Survey of Red Deer Stags for Yersiniosis at Slaughter

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Abstract

The results of a *Yersinia pseudotuberculosis (Y. pstb)* survey of 170 adult red deer stags (*Cervus elaphus*) from 21 commercial deer farms in the lower South Island of New Zealand are presented. After slaughter samples of liver, lung, spleen, small and large intestines, mesenteric and ileocaecal lymph nodes, and faeces were taken from each animal and cultured using *Yersinia*-enrichment systems. *Y. pstb* was isolated from the ileocaecal lymph node of 1 deer and the large-intestinal contents of another. Serum samples were tested for antibodies against 5 *Y. pstb* antigens from serogroups I, II, and III using the macroscopic agglutination test: 51% had titres >1:20 and 17% had titres >1:40.

Keywords: Cervus elaphus, Yersinia pseudotuberculosis, *survey*

Introduction

Until recently yersiniosis has not been recognised as an important pathogen of domestic animals in New Zealand, although it has occurred sporadically. With the advent of deer farming yersiniosis due to *Yersinia pseudotuberculosis (Y. pstb)* has emerged as a serious disease of farmed deer causing severe haemorrhagic enteritis and death (McAllum 1981). In the first national survey of deer farms (Gladden 1981) yersiniosis was the third most common condition diagnosed by veterinarians (only malignant catarrhal fever and lungworm (*Dictyocaulus viviparus*) were more common). Hodges *et al* (1980) reported that *Y. pstb* was isolated from 14% (37/259) of deer specimens submitted for bacteriological examination to the Ruakura Animal Health Laboratory during a 5-month period (June – October 1979). The peak of clinical yersiniosis cases in deer occurs in winter (McAllum 1981) which is typical of the seasonal pattern of occurrence seen in humans, birds, and animals in Europe (Mair 1968).

Clinical yersiniosis appears to be precipitated by stress factors including capture, transportation, handling, high stock densities, nutritional stress, and adverse climatic conditions (McAllum 1981; Beatson and Hutton 1981). However, it is not known whether stress precipitates a latent infection or allows the establishment of a new infection when the deer is exposed to *Yersinia* organisms in the environment. In Europe, rodents, lagomorphs, and birds are considered to be the main reservoirs of infection (Obwolo 1976). It is not known, however, if deer are significant carriers. A recent survey of 3810 deer faecal samples collected from the ground from throughout N.Z. between July and September 1981 yielded 5 isolates of *Y. pstb.* (Henderson and Hemmingson 1983). However, it is not known if the isolation of *Y. pstb* from faecal samples collected in this way is a true reflection of the prevalence of carriers in the deer population. It is possible that infected deer may shed *Yersinia* organisms in their faeces intermittently. It is also possible that deer with latent infections may have culturally negative faeces but carry *Y. pstb* in their spleen, liver, lymph nodes, or gastrointestinal tract as has been demonstrated with *Salmonella* infections in "normal" sheep and cattle surveyed at an abattoir (Nottingham and Urselmann 1961). The serological prevalence of titres of *Y. pstb* might also provide information on the epidemiology of yersiniosis in farmed deer. Therefore, a cultural and serological survey of deer for yersiniosis was carried out at a deer slaughter premises (DSP) where a range of tissues could be obtained from a large number of farmed deer.

Materials and Methods

The DSP at Mossburn commenced operations in autumn 1982 slaughtering deer predominantly from the lower half of the South Island. Almost all deer killed were stags. Normally 60 – 80 deer from 4 or 5 farms (range 1 – 8 farms) were killed per day, twice weekly. Tissue samples were collected at slaughter from as many stags as possible (up to 10) from each farm, during the autumn/winter killing season.

The following samples were taken from each animal: clotted blood, mesenteric lymph node, ileocaecal lymph node, liver, spleen, lung, small and large intestinal contents, and rectal faeces. Care was taken to minimise any cross-contamination between samples within and between animals. Instruments and gloves were rinsed in disinfecting solution (supplier, Mucocit) and water between samples, which were collected strictly in order, with the intestinal contents and rectal faeces sampled last. Samples were collected in winter at low ambient temperatures and were stored in a refrigerator within 12 hours of collection. Most samples were processed the day after collection.

Culture

Samples were pooled according to tissue type within each farm. The remainders of these samples were stored in the refrigerator for reculturing if necessary. The farm pools of liver, lung, spleen, and lymph nodes were macerated in a Colworth stomacher and 2 ml of the resulting suspension was inoculated into each of 20 ml cysteine selenite broth and 20 ml mannitol-bile salts broth. The faeces and small- and large-intestinal samples were not pooled, because it was considered that the number of contaminants present in pooled samples of those types could overgrow the small numbers of *Yersinia* likely to be present should only 1 animal be infected. Therefore, for these samples 2 ml aliquots were inoculated into 20 ml cysteine selenite broth and mannitol-bile salts broth. The cysteine selenite broths were incubated for 48 hours at 26°C and then subcultured onto McConkey agar, which were then incubated at 26°C and read at 48 hours. Mannitol-bile salts broths were incubated at 4°C for 3 weeks (cold enrichment) and then subcultured onto McConkey agar. If any *Yersinia* cultures were obtained, then all the individual tissue samples from that farm were recultured. It was hoped that the pooling of samples would eliminate unnecessary culturing if the prevalence was low.

All *Y. pstb* isolates were subcultured and serogrouped using rabbit anti-serium against type strains.

Serology

Serum was separated from the clotted blood samples and stored at −20°C until tested by the tube agglutination method (Rose and Friedman 1976). The following strains of *Y. pstb* were used:

Type IA strain No.1 Erlangen
Type *IB strain No.1105* Erlangen
Type IIA strain No.7 Erlangen
Type IIB strain No.1779 Erlangen
Type III NHI Acc. No.770 (NCTC 10278)

The minimum serum dilution was 1:20.

Results

Culture

A total of 170 stags from 21 farms from the southern half of the South Island were sampled; the stags were aged 7 months ($n=2$), 1.5 years (14), 2.5 years (16), 3.5 years (20), and unknown (128). Two stags each yielded a single isolate of *Y. pstb*. Each was obtained from a single tissue type, 1 from large-intestinal contents which were individually cultured and the other initially from an ileocaecal lymph node pool. All the individual samples from the animals from both farms, which had been stored at 4°C for 4 weeks, were then separately recultured with the result that again only 2 isolates were obtained: a serogroup I strain from the ileocaecal lymph node of a stag of unknown age from Southland and a serogroup III strain from the large-intestinal contents of a 1.5-year-old stag from the Queenstown area. No isolates were obtained from any other samples from these stags or from other stags from the same farms.

Table 1: The number of titres obtained from 170 stag sera tested against 5 *Yersinia pseudotuberculosis* serotype antigens

	Serotypes					
Titre	IA	IB	IIA	IIB	III	Total
1:20	14	28	16	44	9	111
1:40	12	6	1	12	3	34
1:80	3					3
⩾1:20	29	34	17	56	12	148
⩾1:40	15	6	1	12	3	37

Table 2: The number of stags with titres to the 5 *Yersinia pseudotuberculosis* antigen serotypes and prevalence

	Titre		
	>1:20	>1:40	>1:80
Serogroup I			
IA	15	13	3
IB	20	4	
IA and IB	14	2	
Total	49 (29%)	19 (11%)	3 (1.8%)
Serogroup II			
IIA	1	1	
IIB	40	12	
IIA and IIB	16	0	
Total	57 (34%)	13 (7.6%)	0
Serogroup III			
Total	12 (7%)	3 (1.8%)	0
Prevalence[1]	87 (51%)	29 (17%)	3 (1.8%)

[1] Total number of stags with ⩾1 *Y. pstb* titre

Serology

Serological data are summarised in Tables 1 and 2. Of the 170 stags, 87 (51%) had 1 or more *Y. pstb* titres $\geq$ 1:20 with a ratio of titres to serogroups I:II:III of 4.1:4.8:1 respectively, 29 (17.1%) had titres $\geq$ 1:40 with a ratio of 6.3:4.3:1 respectively, and 3 (1.8%) had titres of 1:80 which were all to serotype IA. The individual farm prevalence of seropositive animals with titres $\geq$ 1:20 ranged from 29 to 100% and with titres $\geq$ 1:40 ranged from 0 to 80%.

The stag from which a serogroup I isolate was obtained was seronegative, and of the 10 stags from this farm only 1 had a serotype IA titre (1:20). The stag which yielded a serogroup III isolate had a serogroup III titre of 1:20, and of the 8 stags from this farm 4 had serogroup III titres (2 of 1:20, 2 of 1:40).

Discussion

All the samples were collected in June and July (mid winter) when seasonal incidence of clinical yersiniosis in New Zealand is highest (McAllum 1981). In addition, although clinically healthy, the stags sent to slaughter were exposed to handling, social, nutritional, and transport stress for 24 – 96 hours prior to sampling. These factors might be expected to result in a high prevalence of infection with *Y. pstb* if deer were common reservoir hosts for this organism. However, only 2/170 stags were identified as carrying *Y. pstb*, and both these animals had culturally negative faeces. Therefore, at the time of slaughter none of the deer could be regarded as disseminators of *Y. pstb*. It is tempting to say that such a low percentage is insignificant epidemiologically. However, it is possible that if such animals were to suffer prolonged stress, the latent infection might be reactivated and they could start to shed large numbers of *Y. pstb* organisms which might in turn precipitate a propagating epidemic. If this is the case, then large herds are very likely to contain 1 or more such latent carriers which may be a potential hazard during periods of prolonged stress. Nevertheless, if the 1.2% prevalence of infection in these stags reflects the overall herd prevalence, then deer are probably far less important reservoirs than lagomorphs, rodents, cats, birds, and soil (Mair 1973; Barre *et al* 1979; Mackintosh and Henderson 1984).

The technique of pooling the samples which were not heavily contaminated saved considerable time and effort without apparently affecting the recovery rate. The failure to culture *Y. pstb* from faeces of the infected animals suggests that faecal culture is a much less sensitive method of detecting deer carrying *Y. pstb*. Although not directly comparable, it is of interest to note that the faecal survey of deer from throughout N.Z. conducted in late winter had an isolation rate of only 0.1% (Henderson and Hemmingson 1983).

The serological results should be interpreted with caution because of possible cross-reactions between other enterobacteria and *Y. pstb* serogroups I and II as reported by other workers and cited by Wetzler (1970). Wetzler also states that the agglutinins to *Y. pstb* are not known to persist for any length of time after infection, although he suggests that persistent titres may be due to latent carriers. Mair (1977) also contends that "agglutinins decline and, as a rule, disappear within 3 or 4 months". The high prevalence of low titres found in this survey may have a number of possible causes: a high rate of recent exposure at that time of the year; persistent infection in lymph nodes undetected by culture (not all abdominal lymph nodes were cultured); considerable cross-reaction with other enterobacteria. It is also possible that low titres persist in deer for a long time, although of the 2 animals from which isolates were obtained 1 was seronegative and the other had a homologous titre of 1:20.

The ratio of titres $\geq$ 1:20 for the 3 serogroups I:II:III was 4.1:4.8:1, serogroup II occurring most frequently. This is quite different from the serogroup ratio (4:1.9:1) of 61 *Y. pstb* strains isolated at the Invermay Animal Health Laboratory from clinical cases of yersiniosis in deer from the southern half of the South Island (Henderson, 1983). However, the ratio of titres $\geq$ 1:40 (6.3:4.3:1) is somewhat similar to this isolation rate, and the overall prevalence appears more moderate (17%). Raising the arbitrary cut-off level to $\geq$ 1:40 in this way eliminates almost all of the cross-reactivity between serotypes IA and IB and serotypes IIA and IIB, and may also eliminate much of the non-specific cross-reaction with other enterobacteria. However, it may also eliminate true residual titres. If all the titres truly reflect previous exposure to *Y. pstb*, then it suggests a very high level of environmental exposure. It is apparent that, for the purposes of interpreting titres, further work must be undertaken to investigate the serological response of deer to infection with *Y. pstb* with respect to the degree, quality, and longevity of the humoral response.

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