

Survey of the Trachea⁵₁ of Feedlot Cattle for Haemophilus somnus and Other
Selected Bacteria

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The fact that "Haemophilus somnus" (H. somnus) can produce a septicemia in cattle has been reported by several investigators.^{1,2,3} They postulated that the respiratory tract could be the portal of entry, the site of initial multiplication and the locus of systemic invasion by the bacterium. Although details are lacking it was reported by Brown et al³ that they "had experimentally produced lesions resembling those seen in typical field cases by either intravenous or intratracheal administration of H. somnus to susceptible calves." Panciera et al² failed to reproduce clinical disease by intratracheal inoculation of a calf whose tracheal mucosa had been artificially disrupted. They did report laryngeal lesions in affected animals and clinical signs of respiratory disease in groups of feedlot cattle, some of whose members died of confirmed H. somnus septicemia. Because of the existence of laryngeal lesions and other evidence of respiratory involvement

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in affected cattle, collection of data on the prevalence of H. somnus in the trachea of cattle was deemed a vital step in defining the pathogenesis of the infection. Further, since it is likely that the pathogenic potential of one bacterium is influenced by the presence of other bacteria, potential bacterial pathogens in addition to H. somnus were also sought. This paper reports the results of survey of bacterial flora obtained from culture of tracheal fluids of healthy cattle, cattle with clinical signs of bovine respiratory disease (BRD), cattle that had been exposed to BRD, and cattle that had recovered from BRD. This survey was conducted in cattle fed in the Oklahoma and Texas Panhandles.

MATERIALS AND METHODS

Specimens of tracheal fluids were obtained by passage of a guarded culture instrument (24 inches long)* through the oropharynx and laryngeal aperture such that collection of representative specimens from the upper one-quarter of the tracheal mucosa was consistently possible. Collected swabs were held at approximately 4°C. Within one-half to two hours after collection of the specimen, the swab was streaked onto brain heart infusion agar (BHI) containing 5% citrated bovine blood, 10% horse serum and 1% yeast autolysate (Albimi). After initial streaking of a segment of the blood agar the swab was inserted into a mycoplasma medium⁴ containing inhibitors (1-2000 thallium acetate and 1000 units per ml. of potassium penicillin) and 0.4% agar. The blood agar surface was then further streaked with a loop so that isolated colonies would be obtained. The surface of the agar was finally cross-streaked with Staphylococcus aureus. Inoculated media was placed in an atmosphere of 5-8% CO₂ and was examined for bacterial growth at 24 hour intervals for a total of three days.

*KI-2000; Kalayjian Industries, 6050 Appian Way, Long Beach, Calif. 90803

Identification of isolated bacteria was made by various diagnostic laboratory keys.^{5,6} The "Haemophilus spp." were placed into the categories of satelliting or nonsatelliting on the basis of their growth in relation to Staphylococcus aureus on BHI-blood agar. The nonsatelliting strains were morphologically and biochemically similar or identical to H. somnus.^{1,2,7} Pasteurellae identified as Pasteurella multocida did not grow on MacConkey's agar and were positive for indole production in tryptophan broth. Pasteurellae identified as Pasteurella haemolytica hemolyzed erythrocytes incorporated into the BHI agar medium, did not produce indole in tryptophan broth, and usually grew on MacConkey's agar. Colonies of bacteria identified as Pasteurella species morphologically resembled Pasteurella haemolytica but did not produce hemolysis. These Pasteurella species were variable in reaction for indole production in tryptophan broth (usually negative) and growth on MacConkey's agar.

The inoculated mycoplasma medium was incubated for 96 hours at 37°C after which the swab was removed and streaked onto a medium of the same composition but containing 1.5% agar. The inoculated medium was cross-streaked with Staphylococcus aureus and the plates incubated at 37°C in a moist atmosphere of 5-8% CO₂ for a total of eight days. The agar surface was examined for colonies of Mycoplasma spp. on the fourth and eighth days. Typical colonies were transferred to mycoplasma agar without inhibitors, the medium was cross-streaked with Staphylococcus aureus, and the plates incubated as above. An additional passage of organisms to mycoplasma medium without inhibitors was made. Colonies that did not revert to bacterial form after the second passage on non-inhibited medium were recorded as Mycoplasma spp. No further identification of the Mycoplasma species was made.

RESULTS AND DISCUSSION

The various bacteria isolated from healthy, clinically ill (BRD), medicated (BRD) and recovered (BRD) feedlot cattle are recorded in Tables I through V. The results show that the individual bacterial species under consideration existed in about the same prevalence in the trachea of healthy, ill, medicated, and recovered cattle. H. somnus biotypes (nonsatellitizing Haemophilus) were isolated from 3.2% of animals. H. somnus septicemia was not recognized in animals represented in Tables I-V nor in their penmates.

H. somnus septicemia did occur in one group of animals surveyed. Isolation data from individuals in this group at four different sampling times, at approximately two week intervals, are presented in Table VI. The rate of isolation of H. somnus from animals in this group was somewhat greater than in the preceding groups. Isolates of the organism were made from three of seventeen animals (18%) on the first sampling, 4 of 17 (24%) on the second, 0 of 16 (0%) on the third, and 2 of 19 (12%) on the fourth which was six weeks after onset of the outbreak. The rate of isolation of Pasteurellae on the other hand was comparable to that from healthy cattle. This suggests that the presence of Pasteurellae did not enhance the invasion of H. somnus.

The rate of isolation of nonsatellitizing Haemophilus sp. (H. somnus) did not show a significant seasonal variation (Table VII). If one were to assume that the presence of the organism in the host and the prevalence of systemic disease were directly related, then one would anticipate disease to occur at an approximately equal rate throughout the year. Systemic disease, however, occurs largely during the fall and winter months.^{1,8} It is therefore suggested that seasonal influences on the occurrence of the septicemic disease are related more to factors that predispose to systemic invasion by H. somnus from its tracheal locus than

to fluctuations in population of the organism in the respiratory tract. The above rationale assumes that systemic spread from tracheal flora is the modus operandi of infection.

The isolation data presented must be interpreted with caution since this is a survey of cattle under "field conditions." Further, some sampling categories contain few cattle. Nevertheless, the data shows a higher rate of isolation of Pasteurella haemolytica and Mycoplasma spp. than other organisms (Table I). There was an increased prevalence of these two organisms in clinically ill cattle (Table III) compared with either healthy (Table II) or exposed (Table IV) cattle. The greater overall prevalence of Mycoplasma spp. in the clinically ill cattle is not surprising since members of the genus frequently occupy a secondary role in respiratory infections.⁹ The frequency with which Mycoplasma spp. was found could be interpreted either as a high degree of transmissibility or prolonged persistence of infection. The rate of isolation of H. somnus on the other hand was low in all classes of cattle sampled which suggests either a low potential for transmissibility or short duration of persistence of infection in the trachea.

It has been stated that the rate of isolation of H. somnus from tissues is decreased after antibiotic treatment of an animal.³ The data presented in Table V indicates that antibiotic treatment had little influence on the recovery of H. somnus from tracheal fluids. Furthermore, there is no definite evidence that the rate of isolation of any of the bacteria was greatly affected by antibiotic therapy. There was only a slight reduction in the rate of isolation of Pasteurella multocida, Pasteurella haemolytica, Mycoplasma spp., and "nonpathogens" in treated animals as compared with untreated ill animals (Table V).

The Streptococcus spp. (not enterococci) that were isolated sometimes satellited Staphylococcus aureus on blood agar the first 24 hours of incubation; after which they no longer exhibited satellitism. This growth characteristic and colonial morphology resembled that of the satelliting Haemophilus spp. Streptococci often were recovered in almost pure culture; the rate of isolation of these organisms was frequently high from a particular lot of cattle. These organisms are being further studied in order to ascertain if they can be used as an indicator of microfloral and functional disturbances in the trachea.

SUMMARY

The prevalence of selected bacterial species isolated from the tracheal fluids of living feedlot cattle was reported. The data was categorized as to isolation of the bacterial species from cattle ill with respiratory disease, healthy cattle, cattle exposed to respiratory disease, and cattle medicated with antibacterial drugs. In addition the rate of isolation of bacteria is correlated with the duration of residence of cattle in the feedlot. Haemophilus spp., Pasteurella spp., and Mycoplasma spp. were isolated from the tracheal fluids of all classes of the above described cattle. The rate of isolation of Pasteurella haemolytica and Mycoplasma spp. without regard to categories of cattle was considerably higher than other bacterial species. Furthermore, the prevalence of these organisms in ill animals was increased compared with prevalence in healthy or exposed animals.

The data indicated that H. somnus was part of the transient, if not indigenous, flora of the respiratory tract. Higher rates of isolation of H. somnus were not noted in cattle with respiratory disease compared

to healthy cattle, nor was significant seasonal variation in the rate of its isolation observed.

The rate of isolation of H. somnus from a serially sampled herd that had experienced a natural infection and disease due to H. somnus was presented.

REFERENCES

- ¹Kennedy, P. C., Biberstein, E. L., Howarth, J. A., Frazier, L. M., and Dungworth, D. L.: Infectious Meningo-Encephalitis in Cattle, Caused by a Haemophilus-like Organism. Am. J. Vet. Res. 21, (May, 1960):403-409.
- ²Pancieria, R. J., Dahlgren, R. R., and Rinker, H. B.: Observations on Septicemia of Cattle Caused by a Haemophilus-like Organism. Path. Vet. 5, (1968):212-226.
- ³Brown, L. N., Dillman, R. C., and Dierks, R. E.: The Haemophilus somnus Complex. Proceedings - 74th Annual Meeting of the United States Animal Health Association. (October 20, 1970):94-108.
- ⁴Adler, H. E., Fabricant, J., Yamamoto, R., and Berg, J.: Isolation and Identification of Pleuropneumonia-like Organisms of Avian Origin. Am. J. Vet. Res. 19(1958):440-447.
- ⁵Cowan, S. T., and Steel, K. J.: Identification of Medical Bacteria. Cambridge, At the Univ. Press (1966):1-217.
- ⁶Carter, G. R.: Diagnostic Procedures in Veterinary Bacteriology and Mycology. Charles C. Thomas - Publisher (1967):3:282.
- ⁷Baile, W. E.: Characterization of Haemophilus somnus (new species), a microorganism isolated from infectious thromboembolic meningoencephalomyelitis of cattle. Ph.D. Dissertation. Kansas State University, Manhattan, Kansas (1969).

⁸Olander, H. J., Gallina, A. M., Beckwith, D., and Morrow, M.: Observations on Thromboembolic Meningoencephalitis (TEM) in Cattle in Indiana Feedlots. Proceedings - 74th Annual Meeting of the United States Animal Health Association (October 20, 1970):589-600.

⁹Corstvet, R. E. and Sadler, W. W.: A Comparative Study of Single and Multiple Respiratory Infections in the Chicken. Multiple Infections (with *Mycoplasma gallisepticum*, Newcastle Disease Virus and Infectious Bronchitis Virus). Am J. Vet. Res. 27(1966):1703-1720.

TABLE I. Rate of Isolation (percent) of Bacteria from the Trachea of Feedlot Cattle in Relation to the Clinical Status of the Animal.

Status	Periods of samplings	Total cattle sampled	Sat. <u>Haem.</u>	Non sat. <u>Haem.</u>	<u>mult. haem.</u> spp.	<u>Pasteurella</u>	<u>Coryne. pyogenes</u>	<u>Strep.</u>	<u>Mycoplasma</u>	Non pathogens	No bacteria
Respiratory: ¹											
Chronic	5	19	6.9	3.4	15.7	26.3	10.5	15.7	47.3	31.5	
Acute	20	172	6.9	3.4	8.1	36.6	11.0	2.3	63.3	38.3	4.6
Recovered ²	1	2				50.0			50.0	50.0	
Exposed ³	8	88	6.8	3.4	20.4	23.8	7.9	5.6	53.4	48.8	10.2
Non-Respiratory: ⁴											
Healthy ⁵	4	34	11.7	2.9	2.9	8.8	14.7	2.9	26.4	50.0	
Total:	8	53	3.7	3.7	11.3	33.9	5.6	3.7	28.3	43.3	
Respiratory	20	281	6.4	3.2	12.5	30.2	10.0	4.3	59.1	41.3	6.1
Healthy	9	87	6.9	3.4	8.0	24.1	9.2	3.4	27.6	46.0	
All	23	368	6.5	3.2	11.4	30.1	9.7	4.0	51.6	42.3	4.6

Sat. Haem.=satellitling (on blood agar) Haemophilus sp.; Nonsat. Haem.=nonsatellitling (on blood agar) Haemophilus sp. (H. somnus); mult.=multocida; haem.=haemolytica; spp.=species; Strep.=Streptococcus spp.; blank spaces in table indicate that organism was not present.

¹Cattle displaying signs of respiratory disease (BRD) at time of sampling.

²Cattle recovered from BRD.

³Clinically normal cattle from pens in which more than 5.0% of penmates afflicted with BRD.

⁴Cattle which had either tympanites, abscessation, or diarrheal disease prior to or at the time of sampling.

⁵Cattle which exhibited no signs of BRD.

TABLE II. Rate of Isolation (percent) of Bacteria from the Trachea of Asymptomatic (Healthy) Cattle

Grouped According to Days Resident in Feedlot.

Periods of samplings	Total cattle sampled	Sat. Haem.	Nonsat. Haem.	<u>Pasteurella mult. haem. spp.</u>	<u>Coryne. pyogenes</u>	<u>Strep. Mycoplasma</u>	Non pathogens bacteria	No bacteria		
4	35	8.6	8.6	25.7	2.8	17.1	2.8	45.7		
1	3	3-4	33.3	66.7	100.0		100.0			
3	23	7-14	4.3	13.0	26.0		39.1	56.5		
1	18	15-21	11.1	5.5	16.6	22.2	38.8	50.0		
2	5	22 or more		20.0	20.0	40.0	40.0	40.0		
TOTAL										
9	84 ¹	4.8	3.6	8.3	25.0	8.3	3.5	7.1	26.2	47.6

Where applicable the same footnotes apply as given in Table I.

¹Total different from that in Table I because no record of days in feedlot was available for three cattle in this group.

TABLE III. Rate of Isolation (percent) of Bacteria from the Trachea of Cattle with Clinical Signs of Respiratory Disease

(BRD) Grouped According to Days Resident in Feedlot

Periods of Total samplings cattle sampled	Days in feedlot	Haem.		Nonsat.		Pasteurella		Coryne.		Strep.		Mycoplasma		Non pathogens		No bacteria		
		Sat.	Haem.	Haem.	mult.	haem.	spp.	pyogenes	Coryne.	Strep.	Mycoplasma	Non pathogens	No bacteria					
1	1	0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
3	5	1-2	20.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	
3	5	3-4	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	
4	15	5-6	13.3	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	
12	66	7-14	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	
9	50	15-21	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	
7	49	22 or more	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	4.1	
TOTAL																		
20	191		6.2	3.1	8.9	35.6	10.9	3.6	13.0	61.7	37.6	4.1						

Where applicable the same footnotes apply as given in Table I.

TABLE IV. Rate of Isolation (percent) of Bacteria from the Trachea of Cattle Exposed to BRD Grouped According to Days Resident in Feedlot.

Periods of Samplings	Total cattle sampled	Days in feedlot	Sat. Haem.	Nonsat. Haem.	mult. haem. spp.	<u>Pasteurella</u> <u>Coryne.</u>	<u>Strept.</u> <u>Strep.</u>	<u>Mycoplasma</u> pathogens	Non bacteria	
1	3	3-4	33.3	33.3	33.3	33.3	66.7	66.7	33.3	
1	8	5-6	12.5	62.5			37.5	25.0	12.5	
4	29	7-14	3.4	34.4	13.7	3.4	6.8	44.8	6.8	
5	27	15-21	11.1	3.7	18.5	37.0	18.5	3.7	7.4	
3	21	22 or more		4.7	14.2	4.7	9.5	71.4	14.2	
TOTAL										
8	88		6.8	3.4	20.4	23.8	7.9	5.6	48.8	10.2

Where applicable the same footnotes apply as given in Table I.

TABLE V. Rate of Isolation (percent) of Bacteria from the Trachea of Symptomatic and Asymptomatic Feedlot Cattle in Relation to Antibiotic Treatment

Status	No. of cattle sampled	Sat. Haem.	Nonsat. Haem.	<u>Pasteurella mult. haem. spp.</u>	<u>Coryne. pyogenes</u>	<u>Strep. Mycoplasma</u>	Non pathogens	No bacteria
Respiratory signs-treated:								
w/1 48 hours ¹	75	5.5	4.1	1.3	1.3	17.3	45.3	8.0
48 hours ¹	47	6.3	4.2	8.5	8.5	6.3	46.8	4.2
not treated	69	7.2	1.4	17.3	2.8	13.0	71.0	23.1
Exposed-Treated:								
w/1 48 hours	5	60.0	20.0	60.0	80.0		80.0	
48 hours	55		3.6	27.2	21.8	3.6	7.2	45.4
not treated	28	10.7		10.7	21.4	3.5	3.5	49.0
							64.2	57.1
								14.2

Where applicable the same footnotes apply as given in Table I.

¹Indicates the period elapsed between the time of treatment and collection of the tracheal sample. The anti-bacterials used were mainly penicillin, streptomycin, terramycin, tylosin and sulfonamides singly or in combination.

TABLE VI. Isolation of Bacteria from the Trachea of Feedlot Cattle
 Sampled Four times after an Outbreak of
Haemophilus somnus Septicemia.

Calf no.	Bacterium isolated ¹						
	Sat. <u>Haem.</u>	Nonsat. <u>Haem.</u>	Pasteurella			<u>Strep.</u>	<u>Mycoplasma</u>
			<u>mult.</u>	<u>haem.</u>	spp.		
184
185						3,4	
186	1,4		2	2		1,2,3	4
187		2,4	3				
188	1					1,2,3,4	
189		2					
190	1,2					3,4	
191	1				4	1,3,4	2,3,4
192						2,4	
193 ²		1,2					2
194			3			2	
195		2,4				2,3,4	
196					1,4	1	
197		1		1	3,4		
198						1,2	
199		1				1	3,4
200	1					1,2,3	4

¹The numbers indicate the isolation of the organism at a particular sampling period, that is, Jan. 25 (1), Feb. 8 (2), Feb. 25 (3), and March 6 (4).

²Not sampled on March 6.

TABLE VII. Relationship of the Isolation (percent) of Haemophilus spp.
and the Month of the Year.

Month ¹	No. cattle sampled ²	Sat. <u>Haem.</u>	Nonsat. <u>Haem.</u>
January	53		1.8
April	21		
May	86	5.8	1.1
June	95	8.4	3.1
July	21	23.8	9.5
September	27	3.7	3.7
November	65	7.6	6.1

¹May, 1970, through June, 1971

²Cattle comprise those used in Tables I through V.