



UNIVERSITI PUTRA MALAYSIA

**A BACTERIOLOGICAL SURVEY OF THE NASOPHARYNX
AND PHARYNX OF CATTLE AND BUFFALOES SLAUGHTERED AT
SHAH ALAM ABATTOIR WITH EMPHASIS ON *PASTEURELLA
MULTOCIDA***

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ABSTRACT

A bacteriological survey of the nasopharynx and pharynx of nine buffaloes and forty cattle with emphasis on *Pasteurella multocida* was conducted.

The major organisms isolated from the buffaloes were *Staphylococcus aureus*, *Streptococcus* species and *Moraxella* species while from the cattle *Acinetobacter* species, *Staphylococcus aureus* and *Moraxella* species. The isolation of *E. coli* and *Bacillus subtilis* were considered as fecal contaminant.

Two buffaloes had *Pasteurella multocida* in their nasopharynx and pharynx while four cattle had *Pasteurella multocida* in their pharynx.

Pasteurella multocida was isolated from the retropharyngeal lymph node of six buffaloes and seven cattle by the mouse inoculation technique but none of their organism was found in the nasopharynx and pharynx by the same technique.

Results of the serological test suggested that there were no relationship between the immune level and the isolation of *Pasteurella multocida*.

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INTRODUCTION

Respiratory tract diseases continue to be a problem in the cattle industry. The main losses are due to high mortality and morbidity, decrease in live weight gain and high cost of treatment. It is becoming more difficult to obtain the exact etiological agents or correct diagnosis because some of the common diseases appear to be caused by multiple infectious agents rather than a single agent. Due to this problem, the regime of treatment and the method of control are probably supportive and practicing strict hygienic measures. For example, in the calf production, respiratory problems are prominent, especially when calves are reared in the intensive type of management and the premises are unhygienic.

Haemorrhagic Septicaemia (HS) is an important bacterial respiratory disease in cattle and buffaloes in Malaysia caused by *Pasteurella multocida*. The disease has been known to occur for more than fifty years. Mortalities recorded for the period between 1967 and 1976 indicated that buffaloes accounted for 73% of the total and cattle 27% of the total. The average loss amounted to \$200,000 per annum.

Most of the bacterial agents which cause respiratory diseases are ubiquitous in the environment, and are present as normal bacterial resident in the nasopharynx of normal animals. Thus, the objectives of this study are:-

1. To investigate the bacterial flora of the nasopharynx and pharynx of healthy cattle and buffaloes.

2. To determine the presence of *Pasteurella multocida* in the retropharyngeal lymph node of healthy cattle and buffaloes.
3. To carry out serological studies on *Pasteurella multocida* in healthy cattle and buffaloes.

LITERATURE REVIEW

The association of bacteria with bovine respiratory disease has been known for many decades (Jennings and Glover, 1952; Omar, 1966; Ide, 1970). However the true role of bacteria, whether they act as primary or secondary invader in pulmonary disease is unclear.

In Sri Lanka, De Alwis (1977) has isolated a Gram negative Diplococcus resembling *Neisseria catarrhalis*; *Staphylococcus*; *Micrococcus*; *Streptococcus* and *Corynebacterium pyogenes* from the nasopharynx of normal calves. Collier (1968) reported that common dust borne bacteria, fungi and enteric bacteria are readily isolated from the nasal mucus of healthy cattle. It is evident that these organisms are there as a result of continuous inhalation of contaminated air and dirty environment. He also reported that *Corynebacterium pyogenes* is regularly associated with caseous exudates found in the crypts of palatine tonsils of healthy cattle. In addition, beta hemolytic *Streptococcus* and *Staphylococcus* occasionally colonize the upper respiratory tract of apparently healthy cattle.

Kumar and Kupusamy (1973) serotyped the *Streptococci* from healthy cattle by the Lancefields technique, and they found most of them belong to the alpha type B and C groups. *Escherichia coli*,

Staphylococcus species, *Streptococcus* species, *Aerobacter aerogenes* and *Pasteurella* species were isolated from the nasal swab of calves before and after weaning by Hamdy and Trap (1967).

Chakrabarti et al (1978) were able to isolate *Staphylococcus*, *E.coli* and *Pseudomonas* species from the nasal swabs of healthy cattle.

Collier (1968) stated that *Pasteurella hemolytica* and *Pasteurella multocida* were commonly isolated from the upper respiratory tract and tonsils of healthy cattle. Magwood et al (1969) considered *Pasteurella hemolytica* to be part of the normal flora of the upper respiratory tract of cattle. Thomson et al (1969) recovered *Pasteurella* species from nasopharyngeal swabs from six to ten month old healthy calves maintained under conditions where shipping fever might be expected. The carrier rate for both organisms was in the region of 75%. Pass and Thomson (1971) found that *Pasteurella hemolytica* was widely distributed over 15 sites on the nasal mucosa of healthy animals examined at post mortem, whereby nasal swabs taken in vivo had been negative. They also found that *Pasteurella hemolytica* was present on the surface but never between or within epithelial cells. The types of *Pasteurella hemolytica* most commonly recovered from both nasopharyngeal swabs from healthy carriers and from pneumonic lung are biotype A. These comprised 80% of strains isolated from post mortem and 87% from nasal swabs in a survey by Wray and Thomson (1971). Hoerlein et al (1961) reported the isolation of *Pasteurella* species from 3% of 200 apparently normal calves and 59.6% of 52 calves clinically affected with shipping fever. R.A. Curtis (1970) isolated

Pasteurella hemolytica and *Pasteurella multocida* from the upper respiratory tract of clinically normal bulls with the percentage of 43.3% and 51.6% respectively.

Haemorrhagic Septicaemia is an important epizootic disease of cattle and buffaloes in South East Asia. Bain (1957) estimated that annual deaths due to HS in Asia exceeded 100,000 susceptible animals. In Malaysia, although losses are not the same pattern as in other countries, it is still an important disease (Joseph, 1979). An outbreak usually occurs following breakdown in resistance of one or more animals harbouring the etiological agent of HS, especially after the rainy seasons or before the padi plantation seasons. The important causal organism in HS is *Pasteurella multocida* Carter's type B (Robert's type 1). Singh (1948) was able to isolate *Pasteurella multocida* from 3.5% of 200 healthy buffaloes. In Sudan, Mustaffa et al (1978) was able to isolate *Pasteurella multocida* from 44.4% of the population of healthy animals in association with an outbreak of HS. Omar et al (1962) reported that healthy buffaloes have been known to harbour virulent strain of *Pasteurella multocida* in the lymph node of upper respiratory tract. Virulent strains of *Pasteurella multocida* have also been isolated from the tonsils of healthy cattle (Chandrasekaran et al 1981).

MATERIALS AND METHODS

ANIMALS

Forty cattle and nine buffaloes of both sexes, aged between five to ten years, slaughtered at Shah Alam Abattoir were involved in this study. The breed were mainly LID and *Bubalus bubalis* from Selangor.

SAMPLE COLLECTION

Nasopharynx swabs were taken from live animals with flexible wire (35 cm. long). Immediately after slaughter, exposed pharynx was swabbed using swab stick, and blood samples were also collected simultaneously. The swabs were then placed in universal bottles containing 5 ml. tryptose broth. Retropharyngeal lymph nodes were obtained and placed in plastic bags. All the samples were kept in a flask containing ice and transported to the laboratory.

CULTURE AND IDENTIFICATION

The media used for the isolation of bacteria were Tryptose blood agar and MacConkey agar. Upon arrival at the laboratory, the samples were incubated for about 2 hours at 37°C. Later the samples were shaken for about 2 minutes using a vortex mixer and then cultured on Tryptose blood agar and MacConkey agar. Giemsa and Methelene blue stainings were carried out to examine for any bipolar organisms under the compound microscope. Identification of bacterial colonies were based on Cowen and Steels Scheme of Identification, (Cowen and Steels, 1974).

MOUSE INOCULATION

Mouse inoculation was done to isolate *Pasteurella multocida* from samples which might contain small numbers of organisms, which might be missed out on culture.

Half ml. of each broth containing nasopharynx and pharynx swabs samples were injected intraperitoneally into mice using 23G needle. Retropharyngeal lymph nodes were homogenised in 5 ml. tryptose broth and 0.5 ml. of the supernatant was injected intraperitoneally into mice as done previously. Control mice injected with 0.5 ml. normal

saline were kept in separate box. Isolation of *Pasteurella multocida* was made from the heart blood of mice that died within 48 hours.

HAEMAGGLUTINATION TEST.

The test was performed as described by Carter, (1955).

RESULTS

The results of bacterial examination of the nasopharynx and pharynx from forty cattle and nine buffaloes slaughtered at Shah Alam Abattoir are summarised in Table 1 and 2. Bacterial isolates in the nasopharynx of cattle and buffaloes were similar except that *Aeromonas* species, *Enterobacter aerogenes*, *Serratia marcescens*, *Pseudomonas* species and *Pasteurella multocida* Type A and T were present in the cattle but not in the buffaloes. *Flavobacterium* species and *Proteus* species were present in the buffaloes but not in cattle.

In the buffaloes, prevalence of *Staphylococcus pyogenes* in the nasopharynx was 77.8% (seven out of nine animals) whereas in cattle the prevalence was 37.5% (fifteen out of forty animals). The prevalence of *Acinetobacter* species in buffaloes was 33.3% (three out of nine animals) and in cattle 57.5% (twenty three out of forty animals).

Some of the bacterial isolates that were present in the nasopharynx were absent in the pharynx. In cattle, *Bacillus subtilis*, *Aeromonas* species, *Enterobacter aerogenes*, *Serratia marcescens*, *Pasteurella* species and *Pasteurella hemolytica* Type A were present in the nasopharynx but were absent in the pharynx. In the pharynx, *Pseudomonas* species, *Pasteurella hemolytica* Type T and *Pasteurella*

multocida were present but absent in the nasopharynx. In buffaloes, *E. coli*, *Bacillus subtilis*, *Flavobacterium* species, *Neisseria* species and *Proteus* species were absent in the pharynx but present in the nasopharynx.

The prevalence of *Corynebacterium pyogenes* were higher in the pharynx in both cattle and buffaloes. Thirty percent of cattle (twelve out of forty animals) and 44.4% of the buffaloes (four out of nine animals) harbouring this organism.

Pasteurella multocida was found in the nasopharynx and pharynx from two of nine buffaloes. Four out of forty cattle had *Pasteurella multocida* in their pharynx. One of the cattle had *Pasteurella hemolytica* Type A in the nasopharynx and one cattle had *Pasteurella hemolytica* Type T in the pharynx. One of the *Pasteurella* species from the nasopharynx as shown in Table 3 could not be identified.

The prevalence of *Pasteurella multocida* in the nasopharynx, pharynx and retropharyngeal lymph node of cattle and buffaloes by mice inoculation technique are shown in Table 4. Sixty six point six percent (six out of nine buffaloes) and 17.5% (seven out of forty cattle), harboured *Pasteurella multocida* in their retropharyngeal lymph node, but we failed to isolate this organism from their nasopharynx and pharynx when samples were inoculated into mice.

The relationship of *Pasteurella multocida* isolated from the nasopharynx, pharynx and retropharyngeal lymph node of cattle and buffaloes with serum titre were shown in Table 5. All the serum

samples tested from the buffaloes had a negative titre to *Pasteurella multocida*. However, two buffaloes had *Pasteurella multocida* in their nasopharynx and pharynx and six buffaloes had the organism in the retropharyngeal lymph node. In cattle, four out of forty had a titre 1:40, with three of the four cattle harbouring *Pasteurella multocida* in their pharynx and none from retropharyngeal lymph node. Seven of the cattle harboured the *Pasteurella multocida* in the retropharyngeal lymph node of which one animal also had the organism in its pharynx but with absence of titre.

TABLE 1: PREVALENCE OF BACTERIAL ISOLATES, FROM NASOPHARYNX AND PHARYNX OF 9 BUFFALOES

Organisms	Nasopharynx		Pharynx	
	No. isolated	Prevalence (%)	No. isolated	Prevalence (%)
<i>Staphylococcus pyogenes</i>	7	77.8	5	55.6
<i>E. coli</i>	6	66.7	-	-
<i>Streptococcus species</i>	6	66.7	2	22.2
<i>Moraxella species</i>	5	55.6	4	44.4
<i>Bacillus subtilis</i>	3	33.3	-	-
<i>Corynebacterium pyogenes</i>	3	33.3	4	44.4
<i>Acinetobacter species</i>	3	33.3	2	22.2
<i>Staphylococcus epidermidis</i>	2	33.3	2	22.2
<i>Pasteurella multocida</i>	2	22.2	2	22.2
<i>Klebsiella pneumoniae</i>	2	22.2	3	33.3
<i>Flavobacterium species</i>	2	22.2	-	-
<i>Neisseria species</i>	1	11.1	-	-
<i>Micrococcus species</i>	-	11.1	2	22.2
<i>Proteus species</i>	1	11.1	-	-

TABLE 2: PREVALENCE OF BACTERIAL ISOLATES, FROM NASOPHARYNX AND PHARYNX OF 40 CATTLE

Organisms	Nasopharynx		Pharynx	
	No. isolated	Prevalence (%)	No. isolated	Prevalence (%)
<i>Acinetobacter</i> species	23	57.5	8	20.0
<i>E.coli</i>	19	47.5	4	10.0
<i>Staphylococcus pyogenes</i>	15	37.5	17	42.5
<i>Moraxella</i> species	13	32.5	5	12.5
<i>Staphylococcus epidermidis</i>	11	27.5	1	2.5
<i>Streptococcus</i> species	11	27.5	13	32.5
<i>Corynebacterium pyogenes</i>	9	22.5	12	30.0
<i>Klebsiella pneumoniae</i>	9	22.5	4	10.0
<i>Bacillus subtilis</i>	9	22.5		
<i>Micrococcus</i> species	7	17.5	1	2.5
<i>Neisseria</i> species	4	10.0	5	12.5
<i>Aeromonas</i> species	4	10.0		
<i>Enterobacter aerogenes</i>	3	7.5		
<i>Serratia marcescens</i>	1	2.5	-	
<i>Pasteurella</i> species	1	2.5		
<i>Pasteurella hemolytica</i> Type A	1	2.5		
<i>Pasteurella hemolytica</i> Type T	-	-	1	2.5
<i>Pasteurella multocida</i>	-		4	10.0
<i>Pseudomonas</i> species			1	2.5

TABLE 3: PREVALENCE OF PASTEURELLA SPECIES FROM THE NASOPHARYNX AND PHARYNX OF 40 CATTLE AND 9 BUFFALOES

	NASOPHARYNX		PHARYNX	
	NO. ISOLATED	PREVALENCE (%)	NO. ISOLATED	PREVALENCE (%)
BUFFALOES:				
<i>Past. multocida</i>	2	22.2	2	22.2
CATTLE				
<i>Past. multocida</i>			4	10.0
<i>Past. hemolytica</i> Type A	1	2.5	-	-
<i>Past. hemolytica</i> Type T			1	2.5
<i>Past. species</i>	1	2.5		

TABLE 4: ISOLATION OF PASTEURELLA MULTOCIDA FROM NASOPHARYNX, PHARYNX AND RETROPHARYNGEAL LYMPH NODE OF 40 CATTLE AND 9 BUFFALOES BY MOUSE INNOUCLATION TECHNIQUE

	NASOPHARYNX & PHARYNX		R/PHARYNGEAL L.N	
	NO. ISOLATED	PREVALENCE (%)	NO. ISOLATED	PREVALENCE (%)
BUFFALOES:				
<i>Past. multocida</i>	0		6	66.6
CATTLE				
<i>Past. multocida</i>	0		7	17.5

TABLE 5: SERUM TITRE IN RELATION TO ISOLATION OF PASTEURELLA
MULTOCIDA IN 40 CATTLE AND 9 BUFFALOES

	ANIMAL WITH TITRE (1:40)	PASTEURELLA MULTOCIDA FROM:-	
		N/PHARYNX & PHARYNX	R/PHY. L.N
BUFFALOES:			
<i>Past. multocida</i>		2	6
CATTLE:			
<i>Past. multocida</i>	4	4	7

DISCUSSION

Staphylococcus pyogenes was the species most frequently isolated from the buffaloes and *Acinetobacter* species from cattle. High prevalence of *Staphylococcus pyogenes* could be due to the wallowing habit of the buffaloes, where this organism which was present in the water and soil can easily enter the nasopharynx. The presence of *Acinetobacter* species in the nasopharynx and pharynx of cattle and buffaloes at present can be considered as transient flora (Edwin et al, 1974). There is a report in which similar organism has been isolated from pneumonic lung of calves (Allan, 1978). Other bacteria isolated were *Aeromonas* species, *Flavobacterium* species, *Proteus* species, *Enterobacter aerogenes*, *Serratia marcescens* and *Pseudomonas*.species.

In a study carried out by De Alwis (1977), four groups of clinically normal calves, totalling 289, were examined. The percentage of calves in the groups from which *Corynebacterium pyogenes* was isolated ranged from 25.8 to 36.0%. When buffalo calves of two

different age groups were examined, 2.0% of the calves in the pre-weaning group out of 50 animals and 45% of weaned calves out of 80 animals yielded *Corynebacterium pyogenes* from the nasopharynx. In the present study, there was a high prevalence of *Corynebacterium pyogenes* in the pharynx of cattle and buffaloes, thirty percent out of forty cattle and 44.4% out of nine buffaloes harboured this organism, compared to 33.3% out of 40 cattle and 22.5% out of 9 buffaloes harboured the organism in their nasopharynx. The pathogenesis of the variety of disease conditions associated with *Corynebacterium pyogenes*, therefore tends to further support the view that this organism, harboured in the nasopharynx of healthy animals, might be the source of infection to other sites. Other workers, Thrope et al (1944); Carter and Rowsell (1958); Magwood et al (1969); Corsvet et al (1973) and Bitsch et al (1976) considered *Corynebacterium pyogenes* as a potentially pathogenic bacteria but whether this organism is the primary pathogen or not is still unclear. Gunner (1976) also found *Corynebacterium pyogenes* from the tonsils of healthy cattle.

Escherichia coli and *Bacillus subtilis* were considered as contaminants by Hamdy and Trap (1967) and Collier et al (1962). Thus, the high incidence of *E. coli* and *Bacillus subtilis* isolated from the nasopharynx is not surprising since almost all the cattle and buffaloes had fecal material in and around their nostril.

Klebsiella pneumoniae, *Moraxella* species, *Neisseria* species, *Streptococcus* species, *Pasteurella hemolytica*, *Pasteurella multocida* and a species of *Micrococcus* were considered as commensals, which under favourable circumstances may become pathogenic (Eamens, 1979;

Soultys, 1982; Morzaria et al, 1978). In this study, these organism have also been isolated. *Pasteurella hemolytica* was not isolated from the buffaloes. There has been no record in the literature on the isolation of this organism from buffaloes. Soultys (1982); Blood et al (1983) stated that, this organism can be found from the sheep, goat and cattle. Magwood et al (1969) considered *Pasteurella hemolytica* to be part of the normal nasal flora of cattle. In the present, study, one isolate of *Pasteurella hemolytica* Type A and one isolate of Type T were recovered in their nasopharynx and pharynx from two separate animals.

We failed to isolate any *Pasteurella multocida* from the nasopharynx of cattle and buffaloes by mouse inoculation technique, although some of the mice died within 48 hours. The main reason for this might be due to overgrowth of contaminated bacteria such as *E. coli* and *Bacillus subtilis* while the sample were incubated for about 2 hours at 37°C before there were cultured on Tryptose Blood agar and MacConkey agar, shock due to rough handling or faulty injection technique.

There is a high prevalence of *Pasteurella multocida* isolated from the buffaloes. Two out of nine buffaloes (22.2%) had the organism from the nasopharynx and pharynx and six out of nine buffaloes (66.6%) from the retropharyngeal lymph nodes, which would suggest that there is a higher rate of healthy carriers among the buffalo population as compared to cattle with only 7.5% carrier rate. Singh (1948) was able to isolate *Pasteurella multocida* from 7.0% of 200 slaughtered buffaloes.

In this study, the isolation of *Pasteurella multocida* from the pharynx of four out of forty cattle (10.0%) was in agreement with the work of Wijewanta and Karunarante (1968). They found 15% of 300 cattle at slaughter carried *Pasteurella multocida* in the nasopharynx and pharynx. From the serum of 40 cattle, only four showed evidence of agglutination against *Pasteurella multocida* although *Pasteurella multocida* was also recovered from the pharynx of three of the four cattle. In contrast, no buffalo serum showed any positive titre eventhough *Pasteurella multocida* was isolated from their nasopharynx, pharynx and in their retropharyngeal lymph node. It is assumed that, the titre detected in cattle were as a result of vaccination.

The absence of titre in the buffalo population support the observation that buffaloes are more susceptible to the disease (Joseph, 1979). The susceptibility would be further enchanced by the presence of higher rate of healthy carriers. Any stress would cause the organism to multiply and causing disease in other susceptible individual around.

CONCLUSION

From this study, most of the bacterial species isolated from the nasopharynx and pharynx of cattle are quire similar to buffaloes. Not all the bacteria isolated from the nasopharynx are present in the pharynx and vice versa.

Some cattle and buffaloes do harbour *Pasteurella multocida* in their nasopharynx, pharynx and retropharyngeal lymph nodes.

There is no correlation between the isolation of the organisms with titre detected.

RECOMMENDATIONS

Since the number of animals involved in this study was rather small, further study should be conducted to get a better picture of the bacterial flora especially the potential pathogens and the epidemiology of *Pasteurella multocida* infection in the country.



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