Influence of Immunization Procedures on Upper Respiratory Tract Immunity in Cattle

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SUMMARY

Aspects of respiratory tract immunity have been investigated in the bovine species. Using Past. hemolytica type I as the antigen for this model the relationship of nasal and serum antibody production to the route of vaccination and type of vaccine was investigated in a series of 15 dairy calves from two to four months of age. Experimental results indicated that an aerosol vaccination with live Past. hemolytica resulted in a significant nasal antibody response while parenterally vaccinated gave calves with equivalent serum titers had no significant nasal antibody response.

RÉSUMÉ

Les auteurs ont étudié plusieurs aspects de l'immunité respiratoire chez le boeuf. Utilisant Past. hemolytica type I comme antigène, et un groupe de 15 veaux de type laitier âgés de deux à quatre mois, ils ont étudié la relation entre la production d'anticorps nasaux et sériques, le mode de vaccination et le type de vaccin. Les résultats obtenus démontrent que la vaccination réalisée à l'aide de Past. hemolytica en aérosols détermine une formation importante d'anticorps nasaux, alors qu'une injection parentérale de vaccins qui donne des taux sériques équivalents, n'entraîne pas la formation d'anticorps nasaux en quantité valable.

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INTRODUCTION

A general review of literature on nasal antibody revealed that investigations were confined to laboratory animals and man with no information on specific local immunity of the respiratory tract in domestic animals (5). Since respiratory diseases are of great importance to cattle (9), this work was carried out to evaluate the presence of antibody in nasal secretions of cattle and the production of local respiratory immunity by aerosol vaccination.

MATERIAL AND METHODS

Calves were purchased from the local area at three to five days of age and confined in pairs in semi-isolation under routine husbandry conditions until two to four months of age. The pairs were allotted at random to experimental groups.

A lyophilized stock culture (#2636) of *Pasteurella hemolytica* type I and a strain of *Pasteurella multocida* type A[1] were used for the preparation of the vaccines. The strain of *Past. hemolytica* had been isolated from a fatal case of bovine fibrinous pneumonia. The culture was lyophilized on the third serial passage after initial isolation and was confirmed as being a type I strain of *Past. hemolytica*[2].

[1]Obtained from the Department of Veterinary Bacteriology, Ontario Veterinary College, Guelph, Ontario.
[2]E. L. Biberstein, Department of Microbiology, School of Veterinary Medicine, California.

TABLE I. Types of Vaccines Prepared

Vaccine	Route	Suspending Media	Experiment
1. Live **Past. hemolytica**	aerosol	1% casamino acid	A
2. Killed[a] **Past. hemolytica**	intravenous and subcutaneous	.15M saline	B
3. Killed[a] **Past. hemolytica**	aerosol	1% casamino acid	A
4. Live **Past. hemolytica**	intravenous and subcutaneous	1/2000 BSA[b] in .15M saline	B
5. Live **Past. multocida**	aerosol	1% casamino acid	A

[a]80°C for 10 minutes
[b]bovine serum albumin

In the preparation of the vaccines, one smooth colony of the organism was inoculated into 4.5 ml of tryptic soy broth, which was incubated for 18 hours at 37°C, and then used to inoculate one blood agar (5% bovine RBC) Roux flask. The growth was placed in the suspending media and diluted to give a suspension containing approximately 1.5 x 10^9 colony forming units per ml. Five different vaccines were prepared (Table I). Dosage of all parenteral vaccines was 5, 5, 10 and 10 ml respectively for each successive vaccination (Table I).

The aerosol generating unit consisted of a nose cone and an aerosol nebulizer[3] which was operated by a portable pressure vacuum pump[4]. The calf was restrained in a standing position with the head extended and the nose cone fitted tightly over the maxillae. Five ml of the bacterial suspension was used in the nebulizer to generate the aerosol into the nose cone from which the calf was forced to breathe for ten minutes.

For collection of nasal secretions, the calves were restrained in a chute with the head firmly secured and the nares in a dorso-ventral plane. The nares and muzzle were wiped with cotton; nasal secretions were collected by washing each nostril with 20 ml of 0.15M sterile saline introduced in successive 2 ml amounts with a plastic catheter connected to a 10 ml Cornwall syringe[5]. The washings from each nostril were collected in a container held against the ventral aspect of the nares. The collection from both nostrils was pooled. This procedure was repeated three times during one day at about two hour intervals, the total nasal washings from the three collections being pooled to constitute one sample. This material was cultured by dipping a sterile cotton tipped applicator stick into the nasal washings. Although this procedure was not considered representative of the nasal flora, it did provide some information about the flora at the time the nasal secretions were collected. The culturing and recording of isolates were carried out in accordance with the techniques developed by Magwood (10) for nasal swabs. The nasal washings were stored at -20°C.

The methods used for the purification and concentration of nasal secretions were a modification of that used by Artenstein *et al* (1). The nasal secretions were thawed, shaken vigorously with glass beads and then passed through a coarse gauze filter to remove any large foreign particles. The filtrate was then centrifuged at 450 x G for one-half hour. A benzidene test (7) was carried out on the supernatant of some samples for the presence of occult blood after centrifugation. The supernatant was decanted and dialized against distilled water for 24 hours at 4°C. The dialysate was then lyophilized and reconstituted to 4 ml with 0.15 sterile saline.

Total protein determinations were carried out by the biuret method (8) and, where necessary, samples adjusted to a final concentration of 250-500 mg per cent of protein by the addition of an appropriate amount of sterile 0.15 M saline. The nasal washings samples with total protein concentrations below 250 mg per cent were recorded. A few samples were unsuitable for protein determination because of hemolysis.

Following concentration the nasal secretions were stored at -20°C until such time as they were thawed for determination of indirect hemagglutinating antibody. Absorption of nasal secretion was carried out with human type O erythrocytes prior to

[3]#40 Nebulizer De Vilbiss (Canada) Ltd., Barrie, Ontario.
[4]ACD-501 Compressor Vacuum Pump, De Vilbiss (Canada) Ltd., Barrie, Ontario.
[5]Peder Pedersen, Guelph, Ontario.

TABLE II. Grouping of Calves for Aerosol Vaccination

Group	Calf	Treatment	Day of Exposure
1	1, 2	Aerosol suspension of live **Past. hemolytica**	1, 4, 8, 11
2	3, 4	Aerosol suspension of heat killed **Past. hemolytica**	1, 4, 8, 11
3	5, 6	Aerosol suspension of live **Past. multocida**	1, 4, 8, 11

testing for indirect hemagglutinating antibody activity by adding 2 ml of washed, packed erythrocytes to 4 ml of reconstituted nasal secretions. This mixture was allowed to stand at room temperature for 20 minutes at which time the erythrocytes were sedimented by centrifugation at 700 x G for 15 minutes and the supernatant recovered.

Serum was collected from each calf on days 10 and 3 pre-inoculation and days 5, 12, 19, 26, 33 and 40 post-inoculation. The serum was heat inactivated at 56°C for 30 minutes and absorption with human 0 type erythrocytes was carried out in the same manner as outlined above for nasal secretions and stored at -20°C.

The method of Biberstein, Gills and Knight (4) for serological typing of *Past. hemolytica* using the indirect hemagglutination (IHA) test was used except human type 0 erythrocytes instead of bovine were used. The same strain of *Past. hemolytica* was used for vaccine production and for serological tests.

Sensitized erythrocytes (0.2 ml of a 0.5% suspension) were added to doubling serial dilutions (1:2 to 1:1024) of serum or nasal secretions in veronal buffer. The tubes, including standard controls, were mixed by shaking and allowed to stand for two hours at room temperature. Agglutination was graded from — to ++++ upon agitation of the settled cells with a reading of ++ or greater being considered positive.

EXPERIMENT A

The first experiment was designed to compare the immunogenicity of an aerosol of live *Past. hemolytica* in contrast to killed *Past. hemolytica* and to evaluate the specificity of the IHA test by determining if another organism could induce a local and humerol response to *Past. hemolytica* (Table II).

EXPERIMENT B

The second experiment was designed to determine the effect of parenteral administration of vaccines of *Past. hemolytica* on the development and distribution of IHA antibody to *Past. hemolytica* in nasal secretions and serum (Table III).

When signs of respiratory distress were evident shortly after the intravenous inoculation of live or killed bacterial suspension, affected calves were treated with antibodies and antihistamines.

RESULTS

EXPERIMENT A

The effect of live aerosol vaccination was a rise in both serum and nasal antibody, whereas the killed aerosol vaccine

TABLE III. Grouping of Calves for Parenteral Vaccination

Group	Calf	Treatment	Day of Vaccination
1	11, 12, 13	Four subcutaneous vaccinations with heat killed **Past. hemolytica**	1, 4, 8, 11
2	14, 15, 16	Four intravenous vaccinations with heat killed **Past. hemolytica**	1. 4. 8, 11
3	17, 18, 19	Four intravenous vaccinations with live **Past. hemolytica**	1, 4, 8, 11

caused a rise predominantly in the serum antibody (Fig. 1). Isolations of *Past hemolytica* from nasal washings were made only from calves 1 and 2 after vaccination.

The results of an analysis of variance conducted on IHA antibody levels in serum and nasal washings for Groups 1, 2 and 3 indicated that aerosol vaccination significantly increased the nasal titer with no significant effect on the serum titer. A rank correlation indicated that the nasal titer from live aerosol vaccination was significantly greater than the controls while the killed aerosol group was not.

Experiment B

The response to parenteral vaccination was a rise in the level of serum antibody (Fig. 2 and 3). However, an analysis of variance indicated that the rise in serum titer was not significant.

Calf 12 died on post-inoculation day 4 of polioencephalomalacia and terminal intestinal intussusception. Calf 15 died on post-inoculation day 9 with bacterial meningitis and *Past. hemolytica* was isolated from the meninges in pure culture. Calf 13 developed clinical signs of pneumonia on post-inoculation day 2 and meningitis on day 4. It was maintained by supportive therapy and all samples were collected at the designated intervals.

Past. hemolytica was isolated from nasal washings of calf 10 on day 12 and calf 13 on day 33 while all others were negative. In addition calf 10 was the only one to have a rise in both serum and nasal antibody prior to vaccination.

DISCUSSION

Experiments A and B were conducted to investigate the effect of route and type of vaccine upon the responses of nasal and serum antibody. The administration by aerosol of the vaccine used in Experiment A was effective in inducing a significant nasal antibody response with no significant effect on the serum antibody response.

Fluctuating levels of serum antibody occurred at a low level in the control group receiving the *Past. multocida* aerosol vaccination. Explanations for this response are not clear. A low level of natural infection may have been present and not capable of inducing a detectable antibody response but

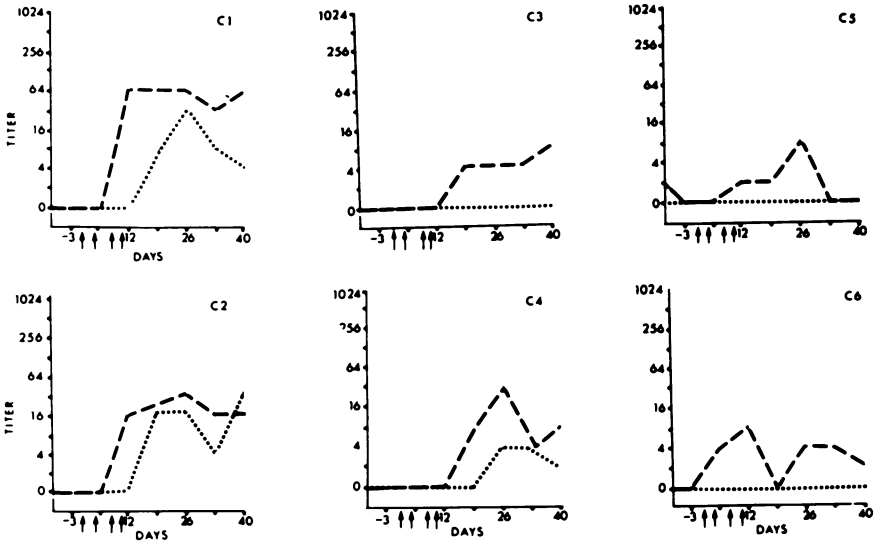

Fig. 1. Serum and nasal washing IHA antibody titers to Past. hemolytica from calves vaccinated with a live (C1 and C2), and heat killed (C3 and C4) aerosol of Past. hemolytica and a live aerosol of Past. multocida (C5 and C6).

dash line — serum antibody titer
dotted line — nasal washing antibody titer
arrow — aerosol vaccination

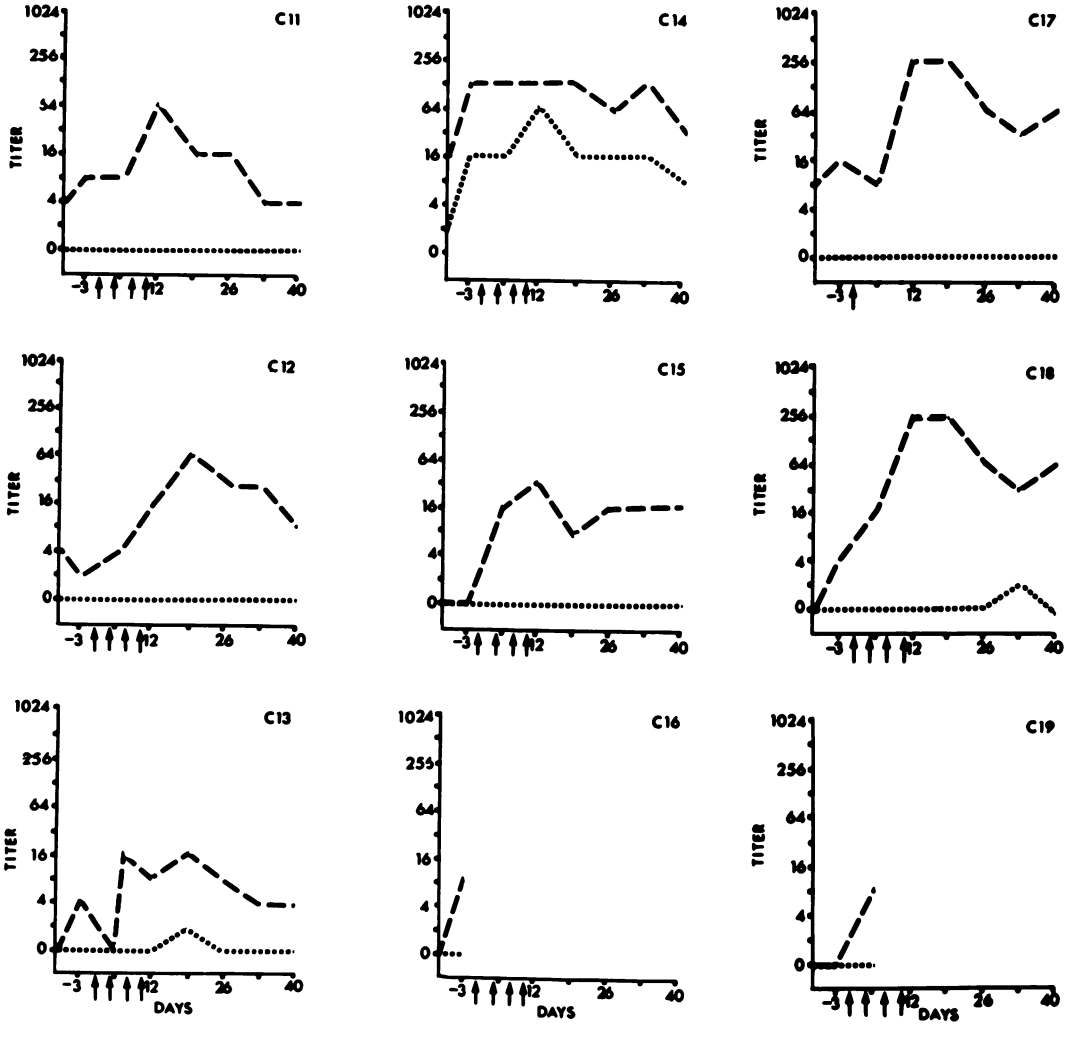

Fig. 2. Serum and nasal washing IHA antibody titers to Past. hemolytica from calves vaccinated subcutaneously (C11 to C13) and intravenously with killed Past. hemolytica (C14 to C16) and live Past. hemolytica (C17 to C20).

dash line — serum antibody titer
dotted line — nasal antibody titer
arrow — parenteral vaccination

since *Past. hemolytica* was not isolated from weekly nasal washings, this was unlikely. Probably these calves had a previous exposure to *Past. hemolytica* type I as Biberstein *et al* (4) found a high degree of specificity in typing individual strains of *Past. hemolytica* and Marshall and Hansen (11) found that antisera prepared against *Past. hemolytica* and *Past. multocida* (polyvalent) were specific for the species against which they were produced.

By contrast the parenteral administration of *Past. hemolytica* (Experiment B) by the subcutaneous or intravenous route caused a non-significant increase of serum antibody and had little or no effect on the nasal antibody response.

Two factors require explanation. First, the rising pre-inoculation serum titers of all groups (Fig. 3) would suggest that the levels of serum antibody could be attributed to natural infection rather than to vaccination. However, the lack of nasal antibody and rising levels of serum antibody (with the exception of calf 10) would indicate that the serum antibody response

was due to vaccination. Secondly, the presence of pre-inoculation serum antibody in the majority of calves would indicate previous natural infection but organisms were either present in very low numbers during the experiment or were completely rejected prior to the experiment. The rising levels of pre-inoculation IHA antibody in the nasal secretions of calf 10 and the isolation of *Past. hemolytica* from its nasal secretions would suggest that the nasal antibody present was due to antigenic stimulation from natural infection via the respiratory tract prior to inoculation and was not attributable to vaccination procedures. The lack of production of antibody in the nasal secretion of other calves following parenteral vaccination and the development of high levels of serum antibody would suggest that the parenteral vaccination procedure had little influence on the production or secretion of local antibody.

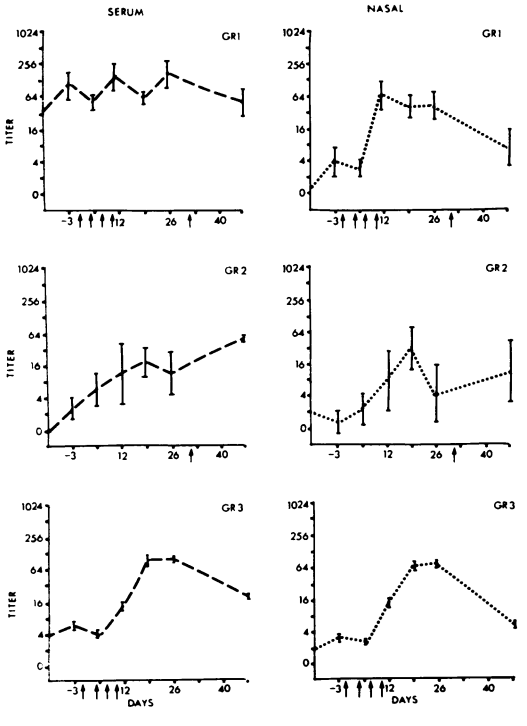

Fig. 3. Mean serum and nasal washing IHA antibody titers to Past. hemolytica from calves receiving live and heat killed Past. hemolytica vaccine by different parenteral routes.

GR 1 — killed Past. hemolytica subcutaneously
GR 2 — killed Past. hemolytica intravenously
GR 3 — live Past. hemolytica intravenously
arrow — parenteral vaccination

The use of intravenous vaccines in this experiment had certain undesirable features. The death of calf 19 can be attributed to the vaccine alone as can the clinical disease induced in calf 17. The death of calf 16 can only be considered coincidental to treatment but is possibly related to stress induced at the time.

The possibility of contamination of nasal washings by components of serum was considered. However, Rossen *et al* (13) found nasal secretion protein to be independent of the presence of occult blood. The absence of detectable nasal antibody in calves with high levels of serum antibody in Experiment B would also confirm the contention (13) that trace amounts of hemoglobulin did not indicate a significant contamination of nasal washing with serum antibody.

A comparison of the results of Experiment A and B would suggest that only the respiratory route of vaccination did increase the levels of antibody formation in the respiratory tract. Eigelsbach *et al* (6) concluded that aerosol administration of a live tularemia vaccine to monkeys afforded greater immunity to aerosol challenge with living virulent organisms than vaccination by the dermal route. In addition, available evidence suggests that significant protective immunity to *F. tularensis* occurs only after vaccination with attenuated strains or following natural infection (12). Bellanti *et al* (3) suggested that innate or induced cellular resistance may be more important than humoral antibody in modifying the pathogenesis of tularemia. Alternatively, antibody present in respiratory secretions may be more important than serum antibody in the prevention of aerosol acquired disease.

Baldwin *et al* (2) stated that their experimental investigations supported the suggestion that respiratory illness can result from intratrachael inoculation of *Past. hemolytica* alone in calves with levels of serum IHA antibody less than 1:10. The absence of a serum or nasal IHA antibody in calves 1 and 2 in Experiment A and the development of clinical signs of respiratory disease would suggest that *Past. hemolytica* alone can cause respiratory illness.

The application of these findings to the control of bovine respiratory diseases would suggest that future investigations should give consideration to local antibody production since it appears to be independent of serum antibody titer if the route of

exposure is other than the natural route. In addition, specific immunity to *Past. hemolytica* may be of importance and should therefore receive the same consideration as that given to the various viral agents implicated in shipping fever.

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