

The prevalence of *Fusobacterium necrophorum* biovar A in animal faeces

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(Accepted 8 October 1992)

SUMMARY

Only a small proportion of animals tested were found to be excreting *Fusobacterium necrophorum* biovar A, the causative organism of necrobacillosis, in the faeces (3 of 69 wallabies, 1 of 66 deer, 2 of 81 cattle). The two positive cattle belonged to a single group of calves on a farm with a history of necrobacillosis and the litter underfoot also readily yielded biovar A organisms. All attempts to demonstrate biovar A in litter on other farms and in soil from an area populated by wallabies and deer failed. Ruminal contents from young beef cattle proved a fertile source of *F. necrophorum* biovar A, 15 of 18 animals giving a positive result. It is suggested that disturbance of the gastrointestinal microflora leads to intestinal multiplication and faecal excretion of the organism, which may then give rise to necrobacillosis of the body surface.

INTRODUCTION

Necrobacillosis, caused by *Fusobacterium necrophorum*, is a common and often serious disease of farm and wild animals, especially cattle, deer, antelope and macropods [1]. The bacterium has long been considered a frequent inhabitant of the alimentary tract of normal herbivores [2].

There are two biovars (biotypes, phases), A and B, of *F. necrophorum* [3–6]. Biovar A strains are haemolytic, haemagglutinating, highly leucocidinogenic, and pathogenic for mice; biovar B strains produce less leucocidin, are haemolytic but not haemagglutinating, and have little if any pathogenicity for mice. Necrobacillosis of animals is caused by biovar A [7], which is however outnumbered in the bovine alimentary tract by biovar B. Thus by means of an *in vitro* technique Kanoe and co-workers [8] found biovar B in the ruminal contents of 40% of 126 cattle but biovar A in only 13%; the intestines yielded only five strains, all of biovar B. Other workers [9] isolated 17 biovar B but only 2 biovar A strains from 15 samples of ruminal fluid. However, by means of an *in vivo* technique in which gelatin capsules containing ruminal contents were surgically implanted in the peritoneal cavity of rats, biovar A was found in samples from 9 of 12 cattle [10].

Hepatic necrobacillosis of cattle and other ruminants is believed to arise from organisms in the contents of the rumen and reticulum which, after infecting lesions of the stomach wall, enter the portal circulation and hence the liver [11]. Bovine

foot rot and other necrobacillosis lesions of the external body surface are thought to arise from contamination of small wounds with infected faeces or litter [12, 13]. Presumably, therefore, faecal excretion of *F. necrophorum* biovar A plays an important epidemiological role. Despite this it has not been closely studied and the work described here was designed to make good this deficiency. Because of the lack of an efficient selective medium use was made of an *in vivo* method capable of demonstrating small numbers of *F. necrophorum* biovar A in grossly contaminated materials such as faeces, gut contents, litter and soil.

MATERIALS AND METHODS

Samples of faeces, gut contents, litter and soil

Faeces samples were obtained from wallabies at Whipnade and Regent's Park (Zoological Society of London), deer of several species in Thetford Forest and at Whipnade, and cattle on farms in Hertfordshire or at slaughter in an abattoir. These samples were either freshly passed or obtained from the rectum during life or *post mortem*. The wallabies were mainly adults of various ages, though a few 'joeys' were included, and the deer were mainly aged 1.5–2 years; the cattle ranged from calves aged < 2 months to adult dairy cows. Samples of bovine ruminal contents, deep or shallow litter from cattle pens, and soil were obtained from, respectively: an abattoir; farms in Hertfordshire; a field and woodland at Whipnade, populated by wallabies and deer. All samples were examined after either (a) a delay of no more than a few hours, or (b) refrigeration at 4 °C for up to 2 days, or (c) freezing at –20 °C for longer periods.

Detection of F. necrophorum biovar A in samples

The method, in an 'original' and 'improved' form (see below), consisted in the subcutaneous injection of a 5% suspension of each sample into mice pretreated subcutaneously with a mixture of clostridial antitoxins to assist in preventing non-specific deaths. In the period of c. 3 years during which the work was done, one major and several minor (probably insignificant) technical modifications were introduced. The major modification was the use of bacterial broth culture ('improved' method) instead of sterile diluent ('original' method) to prepare the 5% suspensions of samples. The bacterial broth culture, usually of *Staphylococcus aureus*, produced by itself only a mild and rapidly healing lesion but reduced the minimum infective dose of any *F. necrophorum* present by a factor of > 10⁶ [13, 14].

The 'improved' method, designed, like the 'original' method, to demonstrate biovar A but not biovar B, was that already fully described [13], with occasional slight modifications. Briefly, a 5% suspension of the sample was made in an undiluted overnight broth culture of *S. aureus*. This suspension was injected subcutaneously (dose 0.25 ml) into two mice pretreated with clostridial antitoxin (0.1 ml) consisting of 0.075 ml of lamb dysentery antiserum (Lambisan; Hoechst Animal Health) and 0.025 ml (12.5 units) of tetanus antitoxin (Wellcome Foundation Ltd). *F. necrophorum* was demonstrated in the advancing edge of characteristic necrobacillosis lesions by examining Giemsa-stained impression smears for the presence of beaded filaments and by culturing the strongly

Table 1. *Prevalence of F. necrophorum biovar A in animal faeces*

Animals	Individuals* in whose faeces <i>F. necrophorum</i> was detected by	
	'Original' method	'Improved' method
Wallabies	0/14	3/55
Deer	—	1†/66
Cattle	2‡/48	0/33

* Of the 216 results, 63 have been reported previously [12, 13].

† Slaughtered because of a digestive abnormality.

‡ Two of 15 calves sampled on a farm with a history of necrobacillosis; 4 of 15 samples of litter also contained *F. necrophorum biovar A*.

haemolytic, Gram-negative, filamentous organism on a semi-selective blood agar containing nalidixic acid and vancomycin. To confirm the identity of the *F. necrophorum* isolates, all those from faeces and litter and 33% of those from ruminal contents were injected subcutaneously (0.1 ml of a 24 h broth culture) into mice to produce typical fatal necrobacillosis.

RESULTS

Prevalence of F. necrophorum biovar A in animal faeces

The results are shown in Table 1. The overall prevalence of the organism in faeces was low. Thus even the 'improved' method identified only 3 (of 55) wallabies, 1 of which was a joey aged 7 months, as faecal excretors; and only 1 of 66 deer, this single excretor being an axis deer at Whippsnade, slaughtered because of a digestive abnormality.

Only 2 of 81 cattle were excretors. However, these 2 animals – identified by the less sensitive 'original' method – belonged to a group of 15 calves sampled on a farm with a history of necrobacillosis. These animals, aged *c.* 3 months, were yarded on deep litter, which was simultaneously shown to contain *F. necrophorum biovar A* (4 positive samples of 15 examined; see below).

Prevalence of F. necrophorum biovar A in bovine ruminal contents

The results obtained from samples collected during two visits to an abattoir are shown in Table 2. Unlike faeces, ruminal contents from young beef cattle proved a fertile source of the organism, 15 of 18 animals giving a positive result. Examination of rectal faeces showed that 6 steers were non-excretors of *F. necrophorum biovar A*, despite the presence of the organism in the rumen of 5 of them.

Prevalence of F. necrophorum biovar A in litter and soil

As stated above 4 of 15 deep litter samples from a yard containing calves (at least 2 of which were faecal excretors) yielded *F. necrophorum*; but 7 samples from a fenced-off section left unoccupied for 40 days were negative. Five of 16 samples collected from the same yard 3 weeks previously had also been shown by the 'original' method to contain the organism.

Table 2. *Prevalence of F. necrophorum biovar A in the alimentary tract of slaughtered beef cattle*

Animals	Age (years)	Individuals with <i>F. necrophorum</i> in contents of	
		Rumen	Rectum
Steers	2	5/6*	0/6*
Bulls	1.5	10/12	—

* The same six animals.

Samples were examined by the 'improved' method.

During the succeeding 2.5 years four further attempts were made to isolate *F. necrophorum* from the environment, all of them unsuccessful despite the use of the 'improved' method. Three of the attempts were made with litter samples, 8–10 being examined on each occasion; these samples came from pens containing calves aged 2–3 months (two attempts, both with shallow litter) and from a pen containing pregnant heifers, aged 22–25 months, on deep litter. The fourth attempt was made with 22 soil samples from woodland (at Whipsnade) heavily populated with wallabies and from an adjoining field containing both wallabies and deer; the majority of these samples were from areas in which the animals tended to congregate.

DISCUSSION

There is no evidence to suggest that *F. necrophorum* is a normal inhabitant of the soil. It is known, however, to occur in the alimentary tract of herbivores, pigs and dogs [2, 15], though in the bovine rumen organisms of the pathogenic biovar A are outnumbered by those of the much less pathogenic biovar B.

It would seem beyond doubt that in necrobacillosis of the body surface the primary source of the causative biovar A organism is faeces, but the present study showed that, in contrast to the high proportion of young beef cattle found to carry the organism in the rumen, a surprisingly small proportion of the animals tested (cattle, deer and wallabies) were faecal excretors. It should be emphasized, however, that although only 2 of 81 cattle tested had positive faeces, both animals belonged to the same group of calves on a farm with a history of necrobacillosis, and the litter underfoot was also readily shown to contain *F. necrophorum* biovar A. All attempts to demonstrate the organism in litter on other farms and in soil from an area populated by wallabies and deer were unsuccessful. It seems likely therefore that important predisposing factors influenced faecal excretion on the farm that yielded positive results. The nature of these predisposing factors is unknown, but it may be relevant that of 66 deer tested the only faecal excretor identified was an animal slaughtered because of a 'digestive abnormality'. The question arises as to whether biovar A organisms that are normally confined to the stomach of ruminant or ruminant-like animals can enter the intestine, multiply and be excreted in the faeces as a result of digestive disturbance. Some support for this hypothesis was recently obtained from mice whose faecal excretion of an orally administered biovar A strain was greatly enhanced by oral antibiotic pretreatment to disturb the gastrointestinal microflora [16].

Future investigations should include the following: (a) a search for biovar A strains in the rumen of animals other than cattle, and in the sacculated forestomach of wallabies, which corresponds to the reticulo-rumen of the Ruminantia [17], (b) an attempt to induce faecal excretion of biovar A organisms in cattle and wallabies by deliberately disturbing the gastrointestinal microflora, and (c) an examination of the possible multiplication of *F. necrophorum* biovar A in manure and litter.

ACKNOWLEDGEMENTS

Thanks are due to Miss L. M. Wallace and Miss S. A. Barton for technical assistance; and, for assistance in collecting material for examination, to Professor D. E. Noakes and Mr J. Thompson (farm samples), Mr R. Whitta (deer samples), and Mr R. H. Morton (abattoir samples).

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