procedures other than the Heineke-Mikulicz pyloroplasty would be superior, although a case has been made for the use of Y-U antral flap advancement pyloroplasty by Bright *et al* (1988).

In the one case in which gastric perforation and death occurred following pyloroplasty, the perforation occurred in the cardia and not in the pyloric region. The pyloroplasty site as observed at autopsy was healing without any complication. This is the first case of this condition, so far as we are aware, in which a perforated ulcer has been reported.

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Enteritis in sheep, goats and pigs due to *Yersinia pseudotuberculosis* infection

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SUMMARY: The features of naturally occurring *Yersinia pseudotuberculosis* serotype III infections in 16 sheep, one goat and 3 pigs, and *Y. pseudotuberculosis* serotype I infections in 3 goats, are described. Affected animals usually had diarrhoea and were in poor condition or emaciated. A number were moribund or dead when submitted for necropsy. Thickening of the caecal and colonic mucosa was the only gross lesion attributable to *Y. pseudotuberculosis* infection, with liver or other visceral abscesses not being seen. Characteristic microabscesses were demonstrated in the intestinal mucosa of 10 sheep, one goat and one pig infected with *Y. pseudotuberculosis* serotype III and one goat infected with *Y. pseudotuberculosis* serotype I.

Sheep, goats and pigs dosed orally with *Y. pseudotuberculosis* serotype III, the serotype isolated most commonly from these species, developed intestinal infection. In sheep and pigs, infection was accompanied by diarrhoea. Haematological changes and specific antibodies were elicited in all 3 species in response to infection. Microabscesses were seen in the intestinal mucosa of all experimentally exposed animals.

The occurrence of field cases and the results of experimental exposure confirm that *Y. pseudotuberculosis* serotype III is an enteropathogen of sheep, goats and pigs. The association of *Y. pseudotuberculosis* serotype I with lesions in a goat, indicates that this bacterium may also be a pathogen of this species.

It is concluded that *Y. pseudotuberculosis* serotype III is an enteric pathogen of a wide range of ungulate species including cattle, buffalo, deer, antelopes, sheep, goats and pigs. Serotypes I and II, while having a more restricted host range, are probably also pathogens of ungulates and, in particular, deer, antelopes and goats.

Introduction

Y. pseudotuberculosis has been recognised as a sporadic cause of outbreaks of a fatal disease in sheep in Australia for many years (Gilruth 1911; Pullar 1932). The disease that these authors described is similar to that recognised in rodents and birds (Bercovier and Mollaret 1984), which is characterised by extensive abscessation in visceral organs such as liver and spleen. Pullar (1932) did, however, note that diarrhoea and intestinal lesions occurred in a percentage of affected animals. Both Gilruth (1911) and Pullar (1932) exposed sheep experimentally to *Y. pseudotuberculosis* but failed to reproduce disease.

More recently, infection with *Y. pseudotuberculosis* has been shown to be associated with enteritis in a range of ungulate species including cattle (Slee *et al* 1988), deer (Henderson 1983; Mackintosh and Henderson 1984; Jerrett *et al* 1990), antelopes (Baskin *et al* 1977), buffalo (Behra *et al* 1984), a goat (Buddle *et al* 1988) and pigs (Morita *et al* 1968; de Barcellos and de Castro 1981). Most of these infections have been due to *Y. pseudotuberculosis* serotype III, although serotypes I and II are reported, particularly from deer (Hodges *et al* 1984; Jerrett *et al* 1990) and antelopes (Baskin *et al* 1977).

Although there is circumstantial evidence that *Y. pseudotuberculosis* is an enteropathogen of ungulates, only cattle have been exposed to experimental infection (Slee *et al* 1988). These authors failed to produce clinically apparent disease in calves exposed experimentally to *Y. pseudotuberculosis* serotype III, but did establish intestinal infection and demonstrated associated haematological changes, antibody production and the intestinal microabscesses characteristic of naturally occurring infection.

Other than the early infection experiments in sheep by Gilruth (1911) and Pullar (1932), there are no reports of the experimental exposure of sheep, goats or pigs to *Y. pseudotuberculosis*. Since this bacterium can be isolated from apparently normal sheep

(Bullians 1987) and pigs (Zen-Yoji *et al* 1974; Blackall 1977), it was considered that experimental exposure of sheep, goats and pigs was necessary to establish the pathogenicity of *Y. pseudotuberculosis* serotype III for these species.

We report a number of field cases of *Y. pseudotuberculosis* infections in sheep, goats and pigs and the results of experimental exposure of these species to *Y. pseudotuberculosis* serotype III.

Materials and Methods

Field Cases

Sick and dead sheep, goats and pigs, as well as formalin-fixed and fresh tissue samples, were submitted to the laboratory for diagnosis. Submissions that did not include intestinal tissues for histology and a clinical history were excluded from consideration.

Diagnostic Methods

Microbiology and parasitology – Intestinal samples and tissues were cultured for *Yersinia* sp on CIN selective agar* incubated for 40 h at 30 °C as previously described (Slee *et al* 1988). *Yersinia* sp isolates were identified by testing for fermentation of cellobiose, melibiose, rhamnose and sucrose and hydrolysis of urea (Slee *et al* 1988). *Y. pseudotuberculosis* isolates were serotyped using a slide agglutination technique (Hodges *et al* 1984).

Intestinal samples and faeces were examined for *Salmonella* sp, nematode and trematode ova and coccidial oocysts. Burdens of 10^4 or more worms of *Ostertagia* sp and *Trichostrongylus* sp were considered to be of pathological significance in sheep and goats.

Pathology – Tissues were fixed in 10% buffered formalin and embedded in paraffin. Sections were cut at 4 μ and stained with haematoxylin and eosin. Sections were examined for the lesions that are considered characteristic of yersiniosis: namely a central colony of ovoid bacteria surrounded by neutrophils and occurring in the intestinal lamina propria or in a visceral organ such as liver (Slee *et al* 1988).

Haematology and biochemistry – Haematological and biochemical investigations, including haemoglobin, total and differential leucocyte count, serum protein, albumin and urea estimations were done when blood was available.

Experimental Infections

Animals – Four 18-week-old Merino wethers, three 18-week-old Angora bucks and 2 male and 2 female 8-week-old Duroc/Large White crossbred pigs were used in challenge experiments.

Preliminary testing – Experimental animals were dosed by mouth with a broad spectrum anthelmintic and faeces were then tested to confirm freedom from enteric pathogens and parasites. Animals were bled to establish pre-treatment haematological and biochemical values and to confirm freedom from antibodies to *Y. pseudotuberculosis* serotype III.

Inoculum and experimental infection – Minimally subcultured isolates of *Y. pseudotuberculosis* serotype III of ovine (2 isolates), bovine (1 isolate) and porcine (1 isolate) origins were used in challenge experiments. These isolates were all from animals with enteritis.

The challenge bacteria were grown on sheep blood Columbia agar at room temperature for 24 h and suspended in sterile whole cows milk. Two sheep were each dosed orally with 3.6×10^{11} viable bacteria of ovine origin in 10 ml of milk. Two goats and 2 pigs were each dosed with 10 ml of milk containing 1.4×10^{11} viable bacteria composed of all of the above isolates. The remaining sheep, goat and pigs, were dosed orally with 10 ml of sterile cows milk and were maintained as unexposed controls.

Exposed and control animals were housed separately in concrete pens. At one to 2 d intervals, faecal consistency was recorded and faeces were examined for bacterial pathogens and parasites. Blood was also collected for haematological and biochemical testing and examination for antibodies to *Y. pseudotuberculosis* serotype III (Slee *et al* 1988).

Exposed and control animals were killed after the exposed animals began to show signs of infection such as diarrhoea or neutrophilia. A gross post-morten examination was done and tissues were collected for histological examination. Swabs were collected from the stomach or abomasum, duodenum, jejunum, ileum, caecum, colon, mesenteric lymph node and liver and were cultured for *Yersinia* sp and *Salmonella* sp. Faeces were also examined for virus particles by transmission electron microscopy.

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Results

Field Cases

Y. pseudotuberculosis was isolated from 16 sheep, 4 goats and 3 pigs. Eleven of the affected sheep were weaners and 5 were mature animals. Goats were from 3 to 24 months old and pigs were from 8 to 12 weeks old. Animals infected with *Y. pseudotuberculosis* serotype III usually had no contact with cattle. The majority of sheep, goats and pigs infected with *Y. pseudotuberculosis* had a watery, non-bloody diarrhoea. Sheep and goats were often in poor body condition and were moribund or dead when received. Many had been treated with anthelmintics and sulphonamides prior to submission to the laboratory. Usually a number of animals were affected in the flock and, in some instances, other deaths had already occurred.

Gross lesions were seen in some of the infected sheep, goats and pigs. Four sheep had thickening of the caecal and colonic mucosa, while sub-mandibular oedema was seen in a further animal. Intestinal intussusception was found in one pig, while a second had thickening of the mucosa of the spiral colon with associated fibrin tags. One goat had extensive thickening, ulceration and fibrin plaque formation in the caecum and proximal colon, but not in the mid or distal colon.

Pathologically significant parasite burdens were demonstrated in 3 of the 14 sheep. No other disease entities were diagnosed.

Lesions typical of yersiniosis were seen in the small and large intestine of 10 of 16 sheep. Two of 4 goats had lesions in the small and large intestine and one goat had a single microabscess in the liver. Microabscesses were present in the small intestine of one of 3 pigs. Autoylsis precluded histological assessment of intestinal mucosa in 2 sheep.

Sheep and pig isolates were all serotype III, while goat isolates belonged to serotypes I (3) and III (1). *Y. pseudotuberculosis* serotypes I and III were both associated with lesions in goats.

Neutrophilia was found in 11 of the 12 sheep tested and band-form neutrophils were present in 5 animals. Low serum albumin concentrations were measured in 9 of 10 sheep tested. Blood was not available from any of the goats. Blood was available from only one pig, one of those without lesions. This pig had neutrophilia and increased band-form neutrophils.

The seasonal occurrence of *Y. pseudotuberculosis* infection was the same for sheep, goats and pigs, being restricted to the winter and spring months. One infection occurred in May, one in June, 8 in July, 8 in August, 2 in September and 3 in October.

Experimental Infections

The 2 sheep experimentally exposed to *Y. pseudotuberculosis* began excreting the bacterium after 2 d. Both developed mild, non-bloody diarrhoea and a left-shift in blood neutrophils (0.3×10^9/l and 0.2×10^9/l) on days 4 and 6. One sheep was producing antibody to *Y. pseudotuberculosis* on day 7. Sheep were killed on days 6 and 7 and a post-mortem examination was performed. Other than slight thickening of the intestinal mucosa and fluid colonic contents in one challenged sheep, no gross abnormalities were seen.

Goats exposed to *Y. pseudotuberculosis* began excreting the organism after 5 d and both had developed a mild left-shift in blood neutrophils (0.8×10^9/l and 0.2×10^9/l) and antibodies to *Y. pseudotuberculosis* by day 9, when they were killed and a post-mortem examination was performed. Other than pneumonic consolidation of the right apical lung lobe of one goat, no gross lesions were seen.

The 2 pigs experimentally exposed to *Y. pseudotuberculosis* began excreting the bacterium on day 8 and had developed a mild, non-bloody diarrhoea, a left-shift in blood neutrophils (20.0×10^9/l and 1.0×10^9/l) and antibodies by day 10, when all 4 pigs were killed and a post-mortem examination was performed. Other than the presence of approximately 100 ml of clear fluid in the

TABLE 1

Isolation of *Yersinia pseudotuberculosis* and presence of lesions in experimentally infected sheep, goats and pigs

Host		*Yersinia* sp isolated / lesions present Abomasum / Stomach	Duodenum	Jejunum	Ileum	Caecum / Colon
Sheep	A	- / +	- / +	+ / +	+ / +	+ / +
	B	- / -	- / +	+ / +	+ / +	+ / +
Goat	A	- / -	+ / -	+ / +	+ / +	+ / -
	B	+ / -	+ / -	+ / -	+ / +	+ / -
Pig	A	- / -	- / +	+ / +	+ / +	+ / -
	B	- / -	- / -	+ / -	+ / +	+ / -

+ = *Y. pseudotuberculosis* isolated / microabscesses seen

peritoneal cavity and obvious vascular congestion in the distal jejunum and ileum of one challenged pig, no gross lesions were seen.

Y. pseudotuberculosis serotype III was isolated from a number of intestinal sites in all exposed animals but not from liver or mesenteric lymph node. Typical microabscesses accompanied by bacterial colonies were seen in the gastro-intestinal lamina propria of all exposed animals. Occasional foci of necrosis and neutrophil infiltration were present in the liver of one sheep. No microabscesses were seen in mesenteric lymph nodes. Sites from which *Y. pseudotuberculosis* was isolated and where lesions were seen are listed in Table 1.

No other bacterial pathogens or parasites were demonstrated in the intestines of sheep, goats or pigs, although viral particles resembling coronavirus were seen in the faeces of the control goat. The control sheep and pigs remained normal throughout the study. No biochemical changes were detected in exposed or control animals. All 3 goats, including the control animal, had intermittent diarrhoea during the trial.

Discussion

The isolation of *Y. pseudotuberculosis* from the intestine of 16 sheep, 4 goats and 3 pigs, the association with diarrhoea and the finding of gross and microscopic intestinal lesions is evidence that this bacterium causes enteritis in these animals. The aetiological role of *Y. pseudotuberculosis* serotype III in the disease in sheep, goats and pigs was confirmed by the development in artificially exposed animals of diarrhoea, haematological changes suggestive of infection, specific antibodies and typical intestinal lesions. Although *Y. pseudotuberculosis* serotype I was associated with lesions in a goat, experimental infection was not attempted with this serotype or with serotype II, serotypes which are implicated as enteropathogens of deer (Henderson 1983; Mackintosh and Henderson 1984; Jerrett *et al* 1990) and antelopes (Baskin *et al* 1977).

Clinical signs and gross pathology of *Y. pseudotuberculosis* infections were not sufficiently distinctive to allow a diagnosis of yersiniosis to be made, so that isolation of the bacterium or detection of the distinctive microabscesses is necessary to reach a diagnosis.

Infection was apparently restricted to the intestinal tract in most animals, although a single microabscess was demonstrated in the liver of one goat. That *Y. pseudotuberculosis* infection in sheep may, on rare occasions, produce abscesses in organs such as the liver has been known for many years (Gilruth 1911; Pullar 1932).

A number of animals infected with *Y. pseudotuberculosis* lacked intestinal lesions, possibly reflecting inadequate sampling for histopathology, or indicating that some other undiagnosed disease and not yersiniosis, was present.

There are apparently no previous reports of the association of *Y. pseudotuberculosis* infection with the characteristic intestinal lesions of yersiniosis in either sheep or goats. However, these lesions have been described previously in pigs (Morita *et al* 1968).

Field-acquired *Y. pseudotuberculosis* infection was restricted to the cooler months, May to October, and to younger animals as has been previously reported in cattle (Slee *et al* 1988) and sheep (Pullar 1932). Although *Y. pseudotuberculosis* serotype III is commonly isolated from cattle (Slee *et al* 1988), field infections in sheep, goats and pigs were not dependent on contact with cattle, suggesting that these species may all be capable of maintaining infection with this serotype.

Tetracyclines have been shown to be effective for treating cattle infected with *Y. pseudotuberculosis* (Slee *et al* 1988) and may be useful in treating yersiniosis in other species including sheep, goats and pigs.

Further investigation of possible production losses caused by yersiniosis in sheep, goats and pigs is warranted. Beside direct losses due to deaths, yersiniosis may also cause decreased production or result in the over-use or inappropriate use of anthelmintics and antibiotics. Diarrhoea also predisposes sheep to flystrike. Since *Y. pseudotuberculosis* is a pathogen of human beings (Bercovier and Mollaret 1984), the zoonotic potential of yersiniosis in livestock species also requires further investigation.

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