Production of Transit Fever with Isolates of *Pasteurella Haemolytica* A1

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Introduction

In 1925 in Britain, *Bacillus bovisepticus* (sic) was isolated in large numbers from the lungs of fatal cases of "transit fever" (4). This was described as a sudden onset, severe and rapidly fatal respiratory disease of mature, store cattle which developed soon after they had arrived in north east Scotland. Since then, transit fever has been largely ignored in Britain although in North America, millions of dollars have been spent studying a similar condition, shipping fever. During recent investigations into infectious bovine rhinotracheitis, transit fever was found to be the most commonly diagnosed disease in weaned, suckled calves. Since 1982, 80 animals from 40 outbreaks, 30 of which were transit fever, have been examined at necropsy and almost 1,000 nasopharyngeal swabs from in-contact animals have been examined microbiologically. The only pulmonary pathogens isolated consistently have been *Pasteurellae* species and, overall 93 percent have been *P. haemolytica.* In an attempt to reproduce transit fever experimentally, conventional calves were infected with isolates of *P. haemolytica* biotype A serotype 1.

Materials and Methods

The experimental procedures are summarised, but the full details have been reported elsewhere (3).

Experimental animals

There were 20, healthy, 3 to 4 months old, male, Friesian cross calves considered to be free from *Pasteurellae* infection on the basis of a reciprocal serum antibody titre to *P. haemolytica* A1 (see later) of less than 1:4 on day -14 of the experiment and the absence of *Pasteurella* species from nasopharyngeal swabs taken on days -14 and -7. The calves were divided into groups of five—four infected animals and one control; the latter animal was housed separately.

Infection procedure

The infected calves were inoculated with one of four isolates of *P. haemolytica* A1, all of which had originally been recovered from the lungs of severely ill, untreated cases of transit fever. Infected calves were given 10ml intratracheally and 5 ml into each nostril of a first pass culture on five occasions—three doses of a 2 hour culture (10.00 hrs inocula) and two doses of an 8 hour culture (16.00 hrs inocula). The bacterial counts are given in Table 1. The control calves were given 20 ml of sterile broth in an identical manner.

Clinical examination

The calves were examined at least once daily (10.00 hrs). The food consumption was expressed as "kg eaten per 50 kg liveweight" thus taking into account variation in calf numbers.

Microbiological examination

Two nasopharyngeal swabs were collected from each calf on days -14, -7, pre-initial infection, days +3 and +6. One swab was stored in virus transport medium at -70° while the other was used immediately for the isolation of mycoplasmas and bacteria. At necropsy, the samples of tissue collected from the upper and lower respiratory tracts were examined for the presence of bacteria, mycoplasmas and viruses by methods previously described (2,5).

Serological examination

Blood samples were collected on days -14, -7, pre-initial infection, days +3 and +6. Antibodies to *P. haemolytica* A1, *P. haemolytica* A2 and *P. multocida* type A were detected by the indirect haemagglutination inhibition (IHA) test (6) and to *Mycoplasma bovis* by an indirect immunofluorescence test (1). Sera collected on day -14, pre-initial and on day +6 were examined for antibodies to parainfluenza type 3 virus (PI3), respiratory syncytial virus (RS) and bovine virus diarrhoea—mucosal disease virus at the Central Veterinary Laboratory, Weybridge, England.

Pathological examination

Two calves from each infected group were killed at 10.00 hours on day +3 and the other two at 10.00 hours on day +6 along with the control animals. The samples of tissue, which were taken from sites in the respiratory tract adjacent to

TABLE 1. The type and severity of the pathological lesions seen in calves infected with **Pasteurella haemolytica** Al, the change in antibody titre and the bacterial counts.

	Pathological Lesions							IHA Antibody Titre		Bacterial Counts/ml	
	Macroscopic			Microscopic							
	Consol. %	Pleurisy	Nodules	Fibrin. Pneum.	Septal Oedema	Bronch. Necrosis	Nodules			2 Hrs.	8 Hrs.
Isolate S/B											
Day +3	42	±	—	++	+++	+	—	2	4	$6.6x10^5$	$8.4x10^{10}$
Day +6	33	+	+++	+++	++	—	+++	>2048	256		
Isolate S/L											
Day +3	29	±	—	++	+++	+	—	4	n	$3.7x10^8$	$16.4x10^{14}$
Day +6	43	—	++	++	+	—	+++	512	>2048		
Isolate C/W											
Day +3	30	—	—	±	—	—	—	—	—	$2.8x10^7$	$5x10^9$
Day +6	18	—	±	±	—	—	±	8	64		
Isolate P/G											
Day +3	50	+	—	±	+	+	—	n	n	$2.7x10^8$	$9.2x10^{13}$
Day +6	35	±	++	++	+	±	++	32	128		

KEY:— — = not present; ±,+,++,+++ = severity of lesion; n = neat;
2 = reciprocal of antibody titre
Consol. = consolidation;
Fibrin. pneum. = fibrinous pneumonia;
Bronch. necrosis = bronchiolar necrosis.

those sampled for microbiology, were collected in 10 percent formol saline and processed by standard methods for histopathological examination.

Samples of tissue from the upper and lower respiratory tracts were also examined for the presence of PI3 and RS virus antigens and for *P. haemolytica* A1 by immunofluorescence (7).

Results

Clinical findings

Since each isolate induced a similar clinical response, the results have been combined (Figure 1) and marked differences highlighted. By six hours pii the mean temperature in the infected calves has increased significantly to 39.5°, the mean respiratory rate had increased from 40 to 51 per minute and each group was slightly dull.

The mean temperature of the infected calves rose progressively to reach a maximum of 40.3° at 54 hours post initial infection (pii), ie. six hours after the final inoculation. Thereafter, the mean temperature gradually decreased until, on day +6, it was 39.5°. All bar one of the calves was pyrexic (>39.4°) on at least one occasion and the highest temperature recorded was 41.5°. The mean temperature of the calves infected with Isolate C/W was highest while that of the animals infected with Isolate S/B was lowest.

The respiratory rates increased markedly six hours after each inoculation and then fell during the subsequent 18 hours. The maximum mean respiratory rate occurred at 54 hours pii (57/minute) after which it declined to 41 per minute at day +6. The highest mean respiratory rate was in the group infected with Isolate C/W and the lowest in the Isolate P/G inoculated animals.

By 24 hours pii, nine of the 16 infected calves were heard to cough and four did so frequently. Regular coughing was heard subsequently whenever the calves were disturbed. Twelve calves developed a persistent seromucoid nasal discharge from days +1 to +3 and in one this became purulent.

The infected calves were easily caught after only six hours and at 72 hours pii, all 12 calves in Groups S/B, C/W and S/L were dull but only one in Group P/G; those in Group C/W were very dull. From day +4 to +6, six of the eight remaining calves became more alert. The food consumption per 50 kg liveweight decreased dramatically from 1.54 kg on day -1 to 0.87 kg on day +2 (Figure 1). Thereafter, it gradually increased although on day +6 it was still only 1.44 kg.

The mean temperature of the control group never exceeded 39.4° although two individuals were pyrexic six hours (Isolate C/W) and 48 and 54 hours (Isolate P/G) pii respectively. The mean respiratory rate behaved in a similar manner to that of the infected calves although it was usually slightly lower. All 4 calves remained alert during the experiment and although the food intake was slightly depressed on day -1, it then increased slowly.

Pathological findings

The lesions produced by each isolate in day +3 and +6 were

similar although they differed in extent and severity (Table 1). At day +3, the main finding was anterior lobe consolidation due to an acute inflammatory reaction with areas of fibrinous pneumonia and mild bronchiolor epithelial necrosis; a mild degree of pleurisy was often present. By day +6, the pulmonary consolidation consisted of foci of an obvious fibrous capsule. At this time, *P. haemolytica* antigens were to be found only within these nodules. Similar lesions were not seen in the lungs of the control calves.

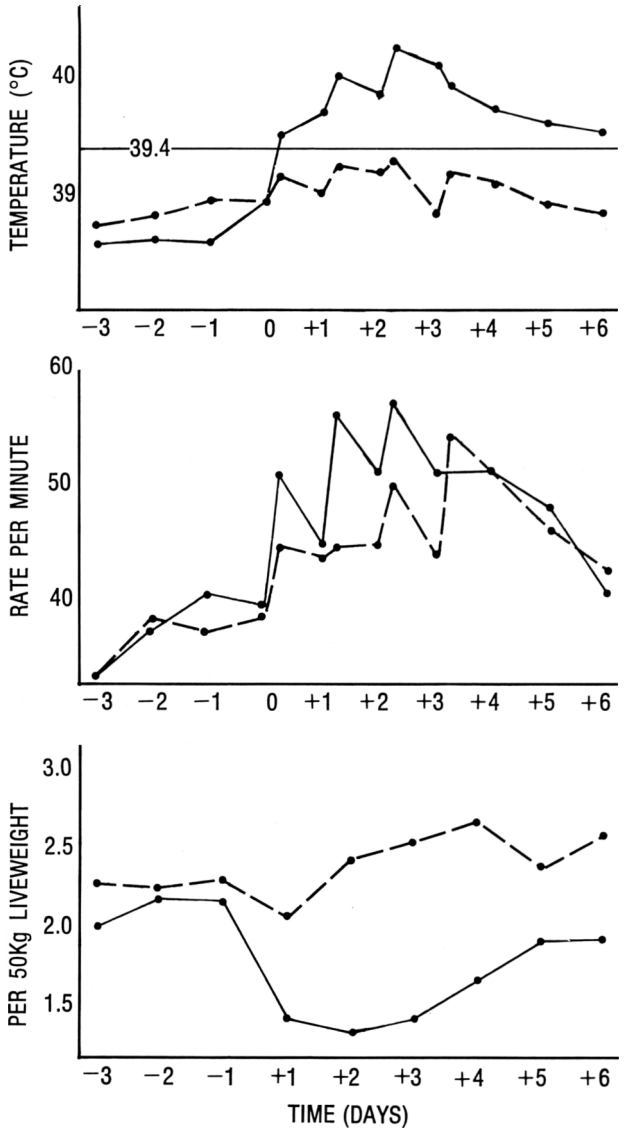

Figure 1. The mean temperature, mean respiratory rate and mean food intake of the control calves and those infected with **Pasteurella haemolytica** Al. (— infected, - - - controls).

Microbiological findings

Pasteurella haemolytica A1 was isolated in large numbers from the upper respiratory tract of all 16 calves on day +3 and from 7/8 on day +6. From the lower respiratory tract, *P. haemolytica* A1 was isolated from 7/8 calves on day +3 and from 5/8 on day +6. In the lower respiratory tract, Isolate S/B was recovered from the most sites in the greatest numbers while Isolate C/W was isolated from the fewest sites and in small numbers. Pasteurellae were not isolated from the lungs of the control calves. No viruses were isolated.

Serological findings

A four-fold or greater increase in antibody titre had developed in all infected calves by day +6 (Table 1). No significant increases in viral antibodies were detected.

Discussion

The clinical signs and pathological lesions in calves repeatedly challenged with *P. haemolytica* A1, were similar to those in field cases of transit fever. The reasons for the success of this work are almost certainly due to the experimental procedures. Firstly, the calves were free from *Pasteurella* species infection as judged by negative serology and Pasteurellae-free nasopharyngeal swabs. Secondly, the infecting strains were known to be pathogenic having been isolated from the lungs of severe, untreated cases of transit fever. Thirdly, relatively high doses of log phase cultures were given on several occasions via the respiratory route. A prior viral infection was not given because field evidence over 2 years had indicated that other infections are not essential for the development of pneumonic pasteurellosis in this country.

It is interesting that even carefully selected isolates differed in their pathogenicity; the isolate which induced the highest temperature response and the dullest calves produced the smallest serological response and the least severe pulmonary lesions. Clearly, the factors which affect the pathogenicity of *P. haemolytica* A1 should be investigated.

References

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