it appears that the ELISA is the most efficient for testing and culling programs.

Prevention of Introduction of Infected Animals Into the Herd

For herds free of Johne's disease, all measures must be used to avoid the introduction of infected animals into the herd by maintaining a completely closed herd or by carefully screening purchased animals. The purchase of cattle is the most common way MAP is introduced into a herd. Purchasing cattle only from herds documented to be free of Johne's disease is preferable to testing specific cattle before introduction because of the low sensitivity of available tests for individual cattle. Dairy herds using typical management practices experience preventable risks of Johne's infection and disease. A dairy herd with 400 cows in milk that introduces 40 cows per year from the general population of dairy cows has an estimated 64% probability of introducing MAP to the herd. This risk could be reduced to 4% through the purchase of cows from herds at level 2 of the U.S. Voluntary Johne's Disease Herd Status Program. A simulation model to assess the risk of introduction of MAP infection into a dairy herd through purchase of female replacement cattle has been used to estimate the probabilities of a producer purchasing an infected lot during a given period of time. The probability of introducing infection is directly proportional to the prevalence of infection in the herds of origin.

All herd replacements should be tested and found negative before being purchased and introduced into the herd. Only test-negative animals from herds with no or few positive animals should be purchased. The goal should be to obtain replacement animals only from herds with a test-positive percentage that is at most half of the test-positive percentage of the buyer's herd.[30]

PREVENTION OF EXPOSURE OF SUSCEPTIBLE ANIMALS TO THE INFECTIOUS AGENT

Dairy Herds

1. **Minimize contact between young and older animals and from fecal contaminated feed and water**:
   - Clean and disinfect maternity and calf pens after each use
   - Calve cows in clean, dry, dedicated maternity pens
   - Remove calves immediately after birth to clean, dry calf pens, stalls, or hutches
   - Harvest colostrum hygienically to avoid contamination with fecal material
   - Feed colostrum only from test-negative cows
   - After colostrum feeding, use pasteurized milk, or use milk replacer
   - Raise calves separate from the adult herd for at least the first year of life
   - Do not allow shared feed or water between adults and young animals;

do not offer feed refusals from adult cattle to young animals
- Avoid vehicular and human traffic from adult animal areas to young animal areas

2. **Prevent manure contamination of feed and water sources:**
   - Use separate equipment for handling feed and manure
   - Design and maintain feed bunks and waterers to minimize risk of contamination with manure
   - Do not spread manure on grazing land
3. **Reduce total farm exposure to the organism:**
   - Immediately cull all animals with clinical evidence of Johne's disease
   - Cull culture-positive animals as soon as possible; for cows with low or moderate fecal culture colony counts, removal at the end of lactation may be acceptable
   - Test adult cattle at least annually by serum or fecal tests; positive serum test results should be confirmed by fecal culture or PCR
   - Purchase replacement animals from test-negative herds

Hygiene

Controlling the disease at a low level of prevalence in the herd requires hygienic precautions to limit the spread of infection. Environmental conditions and manure-handling procedures correlate with prevalence of infection. Overall cleanliness of the farm and especially the amount of fecal contamination resulting from the design, maintenance, location of the housing facilities, and frequency of cleaning are important items for discussion with the producer. Opportunities for exposure of young cattle to adult cattle feces, either because of direct access to water contaminated from adult cow feces or because of the common practice of using the same loader for feeding and manure handling of young stock and adult groups of cattle, are risk factors to be removed or modified. Avoidance of fecal pollution of drinking water and feed by providing troughs in high positions, fencing of marshes and ponds, and closing contaminated pastures for up to 3 years are worthwhile measures.

Strip grazing should be avoided as fecal contamination of pasture is likely to be intense. The provision of piped water supplies to cattle on pasture rather than the use of ponds and ditches has been associated with a decline in the incidence of Johne's disease. Frequent harrowing of pasture fields to disseminate dung pats facilitates destruction of the bacteria by exposing them to sun and drying. Yard and barn manure should be spread only on cultivated fields.

In infected herds, any animal with any signs suggestive of the disease should be isolated until its status has been determined. Adoption of these hygienic precautions has been shown to greatly reduce the prevalence of the disease.

Dairy Calf Health Management

Attention to calf health management practices is a vital component of a control program. A simulation model of the control of the disease in a dairy herd indicates that calf-management techniques that reduce the number of effective cow–calf contacts decreases the prevalence of infection in the herd. It is still advisable to rear calves away from infected cows, and if possible in individual pens to prevent spread among the calves. Newborn dairy calves should be removed from the dam immediately after birth and reared in individual calf pens. Colostrum must be collected with care to avoid contamination with feces. Teats should be thoroughly cleaned before collecting colostrum or letting the calf nurse.

HTST (72°C for 15 seconds) and on-farm pasteurization of raw milk markedly reduces the number of MAP in raw milk from infected cows. Pasteurization of colostrum at 63°C for 30 minutes was found to destroy or at least greatly decrease the number of MAP in colostrum from infected dams.

Dairy cows due to calve should be kept separately from the milking herd and calved in clean calving box stalls. Infected cows should not be allowed in the maternity ward. Calves from cows that are clinically affected should not be reared as herd replacements but grown and fed for beef production. Sucking of dams and nurse cows should not be permitted. Milk for bucket feedings should be collected hygienically, and rearing on milk substitutes should be encouraged. Calves should not have any contact with yearling animals or mature cows that may shed the organism. Postweaning calves should not have contact with the adult herd to avoid infections. In dairy herds with a high prevalence of infection, calves should be moved to calf barns and hutches rather than to pens in the cow barn.

Beef Herds

Control programs for beef cow–calf herds apply the same principles as for those in dairy herds but must adapt the procedures to meet calf health management needs. Some specific control measures for beef herds include the following:

- Avoid manure buildup in pastures and corrals in which late-gestation cows are kept
- Provide a clean calving area, with low cow density
- Move cow–calf pairs to clean pasture as soon as bonding occurs
- Move feed bunks, waterers, and creep-feed areas frequently to avoid exposing calves to manure buildup
- Do not place weaned calves on pasture used by cows
- Blood or fecal test the entire breeding herd annually; avoid calving-out and raising offspring from test-positive animals
- If possible, calve first-calf heifers in an area separate from older cows

Vaccination of Cattle

Vaccination for Johne's diseases with either inactivated or live-attenuated whole-cell–based vaccines have been used since the 1920s. A number of studies collectively confirmed that vaccination reduces the occurrence of clinical symptoms and tissue colonization but does not eliminate infection. Subunit vaccines consisting of sonicated bacteria, bacterial cell fractions, or recombinant MAP antigens were reported to provide a much lower degree of protection. Efficacy of vaccination may depend on the age at the time of exposure versus age at the time of vaccination as well as on the MAP burden on the farm. Vaccination of dairy calves in the Netherlands reduced the number of clinically affected animals by almost 90%. In a cross-section study of 25 vaccinated and 29 nonvaccinated herds, the rate of shedding of MAP was not significantly different between the vaccinated (4.4%) and nonvaccinated herds (6.7%). If legally permitted, a Johne's disease vaccination program can be useful as part of a comprehensive control program but cannot replace concurrent control measures.

Major drawbacks of the use of whole-cell–based vaccines are the interference with the diagnosis of bovine tuberculosis and paratuberculosis, human health risks resulting from accidental inoculation, and s the occurrence of granulomatous lesions at the injection site produced by most oil-based bacterin vaccines. Interference with diagnostic tests used in national tuberculosis eradication programs is the major hurdle affecting approval of MAP vaccines by authorities worldwide.

Vaccination is available on a limited basis in the United States and other countries. In cattle paratuberculosis vaccines are recommended for exclusive use in calves younger than 1 month with the justification that prevention of infection requires vaccination at a very young age and that single early vaccination decreases interference with diagnostic tests for tuberculosis at an older age. The positive test to tuberculin is maximum at 5 weeks after vaccination and has completely disappeared at 18 months. In general terms, vaccination can be recommended in heavily infected, tuberculosis-free herds, but only in areas in which tuberculosis eradication is neither underway nor projected. The comparative tuberculin test can be used to detect tuberculosis in Johne's vaccinated herds. Vaccination of calves from 5 to 40 days of age with an inactivated paratuberculosis vaccine resulted in positive ELISA titers for at least the first 15 months, which could interfere

with the serodiagnosis of the disease in control programs that are based on serologic tests.

Control on a Countrywide Basis

Paratuberculosis in cattle is being recognized with increased frequency in the cattle populations of the industrialized world. The overall prevalence of infection in dairy cattle is about 10%, and no reliable data are available for beef herds. The continued spread of infection in cattle herds, the economic consequences of loss in productivity, and the biological possibility that the organism may be a food-borne disease deserves consideration by the appropriate authorities and research agencies.

Voluntary national guidelines are now available to certify herds as low-risk for paratuberculosis. Voluntary national and regional Johne's disease control programs for dairy and beef cattle herds have been introduced in the United States, Australia, New Zealand, and the Netherlands. Although the disease is notifiable in several European countries such as Austria, Germany, Greece, Ireland, Luxembourg, Norway, Switzerland, Spain, and Sweden, most countries in Western Europe do not have strategically planned control programs. Denmark, the Netherlands, and France have implemented nongovernment industry-supported programs in cattle herds. The emphasis of these programs is to control rather than to eradicate paratuberculosis.

A significant development has been the Voluntary Johne's Disease Control Program (VJDCP) in the United States, the Johne's Disease Market Assurance Program in Australia, or the paratuberculosis control program in the Netherlands.

Johne's Disease Control in the United States

The U.S. **VJDCP** was developed in cooperation between state and federal animal health agencies with industry support in an effort to certify herds free of paratuberculosis. The program was intended as a model for control programs within each state, and the guidelines were considered to be minimal requirements to control the disease in dairy herds. The program consists of three basic elements:

- Education of the producer
- Risk assessment and development of a disease management plan at the herd level
- Herd testing and herd classification

Education of the Producer

Education focuses on providing basic information over Johne's disease; explaining management strategies to prevent, control, and eliminate the disease; and outlining different state program components. The producer must understand the nature and the economic impact of the disease and must be able the recognize risk factors within his operation. Information around Johne's disease is made available to producers at the state level as well as at a national level. The National Johne's Disease Demonstration Herd Project, the National Johne's Education Initiative, and the Johne's Disease Integrated Program are among the best known USDA-funded projects and provide a wealth of information to producers.

Risk Assessment and Disease Management Plan

A risk assessment to identify management practices and facility issues likely to introduce or spread MAP throughout the herd is conducted. A management plan is then developed together with the herd owner with the objective to implement a practical and effective control program customized for the specific herd that the producer understands and to which he can commit. Comprehensive material including a handbook and an instructional guide has been developed to allow the attending veterinarian to conduct a thorough on-farm Johne's disease risk assessment. This risk assessment and the management plan must be reviewed and updated at least every 3 years.

Herd Testing and Herd Classification

Initial testing is required to determine the herd status. The testing strategy can be customized to the needs of the specific herd and the objective of testing. The primary objective of the VJDCP is to identify herds with low prevalence of paratuberculosis. The classification system consists of levels 1 to 6 in which levels 1 to 3 identify herds with low test-positive prevalence and levels 4 to 6 identify herds with two or more years of test-negative results. Levels 1 to 4 require annual testing according to the guidelines of the program, whereas for herds at levels 4 to 6 retesting is required in 2-year intervals.

Johne's Disease Control in the Netherlands

In the Netherlands an industry-driven paratuberculosis control program has been implemented that requires dairy producers to participate to be able to market milk. As part of the program, a paratuberculosis status (A, B, or C) is assigned to each participating herd, based on the results of regular herd screenings. Herd screenings consist of individual animal testing of all cattle 3 years and older either by serum ELISA or by fecal PCR, or by milk ELISA of all milking cows. If no seropositive or fecal PCR-positive animals are identified, then the herd is categorized as status A (low risk of infection). Herds with one or more seropositive animals are categorized as status B, provided positive animals are culled within 1 month of testing. If positive animals are not removed from the herd in a timely manner, then the herd is categorized as status C. Follow-up herd screenings are required in 2-year intervals for herds with status A and in 1-year intervals for herds with status B and C when using the milk or serum ELISA. Two-year testing intervals apply to herds with status B and C when using fecal PCR as a diagnostic test. Status C is maintained as long as positive animals remain in the herd, and status B is maintained until no seropositive animals are identified in one of the regular follow-up herd screenings. Producers can request to have a seropositive result confirmed by fecal PCR. The result of the fecal PCR overrules the result of the ELISA in milk or blood. Serology results are reported as simple dichotomous (positive/negative) results rather than a quantitative OD or S/P ratio.

FURTHER READING

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PARATUBERCULOSIS (JOHNE'S DISEASE): SHEEP, GOATS, CERVIDS, AND CAMELIDS

SYNOPSIS

Etiology *Mycobacterium avium* subsp. *paratuberculosis* (MAP).

Epidemiology Transmitted by fecal–oral route. Prenatal infection occurs in sheep and deer but not confirmed in goats. Source of infection is infected dam or contaminated pasture. Infection more likely soon after birth but age-related resistance to infection is not as pronounced as in cattle. In sheep and goats the incubation period is shorter than for cattle, usually 2–5 years, but increased stress (nutritional and gastrointestinal parasitism) can induce cases earlier. High flock and within-flock prevalence of infection in sheep in many countries. A high prevalence in farmed deer in New Zealand and some other countries. Deer can be infected with both the bovine and ovine stains of MAP, with the former being more infective and pathogenic.

Clinical signs

Sheep Chronic wasting disease of adult sheep; diarrhea not a distinct clinical finding. Common cause of emaciation in ewes, although cases can occur in 10- to 15-month-old sheep in high-prevalence flocks.

Goats Chronic progressive intractable diarrhea and emaciation extending over several weeks and months. Generally, a higher prevalence in milch compared with fiber breeds.

Deer Outbreaks of diarrhea, ill-thrift, and deaths in young deer (8–15 months) or latent infection that causes sporadic cases with weight loss and terminal diarrhea in older deer.

Clinical pathology Culture and direct PCR of feces. Serologic tests (ELISA, AGID, and CF) and bulked fecal culture for flock diagnosis. Low serum protein and marked hypoalbuminemia in affected animals.

Lesions Chronic granulomatous enteritis, regional lymphangitis, and lymphadenitis in sheep and goats; caseous lesions in deer.

Diagnostic confirmation Presence of gross intestinal lesions, culture and PCR of organism from tissues and histopathology, especially terminal ileum, ileocecal valve and lymph node, and mesenteric lymph nodes.

Treatment No treatment of significant value.

Control Identify and eliminate clinical cases and subclinically infected animals. Test flock or herd to identify high-prevalence age groups and make these a priority for culling. Improve management and hygiene to minimize spread of infection with emphasis on avoiding infection of newborn animals. Vaccination of sheep and goats prevents clinical disease but not infection and fecal shedding.

Differential diagnosis list

Diarrhea in adults
- Gastrointestinal parasitism
- Bacterial infections: Yersiniosis and salmonellosis

Chronic weight loss in sheep and goats
- Internal abscesses
- Caseous lymphadenitis
- Caprine arthritis-encephalitis
- Ovine progressive pneumonia
- Dental disease

AGID, agar gel immunodiffusion; CF, complement fixation; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction.

ETIOLOGY

The causative agent of paratuberculosis in ruminants is MAP, a slow growing acid-fast aerobic microorganism forming part of MAC. Although MAP is an obligate intracellular pathogen requiring a host for replication, it can survive for longer than 1 year in the environment. MAP has been subdivided into two main lineages designated as the slow growing type I (or S for sheep) and the faster growing type II (or C for cattle) according to the species from where these lineages were first isolated. Type I strains appear to have a strong host preference for sheep and are more virulent for this species, whereas type II strains are more commonly isolated from cattle and a range of other species. Genome sequencing has confirmed that an intermediate or type III strain is a subtype of the S strain.[1]

Molecular studies of MAP have identified a high degree of genetic similarity within the bovine isolates, regardless of geographic origin, indicating that only a few closely related clones may be responsible for widespread infection in cattle, other ruminants, and possibly wildlife. There is a higher degree of genetic heterogeneity among MAP isolates recovered from ovine sources. C strain more readily infects sheep, goats, and deer, whereas sheep strains tend to be more specific and are less commonly isolated from other species.[2,3] However, cograzing of sheep and beef cattle means that the S strain is being isolated more often from beef cattle, whereas dairy cattle be infected predominantly with the C strain.[4]

EPIDEMIOLOGY

Occurrence, Morbidity, and Mortality

Sheep and Goats

Paratuberculosis occurs worldwide and is of major importance in sheep in temperate climates and some humid, tropical areas. The prevalence is greatest in animals kept intensively under climatic and husbandry conditions, which are conducive to the spread of infection.

The history of ovine Johne's disease (OJD) in Iceland is an example of the spread of this disease from a point source. Briefly, 20 apparently healthy Karakul stud rams were imported from Germany in 1933. After 2 months' quarantine they were distributed to 14 farms, and the first clinical case of OJD was diagnosed in 1938. Gradually, infection spread from five originally infected farms and, after 18 years, 20% to 30% of the farms in the main sheep breeding areas were infected. The annual morbidity of sheep during the epidemic averaged 8% to 9% in affected areas, and was up to 40% on individual farms.

In New Zealand, OJD was first reported in the South Island in 1952, and by 1970 more than 150 farms were confirmed as infected. It was detected in the North Island in 1972, and by 1979 284 farms were known to be infected. More recent estimates of prevalence are 76% of sheep flocks (95% confidence interval [CI] 70%–81%) and 46% of deer herds (95% CI 38%–55%).[5] In Australia, OJD was first confirmed in 1980 and by 1999 had spread to most states. It is presumed that infection originated from sheep imported from New Zealand in the 1970s, and it is estimated that at least 40% of flocks are now infected in some areas.[6] In South Africa, the disease was unknown until an infected Merino ram was imported in 1967 and it then spread among sheep farms in the Western Cape and Eastern Cape provinces in the 1990s. Infection is also confirmed in South America, North America, and Europe in which 74% of 38 dairy sheep flocks in the Marche region of Italy tested positive to a commercial ELISA for Johne's disease.[7]

Clinical signs are progressive emaciation, with intermittent diarrhea in some sheep.

Sheep are easily infected experimentally, with doses from 10^4 to 10^7 viable bacteria reliably inducing infections in 12- to 16-week-old Merino lambs. Infected animals can excrete large numbers of the organism, up to 10^7 to 10^9 per gram of feces, but some can spontaneously recover from infection.[8]

Mortalities can vary considerably between farms, but OJD can cause significant financial losses. For example, in an Australian study the disease was associated with mortality rates from 2.1% to 17.5% and a decrease in farm gross margin of from 2.2% to 15.4%.[6] On average, these losses were estimated to cost affected farms at least $13,700 US$10,500 per year. In Cyprus, where sheep are farmed semi-intensively for milk to produce cheese, ewe mortalities can be as high as 4% per year. The disease is being recognized with increased frequency in goats and can cause large losses. In Australia, Johne's disease occurs in dairy goat breeds with endemic foci of infection in southeastern Australian states.[9]

Deer, Camelids, and Exotic Species

MAP has a broad host range, with deer, alpacas, llamas, camels, and captive and free-living wild ruminants, including bighorn sheep, Rocky Mountain goats, aoudads, mouflon sheep, reindeer, antelope, and yaks being susceptible. A high prevalence was detected in farmed alpacas in Australia in the 1990s, but a concerted control program has virtually eliminated this disease from Australian alpaca flocks.[10]

Outbreaks of Johne's disease have occurred in farmed red deer, and the incidence is increasing in some regions. For example, Johne's disease was recognized in farmed deer in New Zealand in the 1980s and by 2000, the disease had been diagnosed in 299 herds, or 6% of the commercial deer herds in New Zealand. Over 90% of these farms were identified from lesions in mesenteric and ileocecal lymph nodes at meat inspection, whereas only 6% were detected through the presence of clinically affected animals. The disease is now regarded as endemic in farmed deer in New Zealand (46% of herds) and has also been detected in farmed red deer in the UK, Belgium, Holland, and the Czech Republic. Young deer infected with MAP can develop disease within 5 to 7 months, with outbreaks affecting up to 20%, or can remain latent for many years.[3,11] Thus many infected deer will be culled before they show any clinical signs.

The epidemiologic implications of deer, cattle, and wildlife comingling on the same pasture are not fully known, but the rate of infection can be similar in both domestic species and so each can be a source of C-strain MAP for the other.

Prevalence and Source of Infection

The prevalence of Johne's disease infection in flocks or herds within a region is difficult to estimate because of the relative insensitivity of screening tests, uncertainty of antemortem diagnosis, and the failure to report cases unless a specific survey or eradication program is undertaken.

Sheep

OJD was first diagnosed in in Australia in central New South Wales in 1980. The disease has a highly clustered distribution indicating spread between neighboring properties and by sheep trading. In 2000, surveys found that the 95% probability limits for flock prevalence in low-, moderate-, and high-prevalence regions in New South Wales were 0.04% to 1.5%, 8% to 15%, and 29% to 39%, respectively, whereas all other states had an upper 97.5% probability limit of 1% or less. Based on these estimates, from 6% to 10% of flocks in New South Wales and 2.4% to 4.4% of flocks Australia-wide were estimated to be infected. Over 80% of affected flocks were located in a relatively small geographic area of New South Wales, whereas Queensland and Western Australia had a flock prevalence of less than 1%. Subsequently, a review of the OJD control strategy from 2007 to 2012 found that although the transmission of infection from some low-prevalence areas had been restricted, the disease had spread widely, and many areas that were classified as having a low prevalence in 2000 now had a medium or high prevalence of infected flocks.[12]

Methods of Transmission

Spread of the organism from farm to farm is usually caused by trading of livestock, which are unknown infected carriers and shedders of the organism. This results in clusters of infected flocks. Lateral spread between flocks, through contact between infected and uninfected sheep in common areas such as yards or roads, or the movement of feces across boundary fences, can then occur.

Intrauterine infection has been confirmed in sheep and deer, but most infection with MAP occurs by the fecal–oral route. This can occur by neonates suckling from an infected dam via contaminated teats or ingestion of fecally contaminated pasture.

Sheep

Clinically affected sheep excrete a large number of organisms, often over 10^9 viable MAP per gram of feces. Thus the output of 1 to 2 kg of feces from a single clinical animal over 1 day is sufficient to infect many animals, with an infective dose of S-strain MAP being as low as 10^4 organisms.[13]

Ovine trichostrongylid larvae (*Haemonchus contortus, O. circumcincta, T. colubriformis*) may become contaminated with MAP and may play a role in the transmission of the organism, although this is likely to be far less important than direct exposure to pasture contaminated with infected feces. Fetal infection can occur, with a much higher proportion of infected fetuses identified from clinically affected ewes (83%) compared with 1.6% from subclinically affected and none from uninfected ewes.

Deer

Deer can be infected with either the cattle or sheep strain of MAP, but the cattle strain appears to be of higher infectivity.[3,14] In New Zealand and elsewhere deer are cograzed with both sheep and cattle. However, modeling of the dynamics of Johne's disease in farmed deer found that if mixed strains of MAP were present, a reduction in infectivity of 30% would be sufficient for a dominant strain to outcompete a less infective one. This suggests that mixed infections with C and S strains of Map in a deer herd might not be common, because the C strain would become dominant.[11,15]

Risk Factors

Sheep and Management

A relative resistance to infection with increasing age is a feature of Johne's disease in cattle but is less pronounced with OJD. For example, experimental infection with a high dose of MAP induced lesions in both lambs and adult ewes; however, the were restricted to focal granulomas within lymphoid tissue in the ewes, whereas they progressed to more widespread lesions in the lambs.[16]

In Australia and New Zealand, fine wool Merino sheep have a higher mortality from OJD than other sheep breeds. Within large wool-producing flocks wethers often have a higher prevalence of infection. This is probably related to higher stocking rates for this class of animal and poorer nutrition, both quality and amount of pasture, relative to the ewe portion of the flock.[17] Poorly controlled infections with internal parasites and undernutrition are both associated with an increased prevalence of infection and clinical disease. For example, in a cross-sectional study of 92 Merino flocks in southeastern Australia, key risk factors associated with a higher prevalence of OJD included sheep whose dams had been in low body condition at lambing time, sheep that had experienced a longer period of growth retardation during their lifetime, and high stocking rates.[17] In this study vaccinating for more than 2 years was associated with a significantly lower prevalence of MAP infection.

Flocks shorn in winter and farms with a high percentage of improved pastures containing subterranean clover (the latter typically associated with higher stocking rates) were also associated with a higher prevalence of OJD in flocks in southeastern Australia. Exposure of young sheep to a high level of pasture contamination with MAP was identified as a risk factor for a higher prevalence of severe OJD lesions and mortalities in this area.[18]

Consistent with these observations, practices associated with intensive management, such as a high proportion of introduced

sheep, or multiple or foreign breeds, have been identified as risk factors for OJD in in Spanish flocks.

Deer

The risk factors in outbreaks of Johne's disease in deer have not been investigated in any detail. However, it is likely that they are similar to other species, namely age at exposure, size of infective dose, the innate immune response of the animal, and environmental factors.[3]

Environmental Risk Factors

Soil Characteristics

An association between high prevalence of MAP infection in ruminants and soil type has been recognized, and the literature on the possible links between the clinical expression of paratuberculosis and deficiency of macronutrients and micronutrients has been reviewed.[19] The evidence implicates regional soil acidification (low pH), excesses of iron and molybdenum, and marginal deficiencies in copper and selenium in a higher prevalence of Johne's disease. In Australia, mortality from OJD was higher on farms with light sandy soils, consistent with studies in dairy cattle in Spain. In contrast, a later study of 92 Merino flocks in southeastern Australia found a positive association between higher organic carbon, clay, and iron content, whereas there was a lower prevalence of OJD on farms with sandy soils.[20] It was suggested that MAP may adhere more closely to the smaller clay particles, compared with larger sand particles, and thus be retained in greater numbers for a longer period in clay soils. The association between low soil pH and occurrence of OJD was inconclusive, although most farms had relatively acidic soil and a narrow range of soil pH compared with other studies.[20] MAP requires iron for survival and replication, but is relatively inefficient at chelating this element compared with many other bacteria. Thus an increased concentration of iron is hypothesized to increase the survival of MAP in soil. The solubility of iron also increases with decreased pH, hence, the frequent association of increased prevalence of Johne's disease in acidic compared with alkaline soils.

Pathogen Risk Factors

MAP is an obligate pathogen and parasite of animals, and in theory it can be eradicated by removal of all infected animals. However, the organism can survive for long periods outside the host, enabling it to persist and spread in a grassland environment and withstand a periodic lack of suitable hosts.

Survival and Dormancy of Organism in the Environment

Both S and C strains of MAP can be extremely persistent in nature, with survival for more than 1 year. Studies of the survival of S-strain MAP in eastern Australia indicate that when the organism in feces becomes mixed with soil, there is a reduction of 90% to 99% in the apparent viable count of the organism.[21] This is thought to be caused by binding of bacteria to soil particles, which are excluded from culture by sedimentation during sample preparation. Survival of the organism in sheep fecal material applied to soil was greatest in a fully shaded environment (55 weeks) and was least where fecal material and soil were fully exposed to weather and where vegetation was also removed. The organism survived for up to 24 weeks on grass that germinated through infected fecal material applied to the soil surface in completely shaded boxes and for up to 9 weeks on grass placed in 70% shade.

Dormancy of the organism appears to be a feature in the Australian environment, with the dormancy characteristics related to genetic elements of MAP that are also present in other mycobacteria. However, survival is finite and significant pasture decontamination can occur within a relatively short period. This reduces exposure to the organism and the prevalence of disease.[21] Pasture decontamination can be hastened by pasture management, such as selective grazing with less susceptible hosts or mechanical slashing to decrease shade.

The organism persists without multiplication in pasture for long periods, and such pastures are infective for up to 1 year. The organism is relatively susceptible to sunlight and drying, to a high calcium content, and to high pH of the soil. Continuous contact with urine and feces reduces the longevity of the bacteria, but the organism can survive for 98 to 287 days in tanks, depending on the composition and alkalinity of the slurry. The alkalinity of the soil may also influence the severity of the clinical signs.

Zoonotic Implications

MAP is potentially of public health significance because, although there is no evidence of a causal relationship between it and Crohn's disease in humans, there is a growing literature on the possible association between MAP and Crohn's disease.[22] This is addressed in more detail in the section on Johne's disease of cattle, but more than 500 scientific papers made reference to this topic from 1972 to March 2014, averaging around 3.5 papers per month since 2009.[23]

The organism has been found in raw goat milk in Norway and conditions in cheese production have little effect on the viability of MAP, with viable bacteria found in hard and semihard cheese 12 days after production. Therefore consumption of cheese manufactured from raw goat milk sourced from herds infected with Johne's disease might lead to human exposure to MAP.

PATHOGENESIS

Following oral ingestion, the organism localizes in the mucosa of the small intestine, its associated lymph nodes and, to a lesser extent, in the tonsils and suprapharyngeal lymph nodes. The primary site of bacterial multiplication is the terminal part of the small intestine and the large intestine. At least three different groups of animals can occur depending on the host–bacteria relationship that becomes established. In the first group, animals develop resistance quickly, control the infection, and do not become shedders (infected resistant). In the second group, the infection is not completely controlled; some animals will partially control the infection and will shed the organism intermittently, others will become intermediate cases that are incubating the disease and will be heavy shedders of the organism. In the third group the organism persists in the intestinal mucosa, and from these animals the clinical cases develop.

The organism is phagocytized by macrophages, which in turn proliferate in large numbers and infiltrate the intestinal submucosa. This results in decreased absorption, chronic diarrhea, and resulting malabsorption. There is a reduction in protein absorption and leakage of protein into the lumen of the jejunum. In sheep, a compensatory increase in protein production in the liver masks the protein loss, so clinical signs of muscle wasting appear only when this compensatory mechanism fails. Within the macrophages, the bacteria remain viable and protected from humoral factors.

Immune Response

The first line of defense against invading MAP in the ruminant intestine involves M cells (special epithelial cells associated with ileal Peyer's patches and lymphoid follicles that actively take up particulate matter from the intestinal contents) and phagocytic macrophages. In early stages of infection, the organism is found in phagocytic macrophages in the intestine. Once inside the phagosome of an infected macrophage, the organism interferes with the normal course of phagosome maturation into phagolysosome, escaping destruction. The infection of inactivated macrophages within the intestine is the first step in establishing persistent infection and the subsequent development of disease. The host immune system begins a series of attacks against MAP-infected macrophages, initially involving CD4+ T cells, the production of IFN-γ, and cytolytic CD8+ cells (a Th1 response). These cells interact with the PI macrophage and each other through a complex network of cytokines and receptors. Despite this response, MAP organisms persist and the immune reaction injures the intestinal epithelial cells.

During the early subclinical stages of infection, the organism elicits a cell-mediated response by the host, characterized by strong delayed-type IV hypersensitivity reactions, lymphocyte proliferation, and production of cytokines by stimulated T lymphocytes. As

the disease progresses from subclinical to clinical, the cell-mediated immune response wanes and a strong humoral response (IgG1 isotype) becomes dominant. This process is not well understood, but competition for antigen between these Th1 and Th2 responses probably contributes to this switching.[24] ATh1 response is needed to keep the infection under control, and antibody against MAP does not protect the host against disease. During the final stages of disease, lack of antigen-specific cell-mediated immune response or complete anergy may result, allowing for rapid dissemination of the infection throughout the host.

There appears to be an immune spectrum, and no serologic or cellular immunity test will identify all animals in the spectrum. There are infected-resistant animals that control their infection but are unable to completely eliminate the organism. These animals do not react in antibody assays, only rarely or never shed organisms, and respond to the lymphocyte transformation test because their circulating lymphocytes are sensitized. In the intermediate stage, the animal fails to control the infection, antibodies appear in the serum, and organisms are shed in the feces. In the stage of clinical disease, the organisms are shed in the feces and the antibody responses and skin tests are variable.

Development of Lesions

In sheep with OJD two distinct histologic types of granulomatous enteritis occur, with a significant relationship between the infiltrating cell type and the degree of intestinal mycobacterial infection. At the two ends of the spectrum of lesions are these two widely differing forms:

- **Tuberculoid extreme** with a strong cell-mediated immune response and lesions consisting of small granulomata composed of epithelioid cells surrounded by many lymphocytes and with few or no bacilli in the lesions
- **Lepromatous extreme** with a strong humoral immune response and lesions composed of accumulations of macrophages containing large numbers of mycobacteria

Between these extremes are "borderline forms," which tend to be associated with the most severe clinical disease. Most sheep with Johne's disease have the multibacillary lesion (lepromatous) with extensive diffuse macrophage infiltrate within the intestinal mucosa and submucosa. In the paucibacillary lesion (tuberculoid) there is a marked lymphocytic and giant cell infiltration of the intestine. In sheep, the local release of macrophage and other lymphocyte-derived cytokines may influence the type of inflammatory and immune response that develops during infection. It is proposed that the elevated production of cytokines, such as IL-10, may suppress Th1 and encourage a Th2-type response.[25] This, along with a failure to clear a heavy burden of bacteria, may be one factor in the development of chronic inflammatory lesions.

In experimental infections of non-Merino sheep with S-strain MAP, clear differences were found in the cell-mediated immune response and outcome of infection according to age (1-month-old lambs compared with mature ewes) and the dose of MAP given (1.6×10^8 CFU compared with 4×10^3 CFU).[16] Lambs given a higher dose developed progressive and widespread intestinal lesions whereas, in ewes given a higher dose, lesions were smaller and confined to lymphoid tissue. Ewes given the low dose were PCR positive after infection, but no microscopic lesions were detected and tissues were culture negative at 110 and 220 days.

As infection progresses, the bacteria are carried by macrophages to other sites, particularly the uterus, the fetus, and the mammary gland. Vaccination against Johne's disease does not prevent infection or shedding of MAP in sheep, but it restricts the cellular response to the intestinal wall and thus prevents the onset of clinical disease.[26] Disease progression is associated with immune dysfunction, and although the exact mechanisms are not fully understood many differences have been described. A Th1 cell-mediated response, with secretion of IFN-γ, is predominant soon after exposure to MAP. If infection progresses to multibacillary lesions, this alters to a Th2 response, with increased expression of IL-4 and IL-10, whereas in sheep with paucibacillary lesions the Th1 response tends to remain predominant. However, the immune response is complex and not "all or none," with a mix of cell-mediated and antibody responses occurring. Changes described in the ileal and jejunal lymph node cells of sheep exposed to MAP, but with no or paucibacillary lesions, include increased secretion of tumor necrosis factor (TNF)-α, increased IL-10 (which suppresses Th1 and enhances Th2 cytokine production), decreased IL-18, and increased expression of toll-like receptor 9.[25,27,28] Longitudinal studies of experimental infections suggest that antigen-mediated lymphocyte apoptosis may contribute to the immune dysfunction that occurs in Johne's disease.[29]

CLINICAL FINDINGS

Sheep and Goats

In sheep and goats the disease is manifested principally by emaciation, with a marked difference in condition between affected animals and their nonaffected cohorts. In sheep the abrupt cessation of wool growth can cause decreased staple strength or shedding of wool. Diarrhea is not as severe or as common as in cattle, but the feces may be soft enough to lose their usual pelleted form. Affected sheep may be partially anorexic and lose weight for 6 to 12 months before they die.[30] Their feces usually appear normal until the terminal stages of the disease when they may become soft and pasty. Depression and dyspnea are evident in goats but are less obvious in sheep.

Other Species (Deer, Camelids, and Bison)

In deer, Johne's disease is unusual in that it can present as outbreaks of acute disease in young animals, with loss of BW, diarrhea, and deaths as young as 8 months of age, or sporadic cases in adults. C-strain MAP is more pathogenic, but the S strain can also cause disease.[3]

Similarly, in alpaca (*Lama pacos*) and lama (*L. glama*) weight loss, emaciation and diarrhea are reported in both young (8–14 months) and older animals. Some infected animals may show no clinical signs of Johne's disease but are positive on fecal culture or serologic testing. Many cases have grossly enlarged mesenteric lymph nodes, which can be confused with lymphosarcoma, and frequently widespread mycobacterial infection in organs other than the intestine.

In American bison (*Bison bison*) the clinical signs and lesions are similar to those in cattle, with gross lesions in the distal small intestine and enlarged mesenteric lymph nodes.

CLINICAL PATHOLOGY

In an infected flock or herd, animals can be in one of the following four groups:

- Clinical disease and be shedding the organism, usually in large numbers
- Subclinical infection and be shedding the organism, often intermittently and in intermediate numbers
- Infected, but neither ill nor shedding enough bacteria to be positive on fecal culture (infected resistant)
- Not infected

To control the disease, diagnostic tests must identify the first (heavy shedders) and second (intermediate) groups. Diagnosis in the live animal is hindered by the paradoxical immune response during various stages of the disease. Subclinical infection is characterized by a strong cell-mediated but negligible antibody response, reducing the usefulness of serologic tests at this stage. In contrast, clinical disease is characterized by a strong humoral immune response and a weak cell-mediated response. During clinical disease, high numbers of MAP are shed in the feces, so a definitive test is culture of the organism from feces.

Diagnostic Tests

Culture or Detection of Organism

Bacteriologic Examination. Several procedures are used to improve the sensitivity of detecting MAP by culture, including decontamination and concentration of the organism from specimens. Conventional MAP culture consists of decontaminating the specimen, concentrating the organisms, and inoculating a growth medium. A

molecular-based confirmatory test, such as PCR, to detect the MAP marker sequence IS900 is typically used to confirm positive specimens after 6 to 12 weeks incubation. The main criteria for differentiating *M. paratuberculosis* from other mycobacteria are its slow growth and dependence on mycobactin for growth.

Fecal culture using a radiometric technique is more sensitive and less expensive compared with conventional fecal culture and DNA probes, but a confirmatory test such as IS900 PCR is still required on positive specimens. The most commonly used radiometric technique was the automated BACTEC system, which was faster and had slightly higher sensitivity than conventional culture. However, the liquid modified BACTEC 12B medium is being phased out because it requires radioisotopes.

Pooled Fecal Samples and Culture. The culture of pooled fecal samples from 50 sheep or 25 goats of a similar age in a flock or herd is a cost-effective means of determining the infection status of a flock or herd. Pooling samples reduces the number of fecal cultures necessary to determine infection, reducing laboratory costs. It is a more highly sensitive and specific flock test for detection of OJD compared with serology using the AGID test. The estimated minimum flock specificity of pooled culture when used for surveillance and assurance testing is 99.1%. Surveillance and assurance programs in Australia are designed to provide a flock sensitivity of 95% at an assumed prevalence of 2% at a much lower cost (around 30% of that for serologic testing). Pooling of samples is possible because of the large numbers of MAP present in the feces of sheep with multibacillary disease, estimated to be 1.1×10^8 organisms per gram of feces. As the analytical sensitivity of similar culture methods has been estimated to 100 CFU/g of feces, the pooling rate can be large.

Microscopic examination of Ziehl–Neelsen stained smears of feces for the presence of typical clumps of acid-fast bacteria has been an attractive alternative to fecal culture because the results are available within an hour, compared with 2 to 3 months for culture. However, the sensitivity and specificity is low except in advanced clinical cases. It may also be difficult to distinguish MAP from other acid-fast organisms that are often present in feces, and with animals that are intermittent shedders it may be necessary to examine smears on several occasions to obtain a positive result.

Genetic Probe. A genetic element unique to MAP is an insertion sequence designated as IS900. Genetic probes for the detection of IS900 in clinical samples such as feces are available as commercial kits using the PCR. Other mycobacterial species contain IS900-like elements in low copy numbers (*M. cookii, M. scrofulaceum,* and *M. marinum*), although these are not reported in Johne's disease and, if necessary, can be distinguished by amplicon sequencing. The advantage of PCR is the speed of reporting (hours or days) and high specificity and ability to detect low amounts of DNA. For example, a real-time (RT)-PCR was able to detect a single copy of MAP IS900 from a range of tissues of cattle and sheep infected with MAP, including ileum, liver, and muscle.[31] One disadvantage is that molecular tests detect both living and dead organisms, so a positive result is possible from an animal that has ingested and is shedding MAP, but is not truly infected. Validation of molecular tests to detect MAP has also been lacking, plus fecal samples are a challenge because of the presence PCR inhibitors and a large amount of nonspecific DNA from other fecal microorganisms and the host.

Subsequently, a direct quantitative PCR (qPCR) for the detection of MAP in ovine feces was shown to have a sensitivity and specificity similar to BACTEC culture, although it was laborious and unsuited for commercial application.[32] This led to the development of a high-throughput direct fecal PCR, which is highly specific. This test, known as the high-throughput-Johne's (HT-J) test, has been validated in sheep and cattle and approved for use as a herd/flock test in Johne's disease control programs in Australia and New Zealand.[33] The HT-J test detected only MAP compared with 51 other mycobacterial isolates, including those with IS900 type sequences, and 99% of samples from unexposed cattle herds and sheep flocks were negative (458 of 460 samples from 8 unexposed cattle herds, 88 of 89 samples from 1 unexposed sheep flock). It was also reasonably sensitive compared with BACTEC culture at the recommended positive/negative cut points (0.001 pg MAP DNA), detecting 67 of 111 samples positive on culture in exposed cattle (60.4%) and 93 of 117 samples positive on culture in exposed sheep (83.8%). Almost all samples with a high level of MAP DNA were culture positive (97%), whereas only 25% of samples with a low level of DNA were culture positive. Thus scope exists to vary the cut points for the test, depending on the purpose of testing. However, the HT-J test detects a subset of infected animals that overlaps with, but is not identical to, those detected by fecal culture.[33]

Biopsy. Surgical biopsy of the terminal ileum and mesenteric lymph node of sheep for detection of MAP has been described, with histologic examination and bacteriologic culture being highly specific and sensitive.[8] Similarly, histopathology of liver biopsy samples had a sensitivity of 96% and 100% specificity for detection of types 3b and 3c ileal lesions in aged ewes.[34] Early detection of animals is one advantage with these techniques. However, the time taken and costs are major disadvantages, so use of biopsy will be restricted to special circumstances, such as valuable pedigreed animals.

Serologic Tests

Serologic tests are usually cheaper and more rapid than fecal culture. Those used in cattle are applicable to sheep and goats, but diagnosis, particularly in individual sheep, is more difficult. The commonly used serologic tests are the CF test, AGID test, and a number of commercial ELISAs. In cattle the CF test has published estimates for sensitivity as high as 90% for clinical cases, but much lower for subclinical infections, from 11% to 54%. This test is too unreliable for routine use in sheep, because of even poorer sensitivity and specificity, hence, an unacceptably high number of false-positive reactions. Despite this, some countries still require a Johne's CF test before the importation of sheep and cattle, often in combination with intradermal johnin testing or fecal culture.

The sensitivity and specificity of ELISA are similar to those in cattle, although cross-reactions to *C. pseudotuberculosis* occur, so absorbing sera with those heat-treated organisms does give improved results. In Australia, in a population of sheep with a high prevalence of subclinical infection, the sensitivity of an absorbed ELISA was 34% to 54% compared with 38% to 56% for the AGID test. The AGID was much better at detecting infected sheep in low body condition than the ELISA, but the latter was superior in detecting infected sheep with localized lesions or those with small numbers of MAP. These tests have also been evaluated and compared in adult sheep culled from severely affected flocks, with sensitivity and specificity evaluated using histopathologic findings as a reference. The sensitivity and specificity of the AGID was 37% and 100%, respectively, whereas the sensitivity of the ELISA was 48%, but its specificity was only 89%.

As discussed previously, in sheep a spectrum of infection is defined by two widely differing forms of the disease: a tuberculoid form, with strong cell-mediated immune response and lesions characterized by small granulomata composed of a few epithelioid cells surrounded by a large number of lymphocytes, and with no or few bacilli in the lesions; and a lepromatous form, with a strong humoral immune response accompanied by lesions with macrophages full of mycobacteria. The sensitivities of ELISA and the AGID test in sheep with lepromatous lesions were 86% and 100%, respectively, but only 10% to 50% and 30% in sheep with tuberculoid lesions. Thus there is a close correlation between serologic response to AGID and the presence of acid-fast bacilli in the intestinal tissues, and the diagnosis of tuberculoid cases remains difficult.

Nevertheless, the AGID is rapid, inexpensive, easily available, and technically easy to perform. Thus it is useful for

flock-screening programs to identify infected age groups of sheep, especially those with advanced OJD lesions and shedding the greatest number of organisms. In goats, the specificity of the AGID and absorbed ELISA tests in an Australian study was 100% and 99.8%, respectively, with the ELISA preferred because of its higher sensitivity.

Tests of Immunity

In vivo tests of cell-mediated immunity included the skin and intravenous johnin tests, but these are no longer used in control programs because of inadequate sensitivity and specificity. An indirect estimate of cell-mediated immunity is the assay of specific cytokines, but none are available for routine use in sheep or goats.

Serum Biochemistry

Sheep with clinical Johne's disease have decreased serum concentrations of calcium, total serum proteins, and serum albumin compared with controls. Serum protein concentrations range from 5 to 49 g/L, compared with controls at 68 g/L, whereas serum albumin concentrations range from 14 to 19 g/L with controls at 29 g/L. Sheep with lepromatous lesions have more severe depletion of calcium and protein than tuberculoid cases.

Deer

Fecal culture, qPCR, and serologic tests, including the CF test, AGID, and ELISA, have been used in deer. An IgG1 ELISA developed specifically for the serodiagnosis of Johne's disease in farmed deer had a specificity of 99.5% and a sensitivity of up to 91%.[35] Sensitivity was estimated using 102 infected animals from 10 deer herds, whereas specificity was determined using 508 uninfected animals from 5 herds without disease. Histologic lesions were detected in 80% of the seropositive deer. The test was less sensitive in animals that were culture positive for MAP but had no detectable pathology (75%) compared with those with JD lesions (>90%). The use of a deer-specific ELISA (Paralisa) in a deer herd, followed by fecal qPCR on positive samples, has been proposed as a cost-effective way of detecting and culling deer that are shedding MAP.[36]

NECROPSY FINDINGS

Sheep and Goats

On necropsy emaciation and subcutaneous edema are usually present, but gross necropsy lesions are often minimal despite severe clinical signs. In sheep there may be a deep yellow pigmentation of the intestinal wall and of the cortex of the draining lymph nodes. The intestinal wall may be thickened, although corrugation of the mucosa is not always obvious. Serosal lymphatics are often very prominent ("lymphatic cording"), and caseation and mineralization of the lymph nodes or enteric tubercles may occur. The pattern of lesions seen in cases of OJD may be classified into two major types, and detailed descriptions of these histopathologic changes are available.

Bacteremia occurs with MAP infection, so granulomatous lesions are sometimes identified in filtering organs such as the liver, lung, and spleen. No lesions occur in an infected fetus, but the organism can be isolated from its viscera and from the placenta and uterus. Traditionally, the most accurate postmortem tests for detecting MAP have been a combination of histopathologic examination and bacteriologic culture. PCR techniques may offer a higher level of sensitivity, but they do not discriminate between infection and the passive presence of MAP DNA. For most clinical cases of OJD, the demonstration of acid-fast bacilli within typical lesions is sufficient to confirm the diagnosis at necropsy. *M. paratuberculosis* can be detected in tissue sections from formalin-fixed, paraffin-embedded blocks with a PCR using IS900 sequence primers. This is more sensitive than acid-fast and immunohistochemical (IHC) staining.

In adult goats with clinical and subclinical paratuberculosis, the lesions have been divided into four categories: (1) focal lesions with small granulomata in the ileocecal Peyer's patches or related lamina propria; (2) diffuse multibacillary lesions with granulomatous enteritis at different intestinal sites (numerous macrophages containing many mycobacteria are usually present, resulting in macroscopic changes in the normal gut morphology); (3) diffuse lymphocytic lesions, in which the lymphocyte was the main inflammatory cell, with some macrophages; (4) diffuse mixed lesions, in which the infiltrate consisted of numerous lymphocytes and macrophages, with small numbers of mycobacteria. The three types of diffuse lesions are often associated with necrosis in the lymph vessels of the mucosa, mesentery, and lymph nodes, and with greater thickening of the jejunum than of the ileum.

Experimental subclinical infection of goat kids with MAP at several weeks of age and killed 2 years later results in lesions predominantly associated with intestinal segments containing persistent organized lymphoid tissue, with the distal jejunum, and proximal ileum being without lesions.

Samples for Confirmation of Diagnosis

- Bacteriology: distal ileum, colon, ileocecal lymph node for culture (with special growth requirements), direct smear using acid-fast stains, and PCR
- Histology: formalin-fixed samples of these tissues (histopathology and PCR)

Rabbits

In natural paratuberculosis in rabbits there are no gross lesions suggestive of Johne's disease, and the histologic lesions are either severe or mild. Severe lesions consist of extensive macrophage granulomata and numerous giant cells, with many intracellular acid-fast bacteria in the small intestine.

DIFFERENTIAL DIAGNOSIS

The characteristic features of clinical Johne's disease include progressive weight loss, and emaciation in a single animal or group of animals within a mob, and chronic diarrhea, which does not respond to therapy. A definitive diagnosis can be obtained by using a combination of serologic tests, fecal culture, and biopsy of intestine.

Sheep and goats

The characteristic features of clinical Johne's disease in sheep and goats are emaciation, weakness, and normal feces with intermittent bouts of mild diarrhea. The other causes of unexplained weight loss in sheep and goats include **caseous lymphadenitis, internal abscesses, gastrointestinal parasitism, caprine arthritis-encephalitis, ovine progressive pneumonia, dietary deficiencies, and dental disease.**

The major difficulty encountered in the diagnosis of Johne's disease is the accurate identification of subclinically infected animals. These are usually negative to the serologic tests but in the intermediate stage of the diseases and excreting the organism in their feces. Thus tests are usually flock-based or herd-based rather than useful for an individual animal. Pooled fecal culture or serologic tests of a cross section of the flock or herd will usually indicate if the infection is present or absent.

TREATMENT

M. paratuberculosis is more resistant to chemotherapeutic agents in vitro than *M. tuberculosis* so prospects for treatment are poor. Because of this lack of efficacy, and the failure of any of the antimicrobials to provide a bacteriologic cure, treatment is not recommended.

CONTROL

The control of Johne's disease is challenging because of the widespread nature of the organism, long incubation period, and the fact that most cases are subclinical. The available tests lack sufficient sensitivity to identify a large proportion of subclinically infected animals, which allows undetected infection to spread within and between flocks. Because of this low sensitivity, it is currently not possible to eradicate the disease other than by complete depopulation of the flock or herd and restocking with noninfected animals. Thus eradication is usually not practical, both for economic reasons and the difficulties in acquiring noninfected animals. Consequently, the preferred option is to limit economic loss by keeping the disease at a very low prevalence, such as by vaccination. However, a large proportion of flocks vaccinating for more than 5 years still had infected

sheep shedding MAP, so vaccination of young sheep or goats needs to be ongoing to reduce this risk.[37]

Successful control requires a long-term commitment by the flock or herd owner. In addition, because of its subclinical nature, producers often fail to practice adequate control measures because they do not recognize the importance of the disease.

The lack of integrated national control programs in countries in which the disease is endemic also allows the disease to spread continuously from herd to herd and region to region.

Control on a Flock Basis

The control of Johne's disease is based on two major principles:

- Identification and elimination of infected animals
- Prevention of new infections

For flocks known or thought to be free of Johne's disease, measures should taken to avoid the introduction of infected animals by maintaining a closed herd or by carefully screening purchased animals. The purchase of stock is the most common way MAP is introduced into a flock or herd. Thus purchasing only from flocks or herds documented to be of low risk of Johne's disease is preferable to testing specific animals before introduction because of the low sensitivity of available tests for individual animals. This is difficult because very few countries or jurisdictions have assurance programs, and few livestock producers participate in these.

The control of Johne's disease in sheep flocks has been widely implemented in Australia. This program is based on flock testing and management procedures, such as secure boundary fencing and introducing sheep only from flocks of similar MAP status. Testing is by pooled fecal culture of 350 animals (50 animals per pool), or negative serologic testing (ELISA or AGID) of a sample of 500 animals from the adult flock, defined as animals 2 years or older. This flock-sampling program has a 95% chance of detecting a 2% or greater prevalence of infection. Its purpose is to identify mobs or age groups of sheep that may have been more heavily exposed to infection. These become a priority for culling before they develop a large proportion of clinical cases, preventing further contamination and exposure of susceptible sheep to MAP.

A disadvantage of bacterial culture techniques is the relatively long incubation period needed to obtain results, typically from 2 to 3 months. Consequently, a high-throughput direct qPCR test (the HT-J test) has been developed and validated and was accepted in 2013 as a flock test suitable for use in Johne's disease control programs in Australia and New Zealand.[33]

Eradication by destocking for at least two summers was attempted in Australia, but the disease was often reintroduced in newly purchased stock. This arose due to a lack of sheep with a known reduced risk of infection and due to relatively low numbers of flocks participating in the Market Assurance Program. Persistence of the organism in the environment, up to 1 year or more, contributes to reinfection. Grazing management to reduce pasture contamination, such as selective grazing with more resistant hosts (e.g., adult cattle) or reducing shade (pasture length) are ways to more rapidly decontaminate paddocks known to be contaminated with MAP.[21]

Vaccination

Vaccination is now a common method of controlling OJD.[26,37] The most common strategy is to vaccinate the replacement ewes. However, in self-replacing Merino flocks with a high proportion of castrate males (wethers) infection can still be propagated from the unvaccinated portion of the flock, with the prevalence of shedding of MAP organisms from unvaccinated sheep six times that from vaccinates (1.27% versus 0.21%).[38] Mortalities and the proportion of sheep shedding MAP are reduced by up to 90% after the flock is vaccinated, although the response is variable.[26] In a longitudinal study involving 37 flocks, there was a decline in the prevalence of fecal shedding, from 2.72% before vaccination to 0.72% following 5 years of vaccination.[39] However, more than 80% of these flocks had detectable fecal shedding.[37,39] Thus it is advisable for them to continue vaccination, otherwise production losses and mortalities could rapidly increase. A higher initial prevalence of fecal shedding and less stringent biosecurity, such as straying or a greater number of introduced sheep, was associated with a higher prevalence of shedding.

The widespread use of a killed OJD vaccine in a mineral oil adjuvant does have the disadvantage of being a significant occupational health and safety problem, because producers have accidentally self-injected and suffered severe and debilitating injuries, often necessitating the amputation of affected digits or extensive debridement of necrotic tissue.[40] However, vaccination in sheep is not impeded by interference with tuberculin testing, although it will produce positive CF test titers that can interfere with serologic testing for export and diagnostic purposes. Newer vaccines may offer a more targeted cell-mediated immune response and less reactivity, but they are yet to be released commercially.[41]

Goats

A high prevalence of clinical Johne's disease in goats has been controlled by vaccination using a commercial inactivated vaccine. In small or intensively managed flocks, attempts to control and eradicate the disease have been made by undertaking pooled fecal culture and blood testing two to four times annually and weighing all goats monthly to detect any individuals losing weight. This is a relatively costly program and has a high risk of failure. Environmental and management changes were also undertaken, including altering trough design to minimize contamination of feed and water, restricting movement of goats between pens, cleaning pens three times weekly, eliminating grazing of pasture, spreading and ploughing manure into fields, isolating young and newly goats from the herd until their test status was determined, and strict attention to disinfection of footwear before entering or exiting barns and pens.

Control on a Countrywide Basis

There are wide variations in how Johne's disease is controlled by national, state, or provincial agencies. In some jurisdictions, the disease is reportable and in others it is not. In many areas, health certificates are required for interstate or intrastate movement of livestock, and most certificates require a statement that the animals are free of certain diseases. However, often the livestock owner or certifying officer has no knowledge of the presence of Johne's disease, or relatively insensitive serologic tests are used, so infected animals are still traded. Voluntary national and regional Johne's disease control programs for sheep and goat flocks have been introduced in Australia, and the disease is notifiable in many other countries including Greece, the Republic of Ireland, Luxembourg, Norway, Switzerland, Spain, and Sweden. Often the emphasis in the early stages of programs is to control clinical disease.

In Australia, an accreditation program for negative sheep and goat flocks, the Johne's Disease Market Assurance Program (SheepMAP, GoatMAP), was launched in 1999. It is a voluntary, audited, quality assurance program based on negative pooled fecal culture (50 sheep per pool) or serologic testing of a sample of the adult flock (animals 2 years and older). Testing is combined with prudent flock management, such as secure boundary fencing, restricting introduced sheep to flocks with a similar flock status, and abattoir monitoring, to assure owners and clients that participating flocks have a very low risk of being or becoming infected with OJD. SheepMAP is part of a national OJD control program, jointly funded by the sheep industries and Commonwealth and State Governments, and managed by Animal Health Australia. In 1999 a control and surveillance program was enacted for 1 year to limit further spread of OJD and to determine the distribution of this disease. Known infected and suspect flocks were subject to movement restrictions, and movements of sheep onto and off known infected farms were traced and investigated. Proposals for development of a market assurance program and zoning according to prevalence of OJD

within a state, as well as advisory and research programs, were developed as part of the National Ovine Johne's Disease Control and Evaluation Program. By 2000, OJD had been confirmed in every Australian sheep-producing state except Queensland. As part of the control program, some states, such as Victoria, provided an industry-funded subsidy to encourage the use of OJD vaccine in known infected flocks. Subsequently, the program was reviewed and modified, with 5-year programs enacted from 2007 to 2012 and 2013 to 2018.[10] At each time, modifications to zoning were made based on the estimated prevalence of the disease, with a reduction in prevalence areas from four in 2004 (high, medium, low, and very low) to three in 2008 (high, medium, and low), and to none in 2013. In addition, an assurance-based credits (ABC) scheme was introduced to facilitate sheep trading. At first, points were allocated based on the location of a flock (the prevalence area), the use of vaccination, and any whole-flock or part-flock testing undertaken, including monitoring at abattoirs. Subsequently, with the recognition that vaccinated animals can still transmit OJD, because vaccination reduces clinical disease but does not eliminate shedding of MAP, and the abolition of officially recognized prevalence areas in 2013, the ABC system is no longer used to support sheep trading.

Despite the efforts invested by the program, OJD has continued to spread in Australia. This was noted in the review of the 2007 to 2012 program and incorporated into the objectives of the national OJD Plan for 2013 to 2018, which were to (1) minimize the risk of MAP infection spreading to properties and regions that currently appear disease free, and (2) reduce the financial impact and adverse animal health and welfare effects of OJD, both for individual flocks and the sheep industry as a whole.

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Viral Diseases of the Ruminant Alimentary Tract

RINDERPEST (CATTLE PLAGUE)

Synopsis of the Disease

Rinderpest, or cattle plague, caused by the rinderpest virus (RPV), was declared globally eradicated in 2011. The disease often occurred as epizootics associated with a very high mortality rate, and its eradication is arguably the greatest veterinary achievement of our time.[1] Death usually resulted from severe diarrhea/dysentery and dehydration. A detailed account of the disease can be found in the 10th edition of this book. Because RPV is related to other members of the morbillivirus group causing disease in humans (measles), small ruminants (peste des petits ruminants [PPR]), dogs (canine distemper), and some marine mammals and wildlife, some lessons can be learned from a knowledge of the processes and historical background leading to rinderpest eradication.

Several authors have reviewed the history of rinderpest since its eradication.[1-5] Long before its etiology was known, cattle plague was recognized as a most devastating epizootic disease that spread from Asia to Europe, the Middle East, and eventually Africa, initially as a sequel to wars and later through trade-related livestock movements and seasonal migrations for water and pasture (nomadic pastoralists). The disease affected not only cattle but also over 40 other domestic and wildlife species. It is described in ancient Chinese writing, historical Asian drawings, and in documents from the Roman Empire.[4] It had been credited with decimation of native African wildlife and the decline of the European bison.[5] The virus does not cause human disease. Nevertheless, rinderpest was indirectly responsible for countless human deaths resulting from agricultural losses that led to famine, poverty, and disease for centuries.[5] In the nineteenth century, an epidemic in Ethiopia caused rapid loss of virtually all of the cattle, buffaloes, elands, and wild swine, as well as many sheep, goats, and wildlife species, such as antelopes, gazelles, giraffes, hartebeest, and wildebeest and resulted in the Great Ethiopian Famine of 1887 to 1892.[5]

The need to combat rinderpest outbreaks was instrumental in the establishment of the world's first veterinary school in 1762 in Lyon, France, and the Office International des Epizooties (OIE) in 1924, also in France. Furthermore, it led to the development of national veterinary institutions in many parts of the world. With a simple transmission chain and the environmental fragility of the virus, rinderpest was always open to control and even eradication within a zoosanitary approach.[2]

Steps Leading to Eradication

The geographic distribution of the disease had been shrinking steadily since the beginning of the twentieth century. Rinderpest never appeared in North America, and single outbreaks in Brazil and Australia were quickly eradicated. The disease was also eradicated from southern Africa, Europe, and China by the middle of the last century but was still endemic in other parts of Africa (as lineages 1 and 2) and in Asia (as lineage 3). African countries successfully initiated the Joint Project 15 from 1962 to 1976