Preliminary Observations on the Effect of Specific Immunity on Nasal Bacterial Flora

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SUMMARY

This investigation has shown that Pasteurella hemolytica could actively colonize the nasal cavity of calves in the presence of indirect hemagglutinating IHA antibody against that particular strain. It was not determined if the level of nasal IHA antibody to Past. hemolytica had an effect on colonization of the nasal passage by Past. hemolytica. Specific immunity to Past. hemolytica would appear to be important in respiratory disease induced by this organism.

RÉSUMÉ

On a établi que Pasteurella hemolytica peut envahir la cavité nasale de veaux, malgré la présence d'anticorps spécifiques provoquant une hémagglutination indirecte. On n'a pu déterminer si le taux des anticorps nasaux avait un effet sur l'envahissement des voies nasales. Une immunité spécifique contre P. hemolytica semblerait jouer un rôle important dans les affections respiratoires causées par ce micro-organisme.

INTRODUCTION

The relationship of local antibody to nasal colonization by bacteria and, conversely, the role of bacteria as normal nasal inhabitants in naturally acquired immunity have not been investigated in cattle. *Pasteurella hemolytica* is considered to be a respiratory tract pathogen of cattle (2) and also part of the normal nasal flora of cattle (6). Previous work has demonstrated that antibody to *Past. hemolytica* may be present and can be measured in bovine nasal secretion (4). Whether or not such antibody influences the colonization of the nasal passage in cattle is the basis of the report.

MATERIALS AND METHODS

Twelve male dairy calves two to three months of age were maintained paired in semi-isolation and rectal temperatures were recorded daily. The calves were allotted into three groups on the basis of the indirect hemagglutinating (IHA) antibody in serum against *Past. hemolytica*. Group 1 was composed of calves with naturally-occurring serum IHA antibody and was inoculated with living *Past. hemolytica* organisms via aerosol to induce an increase in the level of the nasal antibody. Group 2 was composed of calves which did not have serum antibody to *Past. hemolytica* prior to the experiment. Animals in Group 3 were controls to demonstrate the efficacy of antibiotic treatment for clearing the artificially-administered *Past. hemolytica* from the nasal passages of calves in Group 1. Aerosol vaccination, antibiotic treatment to remove *Past. hemolytica* from the flora and subsequent challenge of *Past. hemolytica aerosol* were administered as outlined in Table I.

Blood samples and nasal secretions were collected on days 3 and 10 prior to the aerosol vaccination (Day 1) and on days 5, 12, 19, 26, and 45. Nasal swabs were collected prior to and after aerosol exposure as recorded in Table II. The procedures for aerosol exposure, calculation of the bacterial colony count, processing of nasal secretions, and conducting the IHA test have been described (4). The *Past. hemolytica* culture used in these studies was obtained from a fatal case of fibrinous pneumonia in a steer and had been classified as Type[1].

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[1]Typing carried out by E. Biberstein, University of California.

TABLE I. Experimental Past. hemolytica Aerosol Exposure and Antibiotic Treatment

Group	Calves	Aerosol Vaccination[a]	Antibiotic Treatment[b]	Challenged by Past hemolytica Aerosol[c]
1	1, 2, 3, 4	+	+	+
2	5, 6, 7, 8	−	+	+
3	9, 10, 11, 12	+	+	−

[a]Past. hemolytica aerosol vaccination on days 1, 4, 8, and 11.
[b]From days 17-22, 250 mg dihydrostreptomycin and 200,000 I.U. penicillin were given twice daily; on days 23-26 double this dose was given.
[c]Challenged on day 31.

RESULTS

All calves in group 1 had high pre-inoculation serum titers to *Past. hemolytica* and developed high titers in nasal secretion following vaccination. The levels of specific antibody in serum and nasal secretion in group 2 were lower (Fig. 1).

In group 2, calves 5 and 6 had serum and nasal antibody before vaccination of group 1. Calf 7 developed a serum titer prior to challenge but calf 8 did not. Calves 7 and 8 had negative nasal titers at the time of challenge. Thus only calf 8 fulfilled the criteria initially outlined for this group.

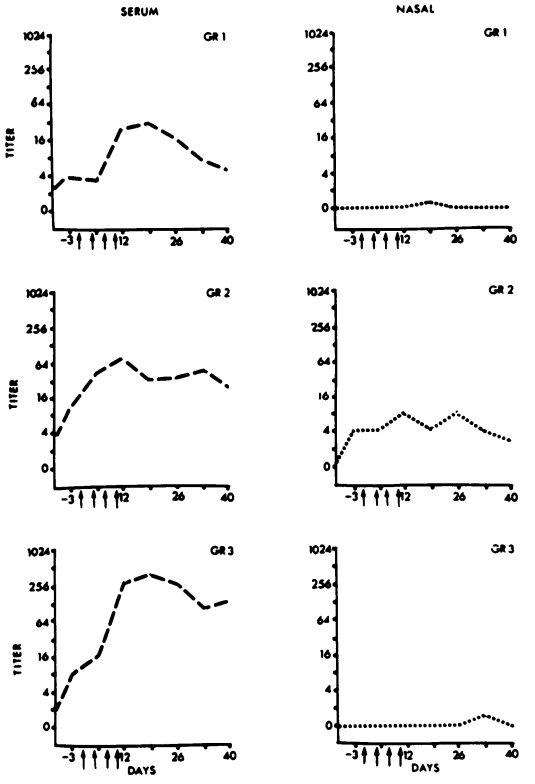

Fig. 1. Mean serum and nasal levels of IHA antibody to Past. hemolytica from calves in groups 1 to 3. Standard error of the mean indicated by vertical bars.
GR 1 — four aerosol vaccinations and one aerosol challenge
GR 2 — one aerosol challenge
GR 3 — four aerosol vaccinations
arrows — aerosol vaccinations on days 1, 4, 8 and 11 and challenge on day 31.

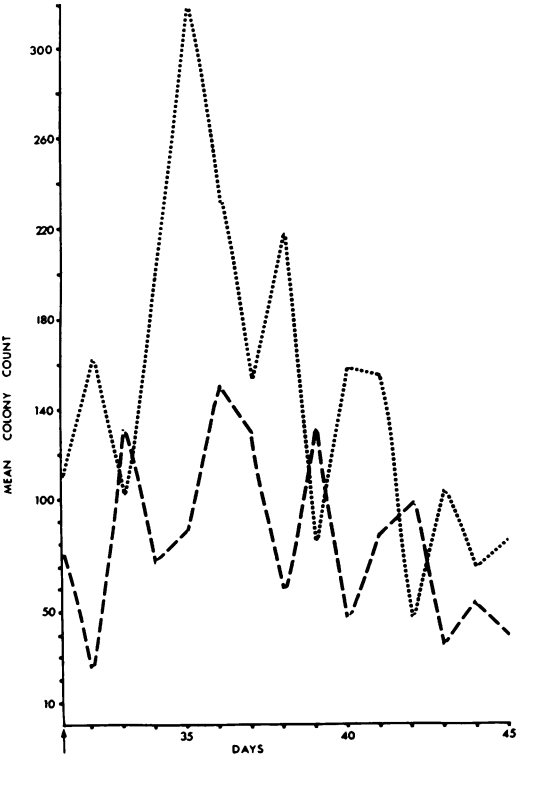

Fig. 2. Daily mean colony count of Past. hemolytica from nasal swabs taken twice daily from both nasal passages for a fifteen day period after aerosol challenge on day 31 with living Past. hemolytica.
dash line — group one
dotted line — group two
arrow — aerosol challenge

TABLE II. Isolations of P. hemolytica from Nasal Washings and Swabs

Group	Calf	Time Intervals: Pre Vaccination Day 3 N(1)[a]	MCC[b]	Vaccination (Gp 1 and 3) Days 5 and 12 N(2)	MCC	Post Vaccination Days 13-16 N(8)	MCC	Antibiotic Treatment Days 17-26 N(18)	MCC	Pre Challenge Days 27-30 N(8)	MCC	Post Challenge Days 31-45 N(60)	MCC
1	1	1	55	2	6	4	55	0	0	0	0	43	135
	2	1	1779	1	55	2	31	0	0	0	0	43	41
VACC.	3	1	2	0	0	0	0	0	0	0	0	18	46
	4	1	6	2	31	5	17	7	12	4	41	40	96
	5	1	1779	1	562	2	17	1	2	0	0	11	50
2	6	0	0	1	55	4	74	2	17	0	0	43	131
NON VACC.	7	0	0	0	0	0	0	0	0	0	0	58	328
	8	0	0	0	0	0	0	0	0	0	0	47	51
3	9	0	0	2	999	8	865	3	177	0	0	0	0
VACC.	10	0	0	2	177	7	40	1	1779	0	0	0	0
CONTROL	11	0	0	0	0	0	0	0	0	0	0	0	0
	12	0	0	2	562	8	648	6	26	0	0	0	0

[a]N = number of positive isolations
( ) = possible number of positive isolations
[b]MCC = mean collony count

Calf 8 developed signs of acute respiratory distress on day 33 and had a febrile response with a rectal temperature above 104.5°F. on days 33, 34, and 35. No febrile responses were noted in any other calves.

The vaccinated group yielded fewer *Past. hemolytica* than the non-vaccinated group during the post-challenge period (Fig. 2). Isolations of *Past. hemolytica* from the nasal passages during the various periods of the experiment were recorded (Table II). Antibiotic treatment was effective in clearing *Past. hemolytica* from all calves in Groups 1 and 2 except calf 4 and *Past. hemolytica* was isolated from calves 5 and 6 prior to challenge.

One colony was selected from one or both nostrils of calves yielding *Past. hemolytica* on culture of nasal swabs on days 23, 33 and 45. When tested against known type I rabbit serum by IHA, all gave a positive result.

An analysis of variance on IHA titers of 26 and mean bacterial colony counts from days 31 to 45 are recorded in Table III. serum and nasal secretions collected on day

DISCUSSION

The results demonstrated that *Past. hemolytica* was capable of colonizing the nasal passages in the presence of specific IHA antibody. However, as a result of natural infection of calves in group 2 before challenge, there was no significant difference between groups 1 and 2 in the mean levels of nasal IHA antibody at challenge (Table III). Therefore, even though the numbers of *Past. hemolytica* isolated from the nasal cavities of the vaccinated group were significantly less than from the non-vaccinated group, the effect of local antibody on the shedding of the organism cannot be clearly assessed.

TABLE III. Analysis of Variance of Pre-exposure Serum and Nasal washing IHA Titers and Past. hemolytica Mean Colony Count from Days 31-45

Source of Variance	Serum Degrees of Freedom	F	Nasal Washing Degrees of Freedom	F	Mean Colony Count Degrees of Freedom	F
Treatment	1	6.08[a]	1	4.24	1	9.66[b]
Error	6		6		28	
Total	7		7		29	

[a]significant five per cent level
[b]significant one per cent level

The relationship of antibody in the upper respiratory tract to the bacteria which constitute the normal nasal flora varies with the host and bacterial species involved. Bull and McKee (1) concluded that in the rabbit specific antibody to *Pneumooccus* was responsible for the nasal rejection of the same strain but specific antibody to *Bacillus bronchiseptica* did not influence its nasal residence. MacLeod, Hodges, Heidelberger and Bernhard (5) observed that immunization of man with certain type specific pneumococcal polysaccharides by a nonrespiratory route significantly diminished the nasal carriage for the corresponding pneumococcal types, but did not influence unrelated types.

Past. hemolytica did not recur in the nasal passages of calves in group 3 which would suggest that the course of antibiotic therapy was effective in clearing *Past. hemolytica* from all except calf 4 in group 1. The failure to clear *Past. hemolytica* from this calf probably did not have any effect upon the final results although it meant a failure in fulfilling one of the prescribed requirements of this experiment. Since all the isolates of *Past. hemolytica* which were typed conformed to the classification of type I *Past. hemolytica,* it does not appear that other types of *Past. hemolytica* were included in the total bacterial or mean colony counts tabulated from nasal swabbing. Following the prechallenge, serum and nasal IHA antibody levels of calves 5 and 6 suggested that natural exposure to *Past. hemolytica* was almost as effective as vaccination in stimulating serum and nasal secretion antibody titers. The results emphasize the necessity of having animals of known status but this becomes difficult when working with organisms that can occur in normal flora.

Dubos (3) stated that the persistence of pathogens as a sub-clinical infection in tissues is so general in occurrence that it can be regarded as the rule rather than the exception. The persistence of any given pathogen in some parts of the body may result in increased resistance to this pathogen (premunition). Acquired immunity was often more effective in controlling the manifestation of disease than in preventing the establishment of infection.

The development of acute clinical respiratory disease in calf 8 in the absence of any of any detectable serum of nasal antibody at the time of challenge concurs with previous findings (4). This would, in our opinion, emphasize the importance of the immune status of cattle in respect to the experimental reproduction of Pasteurella pneumonia and possibly the naturally occurring disease.

If the pathogenesis of Pasteurella pneumonia of cattle was examined on this basis it might be hypothesized that under normal circumstances the host can maintain an attenuated infection of the upper respiratory tract but with a decrease or alteration of disease immunity the organism can proliferate and in turn lead to the development of the disease state. Alternatively, the induction of the disease state may exclude previous infection of the upper respiratory tract with the particular strain thus preventing the induction of immunity by natural means under favorable environmental conditions.

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