susceptibility of the host in cases of winter coccidiosis in Canada when low temperatures would inhibit sporulation and Fitzgerald (1959) found that disease sometimes followed environmental stresses such as may occur at weaning.

The circumstances at Swan’s Lagoon resembled those recorded by Fitzgerald (1962) who recovered sporulated oocysts from only 4 of 53 samples of soil and old faeces recorded by Fitzgerald (1962) who recovered sporulated environmental stresses such as may occur at weaning. He concluded that infective material may occur regularly. He concluded that infective material may other factors may precipitate disease.

We believe that changes associated with weaning precipitate coccidiosis in calves at Swan’s Lagoon. A combination of factors that can be broadly described as environmental including dietary change, weaning stress, and challenge with other infectious agents, possibly immunosuppressive, may operate. In respect of other infections, a concurrent investigation into the occurrence of bovine parvovirus at Swan’s Lagoon using the 40 untreated, sampled calves, indicated that, after weaning, antibody to bovine parvovirus increased 4-fold in more than 60% of the calves; moreover bovine parvovirus was isolated on 12 occasions (P. Durham personal communication).

This study has confirmed that E. zuernii coccidiosis occurs in the dry environment of Swan’s Lagoon, but the reason remains to be clarified. The results obtained during 1981 pose a number of questions and further work is planned.

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References

Anon (1977) — In Manual of Veterinary Parasitological Laboratory Techniques, Minist. Agric. Fish. Food Agric. Dev. adv. Serv., Tech. Bull. No. 18, 2nd edn, Her Majesty’s Stat. Off., London, pp 65-66.

Davis, S. F. M., Joyner, L. P. and Kendall, S. B. (1963) — In Coccidiosis, Oliver and Boyd, Edinburgh and London, pp 46-68.

Davis, L. R. and Bowman, G. W. (1957) — Am. J. Vet. Res. 18: 569.

Fitzgerald, P. R. (1959) — J. Protozool. W (Suppl.): 6.

Fitzgerald, P. R. (1962) — Hungerford, T. G. (1967) — In Diseases of Livestock, 6th edn, Angus and Robertson, Sydney, p 355.

Marquardt, W. C. (1962) — J. Parasit. 48: 270.

Marquardt, W. C. (1976) — J. Protozool. 23: 287.

Niiio, L. (1970a) — Can. J. comp. Med. 34: 20.

Niiio, L. (1970b) — Can. J. comp. Med. 34: 325.

Parkar, R. (1981) — J. Parasit. 67: 724.

Seddón, H. R. (1967) — In Diseases of Domestic Animals in Australia, Part 4, 2nd edn, P. H. E., Serv. Publ. Dept Hlth Aust. vet. Hyg., No. 7, pp 37-38.

Stockdale, P. H. G., Bainbridge, A. R., Bailey, C. B. and Niiio, L. (1981) — Can. J. comp. Med. 45: 34.

Stockdale, P. H. G. and Niiio, L. (1976) — Can. vet. J. 17: 35.

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Colitis in sheep due to a Campylobacter-like bacterium

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SUMMARY: Epidemic diarrhoea was observed in approximately 2,000 of 6,700 sheep on 9 farms. The disease, called weaner colitis, caused mortality of 1%, while morbidity varied from 20 to 75%. Colon contents from affected sheep were inoculated into 17 sheep, 13 of which developed diarrhoea 5 to 7 days after inoculation. Naturally and experimentally infected sheep had mild, erosive typhlitis and colitis. Microscopic examination showed similar bacteria adherent to colonic epithelium of an experimentally infected sheep. Curved, motile bacteria were isolated from 2 naturally occurring cases. One isolate was inoculated into 9 sheep, 2 of which developed diarrhoea. The other isolate was given to 4 sheep without observable effect. The curved bacteria grew only in 80% H2. They were Gram-negative, with a polar flagellum at one or both ends, they did not ferment glucose or give a positive catalase reaction. It is suggested that these bacteria are a new Campylobacter species and that they play a major role in the aetiology of weaner colitis.

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Introduction

In recent years, members of the genus Campylobacter have been recognised as major enteric pathogens. C. jejuni is a cause of diarrhoea in man, dogs, cats, cattle and birds (Russell and Munroe 1982). C. coli and C. fetus subsp. fetus cause diarrhoea in man (Smibert 1978), and the latter species may cause enteritis in cattle (Al-Mashat and Taylor 1983). Two proposed new species, C. sputorum subsp. mucosalis

(Thiaw 1982) and C. hyointestinalis (Gebhart et al 1983), have been described in association with porcine intestinal adenomatosis.

Campylobacter infection is not commonly recognised as a cause of diarrhoea in sheep (Reid 1976). Nevertheless, there have been occasional reports of the isolation of Campylobacter, or related bacteria, from sheep with enteric disease. C. sputorum-like bacteria may be the cause of proliferative regional ileitis of sheep (Hoorens et al 1977; Vandenberghe and Hoorens 1980). In New Zealand, Campylobacter of uncertain species have been isolated from sheep with enteritis (Russell 1955) and from sheep with a syndrome of combined colitis and nephrosis (Jopp and Orr 1980). In addition, several Campylobacter species are frequently isolated from the intestinal tract of normal sheep. They include C. facalis (Firehammer et al 1981), C. fetus subsp. fetus (Smibert 1965) and C. jejuni (Bryner and Johnson 1972; Pressey and Bruin-Mosch 1981).

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Although Campylobacter and related bacteria have been isolated from diseased and healthy sheep, proof of a primary pathogenic role of these bacteria is lacking.

The present report describes an epidemic of diarrhoea in sheep that occurred during the summer of 1981-82. Initial studies showed that colon contents from an affected sheep could be used to transmit the disease to normal sheep, but an aetiologic agent was not identified. Modification of microbiological techniques in the late stages of the epidemic enabled isolation of Campylobacter-like bacteria from naturally infected sheep.

Materials and Methods

Naturally-occurring Disease

The disease was studied on 8 farms where sheep were grazing semi-improved native pasture and on one farm with improved, leucaena, and lucerne pasture. Flock sizes varied from 10 to 2,000. In all cases, veterinary advice was sought because anthelmintic treatment had failed to arrest diarrhoea in the flock.

Pathology — Twenty-one sheep from the 9 farms were obtained alive and killed immediately prior to necropsy. Sections for histopathological examination were stained with haematoxylin and eosin or by the Levaditi method (Luna 1968). Total counts of gastrointestinal parasites were performed on each animal.

Bacteriology — Mesenteric lymph node samples and contents of colon and caecum were cultured on Columbia agar§ containing 6% sheep blood and on MacConkey agar§. Plates were incubated aerobically and anaerobically, using an Anaerobic System$, at 37°C. Colon contents were also incubated anaerobically in a suitable broth* before subculturing on brilliant green agar* for the detection of salmonellas.

Colonic and caecal mucus of all sheep was washed free of ingesta under running tap water, lightly scraped with a scalpel, smeared onto glass microscope slides and stained with 10% crystal violet in ethanol for 20 sec, or by the Gram method. Mucosal smears from the abomasum, duodenum, jejunum, ileum, and colon of each sheep were also examined. Wet preparations of mucosal scrapings were examined by dark field microscopy for motile bacteria.

Up to 5 blood agar plates per sample were lightly streaked with mucosal scrapings from the colon and caecum of all sheep. Mucosal scrapings were also inoculated onto one or more of the following selective media containing antibiotics: Skirrow, modified Butzler (Patton et al 1981), and Lawson and Rowland (1974). In addition, mucosal scrapings from 4 sheep were suspended in phosphate buffered saline, pH 7.2 (PBS), passed through a 0.45 μm filter † and cultured on blood agar or in semisolid, heated serum medium (HSM), prepared by heating 10 ml vials of sterile bovine serum at 80°C until the serum coagulated.

Cultures from the first 16 sheep were all incubated aerobically, and anaerobically, and in an atmosphere of approximately 85% N₂, 10% CO₂ and 5% O₂ (produced by a gas generating kit* for Campylobacter). Cultures from the last 5 sheep were also incubated in an atmosphere of approximately 80% H₂, 10% CO₂ and 10% air (= 2% O₂), without a catalyst, as described by Lawson and Rowland (1974).

Virolology — Colon contents from 16 of the 21 naturally affected sheep, plus the sheep used as a source of inoculum in Experiment 1 (see below), were examined for enteric viruses by electron microscopy as previously described (Tzipori et al 1978).

Experimental Infections

Three experiments were performed and are summarised in Table 1. Merino sheep 3 to 5 months of age, purchased from a local farm, were drenched with fenbendazol† and

| Experiment number | Inoculum | Developed | Necropsied | With curved bacteria | Number of Sheep |
|-------------------|----------|-----------|------------|---------------------|----------------|
| 1                 | Colon    | 17        | 13         | 17                  | 13             |
| 2                 | Supernatant fluid | 3 | 0 | 2 | 0 |
| 3                 | Campylobacter-like bacteria | 2 | 6 | 2 |
|                   | Isolate 1 | 9 | 2 | 6 | 2 |
|                   | Isolate 2 | 4 | 0 | 0 | 0 |
|                   | None      | 6 | 0 | 2 | 0 |

Levamisole**. Control and treated sheep in experiments 1 and 2 were kept in a paddock and allowed unlimited access to green grass and water. Sheep in experiment 3 were held in a cement-floored animal house, and fed lucerne chaff. Necropsies were performed on treated and control sheep at intervals of one to 42 days after diarrhoea began in the treated groups. Pathological and microbiological procedures were as described for naturally affected sheep, with the H₂-based atmosphere being used only in experiment 3.

Experiment 1 — Colon contents from sheep with diarrhoea were inoculated into 4 groups of sheep using serial passage. Inoculum for the first group was obtained from a naturally infected sheep, after which each successive group was challenged with colon contents from a sheep with diarrhoea in the preceding group. Colon contents were suspended in an equal volume of PBS and each sheep received 10 ml orally and 10 ml per rectum. Three controls were given a suspension of normal faeces obtained from cohort sheep, 3 were given saline, and 6 remained uninoculated.

Experiment 2 — Colon contents from a sheep with diarrhoea in Experiment 1 were centrifuged at 10,000 g for 20 min at 4°C. The deposit was reconstituted to the original volume in PBS. The supernate was recentrifuged, the resulting supernate carefully aspirated into a sterile container, and a sample cultured aerobically and anaerobically on blood agar. This procedure was designed to produce a bacteria-free supernate in which any pathogenic viruses would be retained in suspension. Sheep were dosed orally and rectally with 10 ml of the supernate or the resuspended deposit.

Experiment 3 — Two isolates (1 and 2) of Campylobacter-like bacteria from naturally affected sheep were subcultured 4 times on blood agar, then grown for 2 days in HSM. Viability was checked by observing motility. Ten ml of HSM, diluted with PBS, was inoculated orally and rectally into each sheep. Controls were dosed with PBS.

Electron Microscopy

An experimentally infected sheep with diarrhoea and a control sheep were anaesthetised with barbiturate. Segments of colon and caecum were removed at laparotomy and fixed in 4% glutaraldehyde in PBS at 4°C. Tissues were gently shaken to dislodge ingesta, then trimmed, post-fixed in 2% aqueous osmium tetroxide, dehydrated in acetone and embedded in araldite-epon. Sections approximately 0.5 μm thick were stained with methylene blue for light microscopy. Sections of silver interference colour were stained with uranyl

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nitrate and lead nitrate and examined by transmission electron microscopy.

Glutaraldehyde-fixed mucosal scrapings were negatively stained with 3% aqueous phosphotungstic acid, pH 7.1 on carbon-Formvar coated grids and examined by electron microscopy.

Characterisation of Campylobacter-like Bacteria

Isolates were preserved by freezing HSM cultures at -70°C. Cultures on CBA were tested for growth at 25, 37 and 42°C, in aerobic, anaerobic, N₂, and H₂-enriched atmospheres. Growth at 37°C in each atmosphere was tested also on blood agar containing 2.5 mg sodium formate per ml (Lawson et al 1981). All other tests were performed in the H₂-enriched atmosphere at 37°C. Growth on blood agar was compared to that on Columbia agar containing 5% serum. The following blood-free media were used; Columbia agar, brain-heart infusion agar, thioglycolate broth, and semi-defined agar (Mehlman and Romero 1981). Modified rumen fluid agar (Bryant et al 1959) was prepared by adding 20% filter-sterilised rumen fluid to CBA. Tolerance to 1% glycine and 3.5% NaCl were tested by adding each compound to CBA. Oxidase and catalase reactions were performed as described by Cowan and Steel (1965). Ability to ferment glucose and reduce nitrate or nitrite were tested in HSM to which 1% glucose and 0.005 g/l phenol red or 0.01% KNO₃, had been added. Hippurate hydrolysis was tested as described by Leuchtefeld and Wang (1982). Blood agar plates were inoculated to produce confluent growth and one of the following antibiotic containing discs* was placed in the centre of each plate: nalidixic acid (30 µg), cephalothin (30 µg), ampicillin (10 µg), neomycin (10 µg), penicillin (10 U), novobiocin (5 µg), nitrofurantoin (100 µg), tetracycline (10 µg), sulphonamide (100 µg), erythromycin (10 µg) and trimethoprim (1.25 µg).

Results

Naturally-occurring Disease

Of the 6,700 sheep in the flocks studied, approximately 2,000 (30%) had diarrhoea and 75 died. Morbidity rates varied from 20% to 75%. The disease began one to 2 months after weaning, when the sheep were approximately 6 months of age, and continued for 4 months during the summer. Adults were not observed to have the disease.

Physical examination revealed soft, fluid faeces instead of normal pellets. Body condition was generally poor although some sheep with diarrhoea were in good condition. Body temperature, heart rate and respiration were normal.

 Necropsy findings — The only macroscopic finding, in most cases, was increased fluidity of the colon contents. Some severely affected sheep had ascites, subcutaneous, dependent oedema, and loss of adipose tissue. Gross lesions in the intestinal mucosa were not observed. Microscopic lesions were confined to the large intestine and were usually mild. Cases judged to be of more than a week’s duration (by the degree of breech dagging) had focal erosion of the caecal and colonic superficial epithelium and infiltration of the lamina propria by neutrophils. Dilated glands contained necrotic debris and had flattened epithelium. Mucous cells were decreased in number. Milder cases had focal mucosal erosions only. Three sheep had ulcerations extending to half the depth of the mucosa with intense neutrophil and lymphocyte infiltration in the submucosa and lamina propria. The surface of the ulcers was heavily colonised with bacilli. The diagnostic feature common to all 21 sheep necropsied was the close association of faintly basophilic bacteria with the surface epithelium of the caecum and colon (Figure 1). In many sections these bacteria formed an uninterrupted layer along the surface, but they were not seen in the glands. They were best demonstrated with a Levaditi silver stain.

 Parasitology — Numbers of strongyle and coccidian parasites were uniformly low, although up to 200 Trichuris ovis were found in the caecum of each sheep.

Figure 1. Colon mucosa with bacteria visible as a pale-staining, tangle mass (arrow) covering the surface. There is minimal disruption of the epithelium and moderate leukocyte accumulation in the lamina propria. Naturally infected sheep. Haematoxylin and eosin x 400.

Figure 2. Smear prepared from washed colon mucosa, showing numerous curved bacteria. Crystal violet x 1000.

Virology — Coronavirus-like particles were seen in the colon contents of one naturally infected sheep. No viruses were seen in the remaining sheep.

Bacteriology — Stained smears of caecal and colonic mucosa contained many Campylobacter-like bacteria in all 21 sheep. The bacteria were Gram-negative, but were more clearly seen with crystal violet stain. Most were 1 to 2 µm long with a “comma” or “S” shape (Figure 2). The curved bacteria predominated over the other flora, which included only scant bacilli and large spirochaetes. Smears prepared from all levels of the intestinal tract of 5 sheep showed the curved bacteria were confined to the caecum and large colon. Motile bacteria were not seen in mucosal scrapings examined by dark field microscopy.

Campylobacter-like bacteria were isolated from 2 of the 21 sheep. These isolates grew only on blood agar incubated in the H₂-enriched atmosphere. Known enteropathogenic bacteria were not isolated from any of the sheep examined. Many plates inoculated with scrapings from washed mucosa were sterile or had only a light growth of mixed bacteria after 7 days incubation.

Experimental Disease

The results of all experimental inoculations are summarised in Table 1. Diarrhoea in treated sheep began 5 to 7 days after inoculation and persisted, in all cases, until necropsy. Controls were not observed to have diarrhoea at any time. All sheep with diarrhoea remained alert and continued to eat. Body temperature, heart rate, respiratory rate and hae-
are short and disorganised but epithelial cells are otherwise intact. No attachment organelles were seen in the 10 to 20 μm space between bacteria and cells, although a flagellum was often seen in cross section (Figure 4). No intracellular bacteria were seen. Negatively stained mucosal scraping showed numerous curved bacteria with a single flagellum at one or both ends (Figure 5). No pili were present.

**Electron Microscopy**

The colonic epithelium of the control sheep had a regular brush border, with microvilli of uniform length. In the infected sheep, many bacteria were apparently adherent to the apical surface of epithelial cells. The bacteria were aligned in parallel with only their ends in contact with the cell (Figure 3). The epithelial brush border was disrupted, having fewer microvilli, of variable length. However, this was the only sign of damage to the epithelial cells. Bacteria were 1 to 2 μm long x 0.3 μm wide. The structure of the cell wall was typical of Gram-negative bacteria, with an inner cytoplasmic membrane, an outer membrane, and middle periplasmic space. No attachment organelles were seen in any of the 13 control sheep, or 8 sheep unaffected by experimental inoculation, that were necropsied. Larger, spirilla-like bacteria were occasionally seen in mucosal smears of sheep with diarrhoea, but were also seen in controls. The H2-dependent Campylobacter-like bacteria used as inoculum in experiment 3 were not re-isolated from any sheep in that experiment.

**Characterisation of Campylobacter spp.**

Both isolates were identical in all characteristics examined. They were Gram-negative, comma or S shaped and 1 to 2 μm long. Dark field examination of cultures in HSM revealed rapid, darting motion. Initially, the isolates grew only on blood agar or in HSM at 37°C in the hydrogen-enriched atmosphere. Subcultures grew on Skirrow's agar, but no growth was observed at any other temperature or in any other atmosphere. Growth on serum agar was poorer than on blood agar. No growth was seen on media that did not contain blood. Addition of rumen fluid to blood agar resulted in slightly larger colonies after 5 days. Growth on blood agar was first visible after 3 days incubation, as clear, colourless, pinpoint colonies. After 7 days, colonies reached a maximum size of 0.5 mm. Bacterial growth scraped from blood agar was oxidase positive and catalase negative. When grown in HSM, glucose was not fermented and the medium turned alkaline after 48 h. Nitrate and nitrite were reduced. The isolates did not hydrolyse hippurate, and did not grow in the presence of 1% glycine or 3.5% NaCl. Addition of sodium formate suppressed growth in hydrogen and did not allow growth in the nitrogen atmosphere.

Because growth was slow, and testing was done on blood agar, the antibiotic sensitivity results did not strictly relate to the susceptible or resistant category provided for each antibiotic by the manufacturer of the discs. The following results are a guide only, with the diameter (in mm) of inhibition zones given in brackets after each antibiotic — susceptible: nitrofurantoin (70), Tetracycline (60), erythromycin (65), sulphonamide (60), nalidixic acid (26), ampicillin (21); intermediate: cephalothin (18), neomycin (16); resistant: novobiocin (13), penicillin (17), trimethoprim (0).

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Discussion

These clinical, microbiological and pathological features define a syndrome we refer to as weaner colitis. Its hallmark was diarrhea, of high morbidity, in weaned sheep. Mortality was low, but profits from affected flocks were decreased by poor growth rate, cost of treatment and increased need for fly strike prevention.

The major finding in naturally and experimentally infected sheep was the mass of bacteria adherent to the colonic epithelium, with little attendant necrosis. A similar lesion occurs in intestinal spirochaetosis of man (Hovind-Hougen et al., 1982), rodents (Lee and Phillips 1978) and monkeys (Zeller and Takeuchi 1982). However, the cultural characteristics of the spirochaetes isolated from those conditions are quite different from the bacteria isolated from the sheep with weaner colitis. The lesions in sheep also showed similarity to the mild, erosive colitis produced by C. jejuni in gnotobiotic dogs (Prescott et al. 1981). Mucosal ulceration, seen in some naturally infected sheep, may be a more severe, or chronic form of the disease. The pathogenesis of the diarrhea in sheep with weaner colitis is unclear. Bacteria adherent to colonic epithelium may secrete an enterotoxin causing fluid loss from the colon. Alternatively, the disruption of epithelial cell microvilli may physically decrease absorption by the colon.

Campylobacter-like bacteria isolated from naturally infected sheep may be the transmissible etiologic agent of weaner colitis. Evidence in support of this includes experimental reproduction of the disease with pure cultures of the bacteria, lack of reproduction of the disease with commensals of the ruminant gastrointestinal tract, for example, Vibrio, Butyribrio, Succinivibrio and Lachnoclostrida (Skerman 1974), their anaerobic growth and glucose fermentation would distinguish them from our isolates. Within the genus Campylobacter, the negative catalase reaction, H2 dependency, inability to grow in the presence of 1% glucose or 3.5% NaCl, and ability to reduce nitrate align our isolates with C. sputorum subsp. mucosalis (Lawson et al. 1975-1981). However, the small colonies are not typical of Campylobacter. Our isolates were similar to those of Vandenberghe and Hoorens (1980) as each was catalase negative, and did not grow in media containing 1% glucose or 3.5% NaCl. However, their isolates had larger colonies, grew at 43°C and did not reduce nitrite. The Vibrio isolated by Russell (1955) was insufficiently described to compare with our isolates, but the clinical syndromes were similar. The correct taxonomic position of the Campylobacter-like bacteria isolated from weaner colitis must await antigenic and DNA base ratio analysis.

References

Al-Mashar, R. R. and Taylor, D. J. (1983) — Vet. Rec. 112: 54.
Bryant, M. P., Robinson, I. M. and Chu, H. (1959) — J. Dairy Sci. 42: 1831.
Bryner, J. H., O'Berry, P. A., Estes, P. C. and Foley, J. W. (1972) — Am. J. Vet. Res. 33: 2575.
Cowen, S. T. and Steele, K. J. (1965) — Manual for the Identification of Medical Bacteria, University Press, Cambridge.
Dufty, J. H. (1967) — Aust. vet. J. 43: 4.
Firehammer, B. D. (1965) — Cornell Vet. 55: 482.
Firehammer, B. D. and Myers, L. L. (1981) — Am. J. vet. Res. 42: 918.
Gehri, C. J., Ward, G. E., Chang, K. and Kurzt, H. J. (1983) — Am. J. vet. Res. 44: 361.
Hoorens, J., Oyswrt, W., Meyvisch, C., Vandenberghe, J. and Derickx, J. (1977) — Vlaams digerend Tijdsch. 46: 10.
Hovind-Hougen, K., Birch-Andersen, A., Henrik-Nielsen, R., Orholm, M., Federsen, J. O., Teglbjaerg, P. S. and Thaysen, E. H. (1982) — J. clin. Microbiol. 17: 496.
Jopp, A. and Orr, M. B. (1980) — NZ vet. J. 28: 195.
Lawson, G. H. K. and Rowland, A. C. (1974) — Res. vet. Sci. 17: 331.
Lawson, G. H. K., Rowland, A. C. and Wooding, P. (1975) — Res. vet. Sci. 18: 121.
Lawson, G. H. K., Leaver, J. L., Pettigrew, G. W. and Roland, A. C. (1981) — Internat. J. Syst. Bact. 31: 385.
Lee, A. and Phillips, M. (1978) — Appl. Environ. Microbiol. 35: 610.
Leuchtefeld, N. W. and Wang, W. L. (1982) — J. clin. Microbiol. 15: 137.
Luna, L. G. (1968) — Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd edn, McGraw-Hill, New York.
Manninen, K. I., Prescott, J. F. and Dohoo, I. R. (1982) — Infection and Immunity. 33: 27.
Mehman, I. J. and Romero, A. (1982) — Appl. Environ. Microbiol. 43: 615.
Patton, C. M., Brennan, R. L., Potter, M. E. and Kauffman, A. F. (1981) — J. clin. Microbiol. 13: 326.
Prescott, J. F. and Karmali, M. A. (1978) — Can. med. Ass. J. 119: 1001.
Prescott, J. F. and Bruin-Mosch, C. W. (1981) — Am. J. vet. Res. 42: 164.
Prescott, J. F. and Manroee, D. L. (1982) — J. Am. vet. med. Ass. 181: 1524.
Prescott, J. F., Barker, I. K., Manninen, K. I. and Miniatis, O. P. (1981) — Can. J. comp. Med. 45: 377.
Reid, J. F. S. (1976) — Vet. Rec. 98: 496.
Russell, R. R. (1955) — NZ vet. J. 3: 60.
Skerman, V. B. D. (1974) — In Bergy’s Manual of Determinative Bacteriology, edited by Buchanan, R. E. and Gibbons, N. E., 8th edn, Williams and Wilkins, Baltimore.
Skerman, V. B. D., McGowan, V. and Sneath, R. H. A. (1980) — J. int. J. Syst. Bacteriol. 30: 225.
Smibert, R. M. (1965) — Am. J. vet. Res. 26: 315.
Smibert, R. M. (1974) — In Bergy’s Manual of Determinative Bacteriology, edited by Buchanan, R. E. and Gibbons, N. E., 8th edn, Williams and Wilkins, Baltimore.
Smibert, R. M. (1978) Ann. Rev. Microbiol. 32: 673.
Tzipori, S., Smith, M., Makin, T. and McLaughan, C. (1978) — Aust. vet. J. 54: 320.
Vandenberghe, J. and Hoorens, J. (1980) — Res. vet. Sci. 29: 390.
Zeller, J. and Takeuchi, A. (1982) — Vet. Path. 19 (Supp t. 7): 26.

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