Pasteurella haemolytica in the Tracheal Air of Calves

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SUMMARY

Pasteurella haemolytica was shown to be present in the tracheal air of calves and was likely transported in droplet nuclei formed in the nasal passages. The number of colonies of P. haemolytica found in the tracheal air of the calves ranged from 1.9 to 12.5 colonies per cu ft of air. As long as P. haemolytica colonized the nasal passage in numbers detectable in nasal swabs it could be found in the tracheal air but there was no direct correlation between the numbers in the nasal flora and the numbers found in the tracheal air. Of the P. haemolytica which travel via the tracheal air 47.8% were in droplets of the aerodynamic size of from less than one to five microns, the size range which is considered hazardous for lung penetration in man.

The technique used demonstrated the presence of P. haemolytica in the tracheal air of calves and provides a useful tool for monitoring and determining the phase in the colonization of the respiratory tract in which the majority of the potential pathogen P. haemolytica pass from the nose to the tracheal air and presumably to the lung.

RÉSUMÉ

Les auteurs ont démontré la présence de Pasteurella hemolytica dans l'air de la trachée de veaux et son transport probable dans des amas de gouttelettes formées dans la cavité nasale. Le nombre des colonies de P. hemolytica isolées de l'air de la trachée de ces veaux variait de 1.9 à 12.5 par pied cube d'air. Tant qu'a duré la multiplication de P. hemolytica dans la cavité nasale, en quantité décelable sur des écouvillons nasaux, on a pu l'isoler de l'air de la trachée; il n'y avait cependant pas de relation quantitative directe entre sa présence dans la flore nasale et dans l'air de la trachée. Des P. hemolytica véhiculées dans l'air de la trachée, 47.8% se retrouvaient dans des gouttelettes de la dimension aérodynamique variant de moins d'un micron jusqu'à cinq microns, dimension moyenne considérée dangereuse pour la pénétration dans les poumons chez l'homme.

La technique utilisée a démontré la présence de P. hemolytica dans l'air de la trachée de veaux. Elle fournit aussi un moyen utile pour déceler et déterminer la place de l'envahissement des voies respiratoires où la majorité des P. hemolytica éventuellement pathogènes passent de la cavité nasale à l'air de la trachée, et probablement aux poumons.

INTRODUCTION

The importance of *Pasteurella haemolytica* in bovine pasteurellosis has been emphasized by many authors (1, 2, 4, 12) both as a resident of the nasal passages and of pneumonic lung. Magwood, Barnum and Thomson (8) considered *P. haemolytica* to be part of the normal nasal flora of cattle. Thomson, Benson and Savan (12) noted not only a high frequency of isolation but also a great increase in the numbers of *P. haemolytica* present in the nasal passages of cattle shortly after they had been shipped from Western Canada. The frequency of isolation of *P. haemolytica* and

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the relationship of large numbers of this organism in the nasal passage with pneumonia demonstrated by Thomson *et al* (12) were the stimulus for the work undertaken to establish whether *P. haemolytica* travelled from its colonization site in the upper respiratory tract to the lung via the tracheal air and whether there was a relationship between the numbers in the nasal passages and the numbers in the tracheal air.

MATERIALS AND METHODS

Twenty-eight calves were brought from Western Canada in three groups *i.e.* A, B and C (Table I) and housed as previously described (12). Nasal swabs were taken six days per week, from each nostril of each calf after the method of Magwood *et al* (8). Calves were selected for tracheal air sampling according to the numbers of *P. haemolytica* in the nasal swabs and in an effort to have equal numbers of animals in each class. The calves were divided into four classes on the basis of the Mean Colony Count (MCC) (8) of *P. haemolytica* on the days the tracheal air was sampled. The classes were: Class I with a negative count, Class II with low counts, Class III with medium counts and Class IV with high mean counts (Table I). An animal was assigned to a class provided the MCC for *P. haemolytica* of one nostril fulfilled the requirements for that class. This allowed classification of calves with a nasal population of *P. haemolytica* in one nostril and none in the other.

Based upon the nasal MCC of the previous day, two animals were selected and moved indoors to individual isolation wards. The same day they were transferred from the isolation ward to a crate for surgery in a room where dust was minimal.

Surgery was performed to place a 10 mm Portex[1] tracheostomy tube in the trachea to facilitate the taking of tracheal air samples (Fig. 1). The neck area, from above the larynx to about two-thirds of the way towards the thoracic inlet, was clipped and prepared for surgery under local anesthesia. A longitudinal incision 4-5 cm long, was made on the midline of the neck having as its midpoint the space between the eighth and ninth tracheal rings. The lumen of the trachea was tapped by a transverse incision between the eighth and ninth tracheal rings, and the Portex tracheostomy tube was inserted with the inserted end directed to the nares, and secured to the skin with size 3 Supramid sutures[2]. The external opening of the tracheostomy tube was plugged with a suitable rubber stopper until used. The tracheostomy tubes were placed in the trachea either on the day of sampling (approximately two hours before) or the day before (16–17 hours previous to) sampling.

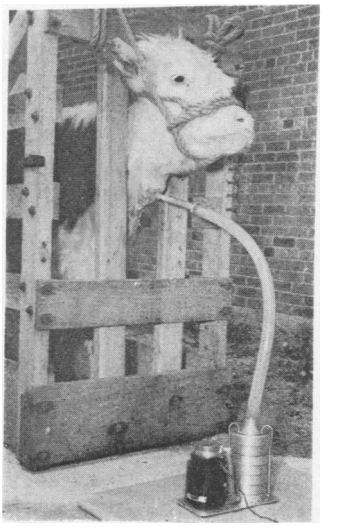

Fig. 1. Sampling the tracheal air. An adapter (close to the external end of the tracheostomy tube) connects the narrow end of the polyethylene tube and sampler to the inserted tracheostomy tube. The picture was taken outside for photographic reasons.

The tracheal air of each animal was sampled with the Andersen sampler[3] which was fitted with a length of polyethylene tubing 76 cm long and 2.5 cm internal diameter. A 15 cm length of tubing 1.75 cm in internal diameter was connected to the free end of the tubing and an adapter provided connection between this and the Portex tracheostomy tubing.

A polyethylene tube attachment was used on one run only each day, and the tubes were thoroughly washed and air dried in preparation for the next day's use. The capacity of the Andersen sampler with the tubing attached was measured at 0.75 cu ft

[1]National Hospital Supply Co., Ltd., 1000 Lakeshore Rd. E., Port Credit, Ontario, Canada.

[2]Supramid, B. Braun, Melsungen, W. Germany.

[3]Andersen Samplers and Consulting Service, 1974 Ash Avenue, Provo, Utah.

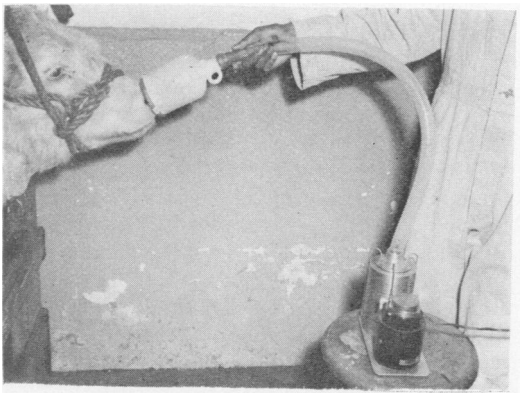

Fig. 2. Sampling the expired air. The cone was held 0.5-1 in from the external nares.

per minute[4].

Six samples (runs) of four minutes duration taken at half hour intervals from the trachea of each calf were made each day for two days and represented 18 cu ft of tracheal air per animal per day.

The room air was sampled prior to the tracheal air at about the height of the head of the calves on days of air sampling with the Andersen sampler and the polyethylene tube attachment. Sampling time was four minutes.

Immediately after the tracheal air was sampled, the external nares and surrounding muzzle were swabbed to eliminate mucus and dirt and the expired air was taken with the Andersen sampler in the manner shown (Fig. 2). Sampling time was four minutes.

Swabs of the lower buccal mucosa in the region of the incisor teeth were taken daily for six days per week including the days of sampling the tracheal air.

Ten ml of blood were taken from each calf by aseptic jugular puncture on alternate days using a sterile needle and syringe and cultured in 100 ml of tryptic soy broth. The blood-broth mixture was incubated for 24 hours. A four mm loopful was plated onto blood agar, incubated and the plates read 24 hours later. All samples were taken at approximately the same time each day.

The plating for both nasal and mouth swabs and the procedures for identification and tabulation of bacterial species and calculation of MCC were the same as previously described (8), except that the colony count class "a" included 0 to 2 instead of 1 to 2 colonies. The five per cent blood agar plates from the Andersen sampler were incubated for 24 hours at 37°C. The colonies of *P. haemolytica* and *P. multocida* were identified and counted on the plates of each stage of the Andersen sampler and recorded separately while all other bacteria were counted as one group. The plates were re-read 24 hours later and any required adjustments made, to take into account colonies whose identification was in doubt after initial incubation.

RESULTS

P. haemolytica was found in the nasal swabs of 21 of the 28 calves, while the tracheal air yielded *P. haemolytica* from 17 of the 28 animals. *P. multocida* was recovered from the tracheal air of one animal, the only one which had this organism in the nasal swab at the time of sampling the tracheal air. Based upon the numbers of nasal *P. haemolytica* there was an uneven distribution of the animals into the four classes; however, Group B had representatives in each class (Table I).

The animals withstood the surgical and tracheal air sampling procedures very well. Breathing was normal over the two days in which the tracheostomy tube was inserted, including the periods when the tracheal air was sampled. At no time in any of the animals was mucus, serum or blood seen in the adapter or polyethylene tube attachment of the sampler during the process of taking the tracheal air. Typical colonies of *P. haemolytica* from the tracheal air of the calves are shown in Fig. 3. The number of

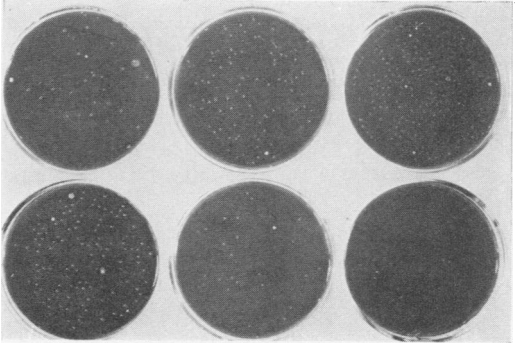

Fig. 3. Typical colonies of P. haemolytica from the tracheal air obtained with other bacteria on the plates of the six stages of the Andersen sampler. Stages one, two and three on the top from the left, and stages four, five and six on the bottom from the left.

[4]Calibration was performed by Mr. Calvin Hutchins, School of Agricultural Engineering, University of Guelph.

colonies of *P. haemolytica* recovered from the tracheal air according to classes (Table II) represents the number of colonies caught in the different stages of the Andersen sampler in 18 cu ft of air on each day and in 36 cu ft over the two days. Wide variations were found in the colony counts per animal from one sample of tracheal air to another. There was a tendency to get a greater number of colonies of the organism on the second day of sampling than on the first. This was so irrespective of whether the calves were sampled within two hours of placing the tracheostomy tube into the trachea or 16 to 17 hours later.

The animals which had no *P. haemolytica* in the nasal swabs (Class I animals) yielded none in the tracheal air. This was consistent for all the groups of animals and for all stages of the Andersen sampler.

When all the groups were taken together (Table III) the number of colonies of *P. haemolytica* collected from the tracheal air was highest for Class III followed by Class II, and was lowest in Class IV animals. Animals in Class III had a nasal MCC for the organism 26 times greater than Class II, yet the mean number of colonies caught per animal in the tracheal air was greater by only 18 colonies. The nasal MCC for the organism for Class III animals was also 12 times smaller than Class IV, yet the number of colonies in the tracheal air per animal for Class III was 89 colonies more. Class IV animals had a mean nasal colony count 327 times greater than Class II yet the number of colonies per animal caught in the tracheal air was 71 colonies less.

Group B was the only group in which

TABLE I. Distribution of Animals into Classes[a]

	Class I	Class II	Class III	Class IV	Total
Group A	2	0	1	5	8
Group B	3	2	3	2	10
Group C	2	0	2	6	10
Total	7	2	6	13	28

[a]Class	Colony Count of P. haemolytica	Count Class	
I	0	0	Negative
II	1 — 2	a)	
	3 — 9	b)	Low
	10 — 30	c)	
III	30 — 100	d)	Medium
	100 — 300	e)	
IV	300 — 1000	f)	High
	1000 — 3000	g)	

TABLE II. Number of Colonies of P. haemolytica in Tracheal Air for All Groups

Class	No. of Animals	Day I S1[a]	S2	S3	S4	S5	S6	Day II S1	S2	S3	S4	S5	S6
							(a) 18 cu. ft. per animal						
I	7	0	0	0	0	0	0	0	0	0	0	0	0
II	2	1	2	3	4	0	0	164	111	97	61	2	1
III	6	146	116	134	50	12	6	289	118	382	150	39	3
IV	13	144	145	240	136	31	2	606	276	251	125	22	0

(b) 36 cu. ft. per animal

Class	No. of Animals	S1	S2	S3	S4	S5	S6
I	7	0	0	0	0	0	0
II	2	165	113	100	65	2	1
III	6	435	234	516	200	51	9
IV	13	750	421	491	261	53	2

[a]S1 — S6 Stages of the Andersen Sampler

TABLE III. Nasal Mean Colony Count and Number of Colonies of P. haemolytica in 36 cu. ft. Tracheal Air for All Groups

Class	No. of Animals	Nasal MCC per Animal	No. of Colonies P. haemolytica Total of All Animals	Average Per Animal
I	7	0	0	0
II	2	2	446	223
III	6	53	1445	241
IV	13	654	1978	152

TABLE IV. Nasal Mean Colony Count and Number of Colonies of P. haemolytica in 36 cu. ft. Tracheal Air for Group B. Animals

Class	No. of Animals	Nasal MCC per Animal	No. of Colonies P. haemolytica Total of All animals	Average Per Animal
I	3	0	0	0
II	2	2	446	223
III	3	68	1356	452
IV	2	1166	381	190

there were animals with nasal *P. haemolytica* counts fulfilling the requirements for all the classes set up, a situation which would reasonably be expected to occur in any calf population (Table IV).

Of the total number of colonies of bacteria which grew from the tracheal air of all the animals *P. haemolytica* comprised 25% for Class II, 17.2% for Class III, and 14.4% for Class IV (Table V).

The number of colonies of *P. haemolytica* found per cu ft of tracheal air per animal was 6.2 for Class II, 6.7 for Class III and 4.2 for Class IV (Table VI).

The frequency of isolation of *P. haemolytica* from the oral cavity in all classes was high. Class I animals, with a negative nasal swab for this organism, harboured them in the mouth. Only one of seven animals in this class with a negative nasal flora had an oral flora negative for *P. haemolytica* over the entire period of sampling. Among the animals with positive nasal swabs, one in Class III and one in Class IV had a negative oral swab over the sampling period. *P. multocida* was not recovered from the mouth of the animal with a positive nasal swab for that organism at any time during the test period.

Neither *P. haemolytica* nor *P. multocida* was isolated from the blood of any of the calves.

TABLE V. Percentage of Colonies of P. haemolytica in 36 cu. ft. of Tracheal Air Per Animal

Class	No. of Animals	All Animals in the Groups			
		Group A	Group B	Group C	All Groups
I	7	0	0	0	0
II	2	—	25	—	25
III	6	6.5	56.2	0.4	17.2
IV	13	25.2	43.7	3.9	14.4

TABLE VI. Number of Colonies of P. haemolytica per cu. ft. Tracheal Air per Animal

Class	No. of Animals	Group A	Group B	Group C	All Groups
I	7	0	0	0	0
II	2	—	6.2	—	6.2
III	6	1.9	12.5	0.3	6.7
IV	13	7.4	5.2	1.2	4.2

P. haemolytica formed part of the flora of the air expired by some of the animals. The organisms were collected only when the animals snorted during the sampling period, and usually were more numerous as the frequency of snorting increased from one to five snorts. Bacteria of rumen origin were often obtained in expired air.

Neither *P. haemolytica* nor *P. multocida* was recovered from the samples of room air and the colonies of other bacteria caught on the days of sampling ranged in numbers from 35 to 1281. Of one hundred colonies picked from the air sampler plates which represented one run of the room air, micrococci numbered 48 colonies, spore forming rods 33 and miscellaneous 19.

DISCUSSION

The *P. haemolytica* found in tracheal air in the present study most likely originated from the nasal passages of animals with positive nasal swabs, as only the calves with positive nasal swabs yielded the organism in the tracheal air, irrespective of the fact that calves with negative nasal swabs had high numbers in the oral swabs. Colonization of the nasal passage in numbers detectable on nasal swabbing seemed a prerequisite for the detection of *P. haemolytica* in the tracheal air. It was unlikely that the source was secretions rolling down and dragged into the tracheostomy tube at sampling, as secretions were not seen in the polyethylene tube attached to the sampler at any time. Further, calculations made of the air velocity through the 10 mm Portex tracheostomy tube was 4.55 metres per second[5] and was within the range of normal breathing as will be shown below from measurements given by various authors. The organism in the oral cavity did not seem to be inhaled directly into the lung, as the organism was not found in the tracheal air of Class I animals which had negative nasal but positive oral swabs for this organism. This finding does not, however, exclude the mouth as a source of infection for the lung. As the organism was not found in the room air, it is unlikely that it came from the environment either in droplet nuclei or on dust particles.

[5]Calculation was performed by Mr. Calvin Hutchins, School of Agricultural Engineering, University of Guelph.

Were the organisms caught in the tracheal air borne on dust particles or in droplets? With dust particles directly from the environment excluded, the possibility of such particles impinging on the nasal mucosa, acquiring a complement of organisms and continuing to the lung in another stage of descent seems unlikely. It was more likely that *P. haemolytica* was carried in droplet nuclei formed in the nasal passages during quiet breathing.

Flugge (3) cited by Hare (5) was the first to show that micro-organisms can be dispersed directly from the human respiratory tract. He calculated that an air velocity of at least 4 metres per second is required and claimed that this was not achieved in normal breathing in the normal respiratory tract. Proctor (10) using an angle metre, measured simulated nasal air flow on models of human nasal cavity, constructed from casts at autopsy. In his measurements, peaks of at least five metres per second occurred in the main airstream flow during quiet breathing.

From the above calculations the authors feel that the velocity in the human upper respiratory tract is sufficiently high to form droplet nuclei from secretions in the nasal passage which can either be expelled to the outside or aspirated into the lung during quiet breathing. Assuming that Flugge's and Proctor's calculations might be applicable to other animals, the organisms caught in the tracheal air of the calves likely travelled in droplets formed in the nasal passage.

The size of particles for the different stages of the Andersen sampler ranges from less than one to 9.2 microns and greater. If the same division used for humans is applied to other animals the percentage of the number of colonies of *P. haemolytica* in the tracheal air in droplets of from less than 1 to 5 microns in size and considered hazardous from the point of view of lung penetration was 47.8%. However, some workers have found that particles much larger than those generally believed capable of penetrating to the terminal airways actually reach there (6).

The results do not necessarily negate transport of respiratory pathogens on dust particles under natural conditions. Jones (7) found that organisms could be isolated from the periphery of the lungs of animals fed dusty hay and oats, whereas in animals fed wet feed and kept away from dusty materials, the lungs were sterile or yielded

fewer organisms. In the present study when the tracheal air of animals was sampled in the building in which the animals were initially housed, organisms borne on dust particles from the bedding were caught in such large numbers that it was impossible to identify any *P. haemolytica* which might have been descending in the tracheal air.

Based on the respiratory parameters given (11), the method of sampling took in but a fraction of the volume of the air breathed in by the calves. The volume of tracheal air taken in by the Andersen sampler from a 527 kg animal would represent approximately 10% of the minute volume or 12.5% of the inspirational flow. It is evident that far more organisms were likely transported by the tracheal air. Once there is nasal colonization by *P. haemolytica* the lungs may be exposed to extremely high numbers of this organism.

There was no direct correlation between the numbers of *P. haemolytica* present in the nose and the numbers caught in the tracheal air. As the samples of tracheal, room and expired air, nasal and oral swabs, and blood for culture were all taken at approximately the same time each day there was little if any environmental variation in the results.

A negative nasal swab does not necessarily mean the absence of the organism in the nasal passage. After intensive swabbing of 15 different areas of the nasal passage of calves at necropsy it was found that calves with a negative nasal swab harboured *P. haemolytica* in many areas of the nasal passage but in small numbers (9). It would appear that the organism has to be in detectable numbers in the nasal passage, as determined by swabs, in order for it to be found in the tracheal air.

The highest yield of *P. haemolytica* in the tracheal air might be expected from animals in Class IV with the high nasal counts, but this class gave the lowest yield. It would appear that *P. haemolytica* descended in the early phases of multiplication, as represented by the nasal MCC of Classes II and III, and descent had diminished in the case of Class IV counts. Possibly droplet nuclei were more readily formed during the early phases of multiplication of the organism in the nasal passages.

A room with relatively low numbers of bacteria in the air was requisite for this work as *P. haemolytica* is easily overgrown by the more vigorously growing organisms in the environment thus making identification difficult; the Andersen sampler was more suitable for handling low yields of bacteria (13).

The mouth could be an important source for expulsion of pathogens provided it were colonized. In the present study 89% of the calves carried *P. haemolytica* in the front of the mouth for varying periods. It was not clear whether this meant a persistent infection or repeated contamination. A likely source of oral infection or contamination in Class I calves was drinking water and feed contaminated by nasal secretions from the other animals. The water supply was not intensively cultured, and no attempt was made to recover *P. haemolytica* from the feed trough. The source of oral contamination or infection for Classes II, III and IV animals could also be directly from the nasal passage with the tongue or from licking contaminated hair coat.

The tracheostomy tube must have been seated somewhere in the vicinity of the mid-tracheal airstream and allowed sampling of the airstream on its way to the lung. The tube was always clear of any exudate which was in contrast to another method of tapping the trachea used in the early stages of development of the technique. In this earlier method a 6 gauge bleeding trochar was used, and this frequently allowed mucus to be drawn into the polyethylene tube attached to the sampler. The velocity at which the Andersen sampler removed the tracheal air was less than that reported for air velocity through the human nasal passages (10). Any turbulence created by the procedure would probably not be greater than that which occurs in normal breathing. The sampler did remove air during the period of inspiration as well as expiration, but as the sampling periods were of relatively short duration (four minutes each) there would be little if any drying out of the respiratory mucosa above or below the level of the tracheostomy tube, which would adversely affect the yields of bacteria collected. Therefore, the equipment used tapped the tracheal airstream and removed the air at a rate within the normal limits of breathing.

The investigation herein is an attempt to elucidate the possible association between large numbers of *P. haemolytica* in the upper respiratory tract and their potential for deposition in the lung. It attempts to elucidate the manner in which pathogens reach the lungs from the upper

regions. Application of the technique used and refinements thereof should facilitate study of the behaviour of particulate matter and droplets in the airways to the lung and the factors which govern the passage of pathogens and potential pathogens from a site in the upper respiratory tract to the lower regions.

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