



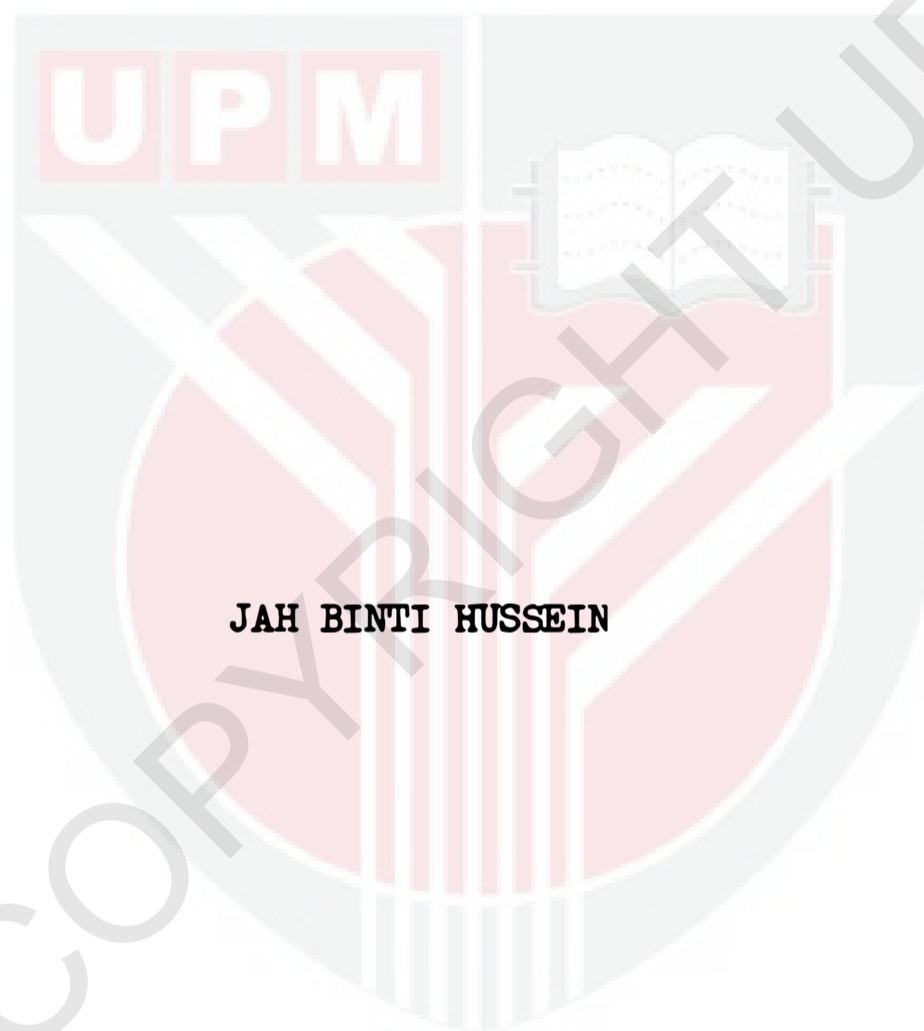
UNIVERSITI PUTRA MALAYSIA

**THE PREVALENCE OF PASTURELLA HAEMOLYTICA IN THE
TRACHEA, LIVER, HEART BLOOD AND SPLEEN OF HEALTHY AND
SICK CHICKENS**

JAH BINTI HUSSEIN

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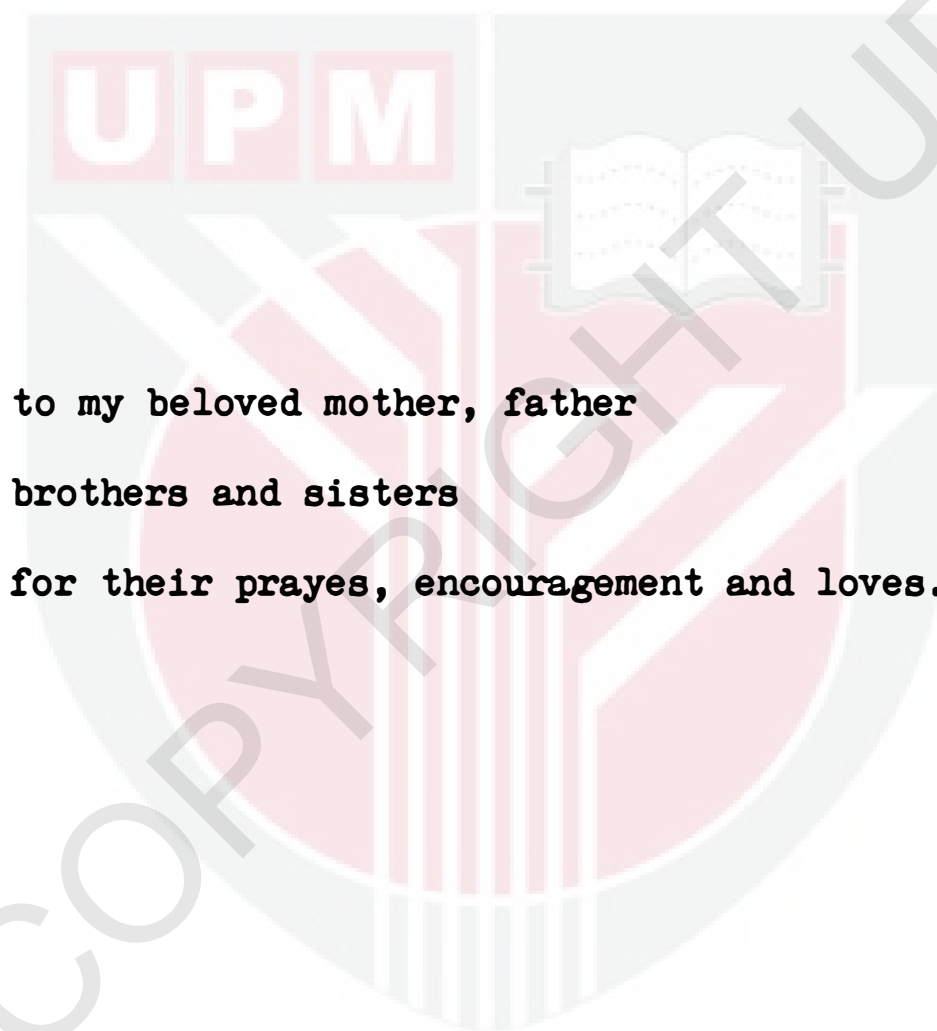
BY

JAH BINTI HUSSEIN

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to my beloved mother, father
brothers and sisters
for their prayes, encouragement and loves.

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ABSTRACT

A study of the prevalence of Pasturella haemolytica from trachea, liver, heart blood and spleen of healthy and sick chickens was carried out. A total of 100 tracheas, 64 livers, 63 hearts and 64 spleens of healthy seven-week-old chickens were obtained from Universiti Pertanian Malaysia, commercial unit. Eighty four tracheas, 83 livers, 85 hearts and 86 spleens of sick chickens were obtained from Universiti Pertanian Malaysia, commercial unit, post mortem room of Fakulti Kedokteran Veterinar and Sains Peternakan and a private poultry farm at Dengkil. Two Percent of P.haemolytica was isolated from tracheas, 4.68% from livers and 1.56% from spleens of healthy seven-week-old chickens. P.haemolytica was isolated from 3.49% of the livers, 2.35% from heart blood and 3.49% from spleens of sick chickens of various age. Nine species of bacteria were isolated from organs of healthy seven-week-old chickens and 21 species were isolated from all organs of sick chickens. Staphylococcus spp. and Streptococcus spp. were frequently isolated from all organs of healthy chickens, while E.coli was the predominant organism isolated from all organs and from all cases of sick chickens regardless of age. Salmonella spp. was also isolated from liver, heart blood and spleen of sick chickens.

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INTRODUCTION

Pasturella haemolytica is an important bacteria which caused pneumonic pasturellosis, a fatal respiratory disease which may result in high mortality in feed lot cattle (5, 10) and lead to septicaemia in sheep (10).

P.haemolytica is a common commensal of nasopharynx of cattle, sheep and goats and will cause respiratory problem under stressful condition (12). Bisgaard (1977) and Mushin et al. (1980) stated that P.haemolytica is a member of the normal flora of respiratory tract of chickens and under condition of stress, this bacterium may acquire the role of an opportunist hence will participate in pathological processes. Harry (1962) and Asnani Pathak (cited by Bisgaard, 1977) reported that P.haemolytica could be isolated from the respiratory tract of healthy adult bird. Invasion of P.haemolytica will result in a variety of pathological manifestation (7, 11, 14, 15, 22). The role of P.haemolytica as secondary invader under stress condition has been suggested by Gilchrist (1963), Hacking and Pettit (1974), Bisgaard (1977) and Mushin et al. (1980).

P.haemolytica have been isolated from poultry submitted for bacteriological examination at the Veterinary Research Institute, Ipoh (1978, 1979, 1980, 1981, 1982, 1983, 1984, 1985), Veterinary Diagnostic Laboratory, Petaling Jaya (1983, 1984) and the bacteriology laboratory Faculty of Veterinary medicine and Animal Science, Universiti Pertanian Malaysia (1983, 1984). Recently, Shah-Majid et al. (1986) isolated P.haemolytica from spleen of chickens which were on Newcastle Disease vaccination trial. The role of P.haemolytica in these spleen with

white focal lesion is not known.

This present study is a continuation of the work conducted by Shah-Majid et al.(1986) and would also compare similar work conducted by Bisgaard (1977) and Mushin et al.(1980) with the following objectives

- i) to determine the prevalence of P.haemolytica in the trachea, liver, heart blood and spleen of healthy and sick chickens.
- ii) to determine the bacterial flora found in the trachea, liver, heart blood and spleen of healthy and sick chickens.

LITERATURE REVIEW

P.haemolytica as described by Wilson et al.(1975) is a gram negative, non motile, short rod which appears as a circular, glistening and convex colonies with a zone of β -haemolysis on blood agar (Fig 1). On MacConkey agar, it appears as small pink colonies (Fig 2).

There are 12 known serotype, designated 1 -12 and two biotypes, A and T of P.haemolytica (12). It is a commensal of nasopharynx of cattle, sheep and goats (12), while Mushin et al.(1980) postulated that P.haemolytica is a member of the normal flora of the respiratory tract of chickens but not of turkeys or ducks.

Chengappa (1982) stated that P.haemolytica and Actinobacillus species are closely related and this is based on guanine-cytosine ratio in DNA of both species and suggested a new species viz

P.avihaemolytica. Using DNA:DNA hybridisation technique, Piechulla et al.(1985) found that Avian Pasturella and Actinobacillus species are closely related. Kohlert (cited by Piechulla 1985) described haemolytic strain from salpingoperitonitis in chickens and named it as P.salpingitidis. Marz and Marz et al.(cited by Piechulla 1985) suggested the transfer of P.haemolytica and P.salpingitidis to the genus Actinobacillus.

Infection due to P.haemolytica may be of an endogenous nature because of its presence as a member of the normal flora of respiratory tract of healthy chickens (17, 22). Apparently some internal or external factors may act as a stimulus for P.haemolytica to become a participant in pathological processes (22). Bisgaard (1977) speculate that chicken with infectious bronchitis or with combination of Mycoplasma gallisepticum may allow P.haemolytica to invade via respiratory tract.

P.haemolytica was not only isolated from trachea with respiratory disease (11, 22) but from a variety of pathological manifestation involving various organs. Greenham and Hill (1962) reported the isolation of P.haemolytica from chicken with enlarged liver and granular lesion on the wall of intestine, while Nicolet and Fey (cited by Hacking and Pettit, 1974) isolated P.haemolytica from young hens with salpingitis and from hens with chronic respiratory disease. Matthes et al.(cited by Hacking and Pettit, 1974) isolated P.haemolytica from 80% of the organs of dead hens with enteritis and drop in egg production, however he did not stated whether P.haemolytica is a primary or secondary pathogen. Isolation of P.haemolytica from lung and oviduct of chicken was reported by Janetschke et al.(cited by Hacking and Pettit, 1974).

Hacking and Pettit (1974) recovered P.haemolytica from liver and heart blood of chickens from eight cases involving decline or abnormality in egg production, peritonitis, salpingitis, enteritis and respiratory problem. An atypical P.haemolytica was isolated by Addo and Mohan (1985) from liver with nodular necrosis while Dhillon (1983) recorded the first isolation of P.haemolytica in parakeets with enlarged mottled liver with multifocal, pale white foci.

Actively growing P.haemolytica biotype A, serotype 1 will produce heat labile leukotoxin (26) and this toxin is cytotoxic to bovine alveolar macrophages (21), cultured blood monocyte (19) and to bovine neutrophil (6,22). Sutherland et al.(1983) stated that cytotoxin produced by P.haemolytica biotype A, serotype 1 only affect phagocytic cell of ovine and bovine origin, however all 12 recognized serotypes of P.haemolytica were toxic to bovine alveolar macrophages and all types appear to differ in their ability to liberate cytotoxin (25).

With the ability to produce and liberate toxin, P.haemolytica not only can act as secondary invader but it could be a potential primary pathogen.

MATERIALS AND METHODS

Tissues samples of healthy chickens were obtained from the Universi Pertanian Malaysia (UPM) commercial unit. A total of 100 tracheas, 64 spleens, 63 hearts and 64 livers were randomly selected from these healthy chickens. Tissues samples of sick/dead chickens were collected from a private poultry farm at Dengkil, dead chickens from UPM

commercial unit and dead chickens submitted for post mortem at the Veterinary school at UPM. A total of 84 tracheas, 83 livers, 85 hearts and 86 spleens were collected from these dead chickens. The age group of the sick/dead chickens varied from two-day-old to seven-week-old while the healthy chickens were seven-week-old.

All tissues were inoculated onto blood agar and Mac Conkey agar. The tracheas were swabbed then inoculated onto these two media. The hearts, livers and spleens were immersed briefly for three to four seconds in a beaker of boiling water in order to reduce surface contamination. The interior portion of these organs were exposed and the inner surface was swabbed and inoculated onto blood agar and Mac Conkey agar.

All plates were incubated aerobically for 24 hours at 37°C. Plates with no visible growth were reincubated for another 24 hours.

Gram stain was performed in order to differentiate the gram positive from gram negative organisms, while catalase test was used to differentiate the gram positive organism particularly Streptococcus spp. and Staphylococcus spp. Biochemical tests such as Triple Sugar Iron Agar test (TSI), Sulphide indole motility test (SIM), urease test, Simmons Citrate, Methyl Red/Voges Proskauer (MR/VP) and oxidase test were used to identify gram negative organisms.

All colonies with β -haemolytic zone resembling P.haemolytica were subcultured onto blood agar and Mac Conkey agar for further identification. Characterization of these isolates were based on gram staining of bacteria, catalase test, TSI, SIM, MR/VP, urease test,

Simmon Citrate and Oxidase test. All other bacteria with the exception of P.haemolytica were identified up to genera level. The identification of organisms based on guidelines in a laboratory diagnostic manual routinely used in the laboratory (18).

The colonies of each bacterial species on each agar plate were counted and the growth pattern were designated as follows: slight growth (+) is 1 - 10 colonies, moderate growth (++) is 11 - 100 colonies, and heavy growth (+++) is more than 100 colonies.

RESULT

The prevalence of P.haemolytica and other bacteria isolated in tracheas, livers, heart blood and spleens of healthy and sick chickens are shown in tables 1, 2, 3, 4. P.haemolytica was isolated from all organs except from trachea of sick chickens and heart blood of healthy chickens. There was no significant difference between the prevalence of P.haemolytica isolated from healthy chickens and sick chickens ($p < 0.05$)

Staphylococcus spp. and Streptococcus spp. were frequently isolated from tracheas of healthy chickens as compared to sick chickens ($p < 0.05$). The frequency of E.coli isolated in trachea is higher in the sick than healthy chickens (table 1)

The frequency of bacteria isolated from liver of healthy and sick chickens is almost identical to those isolated from tracheas except for the isolation of Salmonella spp. from two sick chickens (table 2).

The most predominant bacteria isolated from heart blood and

spleen of healthy chickens are Staphylococcus spp. and Streptococcus spp. ($p < 0.05$), while E.coli is the most predominant isolate in heart blood and spleen of sick chickens ($p < 0.05$), (table 1).

TABLE 1: THE PREVALENCE OF BACTERIA ISOLATES FROM TRACHEA OF HEALTHY AND SICK CHICKENS

ISOLATES	HEALTHY CHICKENS		SICK CHICKENS		CALCULATED χ^2
	NO. EXAMINED	100	NO. EXAMINED	84	
	NO. ISOLATE	%	NO. ISOLATE	%	
Staphylococcus spp.	95	95	14	16.68	117.3445*
Streptococcus spp.	60	60	1	1.19	72.3248*
<u>E.coli</u>	42	42	66	78.57	24.8731*
Micrococci spp.	22	22	3	3.57	13.7987*
Enterobacter spp.	12	12	1	1.19	8.8168*
Bacillus spp.	4	4	1	1.19	1.8068
Citrobacter spp.	2	2	15	17.86	13.1155*
<u>P.haemolytica</u>	2	2			3.7049*
Yeast spp.	1	1			
Klebsiella spp.			9	10.71	10.3514*
Aeromonas spp.			18	21.43	22.7669*
Salmonella spp.					
Actinobacillus spp.			2	2.38	1.7407
Yersenia spp.			1	1.19	0.8702
Edwardsiella spp.					
EF-4			1	1.19	0.8702
Proteus spp.			14	16.66	17.0799*
Aspergillus spp.			1	1.19	0.8702
Acinetobacter spp.					
Pseudomonas spp.			2	2.38	1.7404
Corynebacterium spp.			1	1.19	0.8702

$$p < 0.05, \quad d.f = 1 \quad \chi^2 = 3.841$$

* There was significant difference between the healthy and sick chickens.

TABLE 2: THE PREVALENCE OF BACTERIA ISOLATES FROM LIVER OF HEALTHY AND SICK CHICKENS

ISOLATES	HEALTHY CHICKENS		SICK CHICKENS		CALCULATED χ^2
	NO. EXAMINED 64	NO. ISOLATE %	NO. EXAMINED 83	NO. ISOLATE %	
Staphylococcus spp.	40	62.5	12	14.45	37.0188*
Streptococcus spp.	23	32.98	6	7.23	19.2390*
<u>E.coli</u>	18	28.12	72	86.47	51.5806
Micrococci spp.			2	2.41	0.9074*
Entrobacter spp.			6	7.23	3.9748
Bacillus spp.			2	2.41	0.9074*
Citrobacter spp.			7	8.43	4.8014
<u>P.haemolytica</u>	3	4.68	3	3.49	0.0905
Yeast spp.					
Klebsiella spp.			6	7.23	3.9748*
Aeromonas spp.			10	12.05	7.3750*
Salmonella spp.			2	2.41	0.9047
Actinobacillus spp.			1	1.20	0.2859
Yersenia spp.			1	1.20	0.2859
Edwardsiella spp.			1	1.20	0.2859
EF-4					
Proteus spp.			10	12.05	7.3750*
Aspergillus spp.					
Acinetobacter spp.			1	1.20	0.2859
Pseudomonas spp.			1	1.20	0.2859
Corynebacterium spp.			1	1.20	0.2859

$$p < 0.05, \text{ d.f} = 1, \chi^2 = 3.841$$

* There was significant difference between the healthy and sick chickens.

TABLE 3: THE PREVALENCE OF BACTERIA ISOLATES FROM HEART BLOOD OF HEALTHY AND SICK CHICKENS

ISOLATES	HEALTHY CHICKENS		SICK CHICKENS		CALCULATED χ^2
	NO. EXAMINED 63	NO. ISOLATE %	NO. EXAMINED 83	NO. ISOLATE %	
Staphylococcus spp.	30	47.00	17	20.48	12.9018*
Streptococcus spp.	25	39.00	5	5.88	26.1487*
<u>E.coli</u>	10	15.87	68	80.00	58.8756
Micrococci spp.					
Entrobacter spp.			7	8.23	4.5814
Bacillus spp.			3	3.53	1.5288*
Citrobacter spp.			12	14.12	8.7688
<u>P.haemolytica</u>		-	2	2.35	0.8460
Yeast spp.	-	-			
Klebsiella spp.			8	9.41	5.3905*
Aeromonas spp.		-	7	8.23	4.5814
Salmonella spp.		-	3	3.53	1.5285
Actinobacillus spp.		-	1	1.17	0.2586
Yersenia spp.					
Edwardsiella spp.			1	1.17	0.2586
EF-4			1	1.17	0.2586*
Proteus spp.		-	7	8.23	4.5814
Aspergillus spp.			1	1.17	0.2586
Acinetobacter spp.		-	2	2.35	0.8460
Pseudomonas spp.		-			
Corynebacterium spp.					

$$p < 0.05 \quad \text{d.f} \quad \chi^2 \quad 84$$

* There was significant difference between the healthy and sick chickens.

TABLE 4: THE PREVALENCE OF BACTERIA ISOLATES FROM SPLEEN OF HEALTHY AND SICK CHICKENS

ISOLATES	HEALTHY CHICKENS		SICK CHICKENS		CALCULATED χ^2
	NO. EXAMINED 64	NO. ISOLATE $\bar{\%}$	NO. EXAMINED 86	NO. ISOLATE $\bar{\%}$	
Staphylococcus spp.	26	40.62	15	17.14	10.0604*
Streptococcus spp.	7	10.90			11.0634*
<u>E.coli</u>	1	1.56	67	77.19	85.0533
Micrococci spp.			2	2.32	0.8515
Entrobacter spp.			4	4.65	2.2674
Bacillus spp.			2	2.32	0.8515*
Citrobacter spp.			9	10.46	6.2366
<u>P.haemolytica</u>	1	1.56	3	3.49	0.3441
Yeast spp.					
Klebsiella spp.			4	4.65	2.2674*
Aeromonas spp.			14	16.28	10.5711
Salmonella spp.			3	3.49	1.5372
Actinobacillus spp.					
Yersenia spp.					
Edwardsiella spp.					
EF-4					-
Proteus spp.			9	10.46	6.2366*
Aspergillus spp.					
Acinetobacter spp.			9	3.49	1.5372
Pseudomonas spp.			1	1.16	0.2613
Corynebacterium spp.					

$$p < 0.05, \text{ d.f} = 1, \chi^2 = 3.841$$

* There was significant difference between the healthy and sick chickens.

Comparison of isolates from seven-week-old chickens are shown on table 5, while tables 6,7,8 and 9 shows the number and species of bacteria isolates from tracheas, livers, heart blood and spleens of sick chickens. P.haemolytica was present in liver, heart blood and spleen of sick chickens, while in the healthy chickens it was isolated from trachea, liver and spleen. As the age advanced the number of bacteria isolated from trachea, liver, heart blood and spleen also increased. E.coli was the most predominant bacteria isolated from all organs in the sick chickens regardless of age (tables 6,7,8 and 9).

TABLE 5: NUMBER OF BACTERIAL ISOLATES AND SPECIES ISOLATED FROM TRACHEA, LIVER, HEART BLOOD AND SPLEEN FROM HEALTHY 7 WEEK-OLD CHICKENS.

ISOLATES	TRACHEA	LIVER	HEART BLOOD	SPLEEN
Staphylococcus spp.	95	40	30	26
Streptococcus spp.	60	23	25	7
<u>E.coli</u>	42	18	10	1
Micrococci spp.	22			
Entrobacter spp.	12			
Bacillus spp.	4			
Citrobacter spp.	2			
<u>P.haemolytica</u>	2	3		1
Yeast spp.	1			

TABLE 6: NUMBER OF BACTERIAL ISOLATES AND SPECIES ISOLATED FROM TRACHEA OF SICK CHICKEN RELATED TO AGE.

ISOLATES	AGE (WEEK)						
	0-1	1-2	2-3	3-4	4-5	5-6	6-7
Staphylococcus spp.			1		-	10	3
Streptococcus spp.						-	1
<u>E.coli</u>	5	4	4	7	4	24	18
Micrococci spp.			1		1	1	
Entrobacter spp.				1			
Bacillus spp.			1				
Citrobacter spp.		1			1	8	5
<u>P.haemolytica</u>	-	-					
Yeast spp.							
Klebsiella spp.				1	1	5	2
Aeromonas spp.			1		1	12	2
Salmonella spp.			-			-	
Actinobacillus spp.	-				2		
Yersenia spp.		-					1
Edwardsiella spp.		-	-			-	
EF-4		-	-	-		-	1
Proteus spp.		1	1	1	1	10	
Aspergillus spp.		-	1	-			
Pseudomonas spp.	-				1	1	-
Corynebacterium spp.		-		-		-	

TABLE 7: NUMBER OF BACTERIAL ISOLATES AND SPECIES ISOLATED FROM LIVER OF SICK CHICKENS RELATED TO AGE

ISOLATES	AGE (WEEK)						
	0-1	1-2	2-3	3-4	4-5	5-6	6-7
Staphylococcus spp.	-		2		5	5	3
Streptococcus spp.	1		1	1	2	2	
E.coli	5	3	3	7	1	33	20
Micrococci spp.			1		1		
Entrobacter spp.		-	2	3	1		
Bacillus spp.		-	1	-		1	
Citrobacter spp.				1	1	4	1
<u>P.haemolytica</u>			1	1	1		
Yeast spp.		-					
Klebsiella spp.				2	1	2	1
Aeromonas spp.			1		6		3
Salmonella spp.		-			1	1	
Actinobacillus spp					1		
Yersenia spp.	-						1
Edwardsiella spp.							1
EF-4							
Proteus spp.		1		1		8	1
Aspergillus spp.		-					
Acinetobacter spp.						1	
Pseudomonas spp.				1			
Corynebacterium spp.						1	

TABLE 8: NUMBER OF BACTERIAL ISOLATES AND SPECIES ISOLATED FROM HEART BLOOD OF SICK CHICKENS RELATED TO AGE

ISOLATES	AGE (WEEK)						
	0-1	1-2	2-3	3-4	4-5	5-6	6-7
Staphylococcus spp.	1		1	4	2	5	4
Streptococcus spp.		-	2	1		2	
E.coli	5	3	4	6	3	30	17
Micrococci spp.						-	
Entrobacter spp.				1	1	3	2
Bacillus spp.			1			2	
Citrobacter spp.				1	2	6	3
<u>P.haemolytica</u>			-	1	1		
Yeast spp.	-	-					
Klebsiella spp.			1	4		2	1
Aeromonas spp.					1	4	2
Actinobacillus spp.			1				
Yersenia spp.			-		-		
Edwardsiella spp.	-		-				
EF-4				1	-		
Aspergillus spp.			1				
Acinetobacter spp.						1	
Proteus spp.					5	2	
Corynebacterium spp.			-				
Salmonella spp.	-					3	

TABLE 9: NUMBER OF BACTERIAL ISOLATES AND SPECIES ISOLATED FROM SPLEEN OF SICK CHICKENS RELATED TO AGE

ISOLATES	AGE (WEEK)						
	0-1	1-2	2-3	3-4	4-5	5-6	6-7
Staphylococcus spp.		1	2	1	1	5	7
Streptococcus spp.							
<u>E.coli</u>	5	3	4	6	3	30	22
Micrococci spp.						2	
Entrobacter spp.			-		1	3	
Citrobacter spp.				1	2	4	2
<u>P.haemolytica</u>				1		1	1
Yeast spp.							
Klebsiella spp.				1	1	2	
Aeromonas spp.	1		1			9	3
Actinobacillus spp.							
Yersenia spp.							
Edwardsiella spp.							
EF-4							
Proteus spp.			3		4	2	
Salmonella spp.					1	2	
Aspergillus spp.							
Acinetobacter spp.						3	
Pseudomonas spp.						1	
Corynebacterium spp.							

Tables 10, 11, 12 and 13 shows the bacteria isolated from different disease manifestations which was based on gross findings. E.coli is the predominant bacteria isolated from all cases including those that do not have any significant lesion. Apart from E.coli, Salmonella spp. was isolated from cases of Newcastle Disease and Chronic Respiratory Disease Complex.

TABLE 10: BACTERIA ISOLATED FROM TRACHEA OF SICK CHICKENS
IN A VARIETY OF DISEASE MANIFESTATIONS

ISOLATES	DIAGNOSIS ON GROSS FINDINGS					NSL
	Yolk sac inf.	ND	CRD complex	coccidiosis	congested liver, pulmonary edema	
Staphylococcus spp.		4			9	1
Streptococcus spp.			1			
<u>E.coli</u>	6	20	14	4	18	4
Micrococci spp.		1			2	
Enterobacter spp.				1		
Bacillus spp.					1	
Citrobacter spp.	2	5	1		4	3
<u>P.haemolytica</u>						
Yeast spp.						
Klebsiella spp.		3		2	3	1
Aeromonas spp.	1	10	1	1	4	1
Salmonella spp.						
Actinobacillus spp.		1	1			
Yersenia spp.					1	
Edwardsiella spp.						
EF-4		1				
Proteus spp.	1	7			2	4
Aspergillus spp.					1	
Acinetobacter spp.	1					
Pseudomonas spp.		1				1
Corynebacterium spp.		1				

ND = Newcastle Disease

CRD = Chronic Respiratory Disease

NSL = No significant lesion

TABLE 11: BACTERIA ISOLATED FROM LIVER OF SICK CHICKENS IN A VARIETY OF DISEASE MANIFESTATIONS

ISOLATES	DIAGNOSIS ON GROSS FINDINGS					
	Yolk sac inf.	ND	CRD complex	coccidiosis	congested liver, pulmonary edema	NSL
Staphylococcus spp.		1	1		9	1
Streptococcus spp.	1	1	1		3	
E.coli	6	23	12	3	21	7
Entrobacter spp.					4	
Micrococci spp.		2				
Bacillus spp.					1	1
Citrobacter spp.		5		1		1
P.haemolytica			1		2	
Yeast spp.						
Klebsiella spp.		3			2	1
Aeromonas spp.		7			3	
Actinobacillus spp.		1			1	
Yersenia spp.					1	
Edwardsiella spp			1			
Salmonella spp			1		1	
EF-4						
Proteus spp.	1	4			2	3
Aspergillus spp.						
Acinetobacter spp.						1
Pseudomonas spp.	-					1
Corynebacterium spp.					1	

ND = Newcastle. Disease

CRD = Chronic Respiratory Disease

NSL = No significant lesion

TABLE 12: BACTERIA ISOLATED FROM HEART BLOOD OF SICK CHICKENS
IN A VARIETY OF DISEASE MANIFESTATIONS

	DIAGNOSIS ON GROSS FINDING					
	Yolk sac inf.	ND	CRD complex	coccidiosis	congested liver, pulmonary edema	NSL
Staphylococcus spp.	1	1		3	10	2
Streptococcus spp.		1	1		3	
<u>E.coli</u>	6	20	15	1	18	8
Micrococci spp.						
Entrobacter spp.		5			2	
Bacillus spp.					2	1
Citrobacter spp.		5	1	2	3	1
<u>P.haemolytica</u>			1		1	
Yeast spp.						
Klebsiella spp.		2		1	2	3
Aeromonas spp.		6			1	
Salmonella spp.		1	1		1	
Actinobacillus spp.		1				
Yersenia spp.						
Edwardsiella spp.		1				
EF-4					1	
Proteus spp.		4			1	2
Aspergillus spp.					1	
Acinetobacter spp.					1	1
Pseudomonas spp.						
Corynebacterium spp.						

ND = Newcastle Disease

CRD = Chronic Respiratory Disease

NSL = No significant lesion

TABLE 13: BACTERIA ISOLATED FROM SPLEEN OF SICK CHICKENS IN A VARIETY OF DISEASE MANIFESTATIONS

ISOLATES	DIAGNOSIS ON GROSS FINDINGS					
	Yolk sac inf.	ND	CRD complex	coccidiosis	congested liver, pulmonary edema	NSL
Staphylococcus spp.		2	1		9	3
Streptococcus spp.						
E.coli	6	22	12	2	17	
Micrococci spp.		2				
Enterobacter spp.		4				
Bacillus spp.					1	1
Citrobacter spp.		2	1	3	3	
<u>P.haemolytica</u>			2		1	
Yeast spp.						
Klebsiella spp.		2		1	1	
Aeromonas spp.	1	9			4	
Salmonella spp.		1	1		1	
Actinobacillus spp.						
Yersenia spp.						1
Edwardsiella spp.						
EF-4						
Proteus spp.		5			1	3
Aspergillus spp.						
Acinetobacter spp.						3
Pseudomonas spp.		1				
Corynebacterium spp.						

ND = Newcastle Disease

CRD = Chronic Respiratory Disease

NSL = No significant lesion

DISCUSSION

The incidence of P.haemolytica from trachea of apparently healthy chickens in this study is low (2%) as compared to the high isolation rate of 61% and 97% reported by Bisgaard (1977) and Mushin et al. (1980) respectively. In their study they examined more trachea from a number of flocks of chickens with different age group, while our study was limited to 100 tracheas from one flock of chickens of seven- week-old.

In contrast we did not isolate P.haemolytica from trachea of sick chickens while Bisgaard (1977) isolated 48% from chickens with infectious bronchitis virus. Perhaps, if the dead chickens in this study could have been sampled immediately, the organisms isolated may be different. Chickens less than seven-week-old harbour fewer P.haemolytica as compared to adult (Bisgaard, 1977), hence there is a possibility in this study that it was overgrown by other bacteria.

The tracheal flora of healthy chickens consist of gram positive organisms (7, 27) and we found similar result in this study. In fact, the bacterial flora of liver, heart blood and spleen of healthy seven-week-old chickens in this study were also predominantly gram positive microorganisms.

Isolation of P.haemolytica from liver and heart blood have been reported by Harboune (1962), Greenham and Hall (1962), Hacking and Pettit (1974) and Addo and Mohan (1985). In this study P.haemolytica was isolated from these two organs along with gram positive and gram negative microorganisms, including *Salmonella* spp.. In this study

we found P.haemolytica in Chronic Respiratory Disease Complex and from cases of chickens with congested liver, pulmonary oedema and congestion.

P.haemolytica in combination with Mycoplasma gallisepticum may allow invasion via respiratory tract and act as secondary invader (7). The presence of P.haemolytica in the liver and heart blood indicate that there was bacteraemia. Isolation of P.haemolytica from spleen of chickens have been reported by Shah-Majid et al. (1986). Addo and Mohan (1985) considered P.haemolytica isolated from nodular necrotic liver to be highly pathogenic, however, the role of P.haemolytica in the spleen of chickens is not known.

In this survey the number of microorganisms isolated from various organs of sick chickens were more than those isolated from healthy chickens. Since the study was limited to P.haemolytica, the presence of other organisms in these organs will not be discussed.

CONCLUSION

1. P.haemolytica was present in healthy and sick chickens.
2. The number of microorganisms isolated from organs of sick chickens were more than those isolated from healthy chickens.
3. Staphylococcus spp. and Streptococcus spp. were frequently isolated from all organs of healthy chickens.
4. E.coli is the predominant organism isolated in all organs of sick chickens.

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APPENDIX I

GROWTH PATTERN OF BACTERIAL ISOLATED FROM TRACHEA OF HEALTHY AND SICK CHICKENS

ISOLATES	HEALTHY CHICKENS*				SICK CHICKENS †			
	+	++	+++	TOTAL	+	++	+++	TOTAL
Staphylococcus spp.	26	68	1	95	10	3		13
Streptococcus spp.	41	19		60	1			1
<u>E.coli</u>	34	8		42	8	35	23	66
Micrococci spp.	22			22	2	1		3
Entrobacter spp.	10	2		12	1			1
Bacillus spp.	4			4	1			1
Citrobacter spp.	2			2	12	3		15
<u>P.haemolytica</u>	2			2				
Yeast spp.	1			1				
Klebsiella spp.					6	3		9
Aeromonas spp.					5	13		15
Salmonella spp.								
Actinobacillus spp.					2			2
Yersenia spp.					1			1
Edwardsiella spp.								
EF-4					1			1
Proteus spp.					14			14
Aspergillus spp.								
Acinetobacter spp.					1			1
Pseudomonas spp.					1			1
Corynebacterium spp.	-					1		1

* Total number of samples = 100

† Total number of samples = 84

Growth pattern

+ (1-10 colonies)

++ (11-100 colonies)

+++ (> 100 colonies)

APPENDIX II

GROWTH PATTERN OF BACTERIA ISOLATED FROM LIVER OF
HEALTHY AND SICK CHICKENS

ISOLATES	HEALTHY CHICKENS*				SICK CHICKENS‡			
	PATTERN OF ISOLATES				PATTERN OF ISOLATES			
	+	++	+++	TOTAL	+	++	+++	TOTAL
Staphylococcus spp.	20	20		40	10	2		12
Streptococcus spp.	14	9		23	3	3		6
E.coli	16	2		18	17	30	25	72
Micrococci spp.					2			2
Enterobacter spp.					6			6
Bacillus spp.					1	1		2
Citrobacter spp.					7			7
<u>P. haemolytica</u>	2	1		3	1	2		3
Yeast spp.								
Klebsiella spp.					5	1		6
Aeromonas spp.					7	3		10
Salmonella spp.					2			2
Actinobacillus spp.					1			1
Yersenia spp.					1			1
Edwardsiella spp.					1			1
EF-4								
Proteus spp.						10		10
Aspergillus spp.					1			1
Acinetobacter spp.					1			1
Pseudomonas spp.					1			1

* Total number of samples 64

‡ Total number of samples 83

Growth pattern

+ (1-10 colonies)

++ (11- 100 colonies)

+++ (> 100 colonies)

APPENDIX III

GROWTH PATTERN OF BACTERIA ISOLATED FROM HEART
BLOOD OF HEALTHY AND SICK CHICKENS

ISOLATES	HEALTHY CHICKENS*				SICK CHICKENS [†]			
	PATTERN OF ISOLATES				PATTERN OF ISOLATES			
	+	++	+++	TOTAL	+	++	+++	TOTAL
Staphylococcus spp.	25	5		30	14	2		17
Streptococcus spp.	18	7		25	4	1		5
<u>E. coli</u>	9	1		10	17	26	25	68
Micrococci spp.								
Entrobacter spp.					6	1		7
Bacillus spp.					3			3
Citrobacter spp.					10	2		12
<u>P. haemolytica</u>					1	1		2
Yeast spp.								
Klebsiella spp.					6	2		8
Aeromonas spp.					4	3		7
Salmonella spp.					1		2	3
Actinobacillus spp.					1			1
Yersenia spp.								
Edwardsiella spp.					1			1
EF-4					1			1
Proteus spp.						7		7
Asperagillus spp.					1			1
Acinetobacter spp.					2			2
Pseudomonas spp.								

* Total number of samples 63

† Total number of samples 85

Growth pattern

- + (1-10 colonies)
- ++ (11-100 colonies)
- +++ (> 100 colonies)

APPENDIX IV

GROWTH PATTERN OF BACTERIA ISOLATED FROM SPLEEN OF
HEALTHY AND SICK CHICKENS

ISOLATES	HEALTHY CHICKENS*				SICK CHICKENS [†]			
	PATTERN OF ISOLATES				PATTERN OF ISOLATES			
	+	++	+++	TOTAL	+	++	+++	TOTAL
Staphylococcus spp.	25	1		26	13		2	15
Streptococcus spp.	7			7				
E. coli	1			1	22	19	26	67
Micrococci spp.					2			2
Enterobacter spp.					3	1		4
Bacillus spp.					2			2
Citrobacter spp.					7	2		9
<u>P. haemolytica</u>	1			1	3			3
Yeast spp.								
Klebsiella spp.					3	1		4
Aeromonas spp.					13	1		14
Salmonella spp.						1	2	3
Actinobacillus spp								
Yersenia spp.								
Edwardsiella spp.								
EF-4								
Proteus spp.						9		9
Aspergillus spp.								
Acinetobacter spp.					3			3
Pseudomonas spp.					1			1

* Total number of samples 64

[†] Total number of samples 86

Growth pattern

- + (1-10 colonies)
- ++ (11-100 colonies)
- +++ (> 100 colonies)

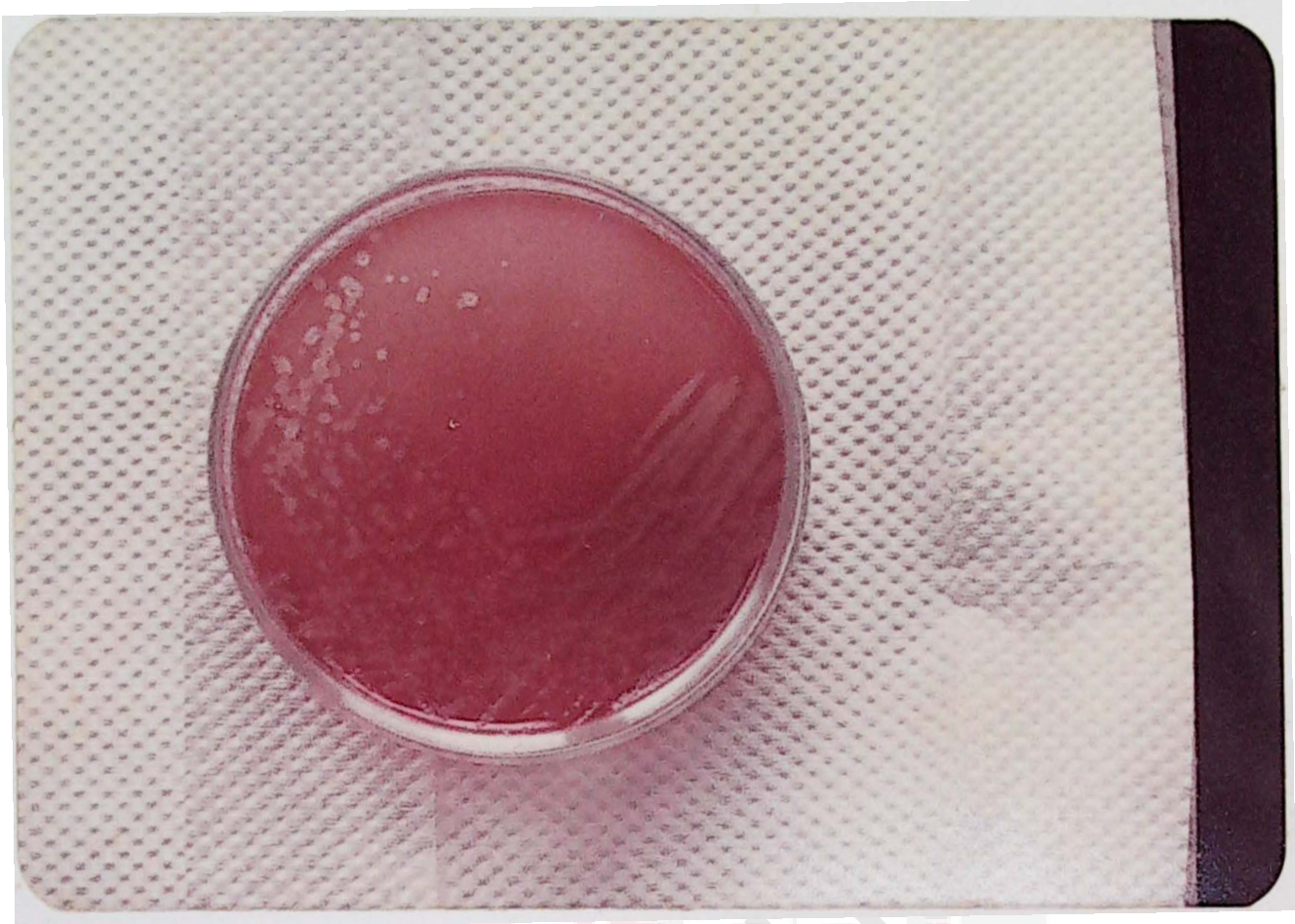


Figure 1 P. haemolytica colonies on blood agar late

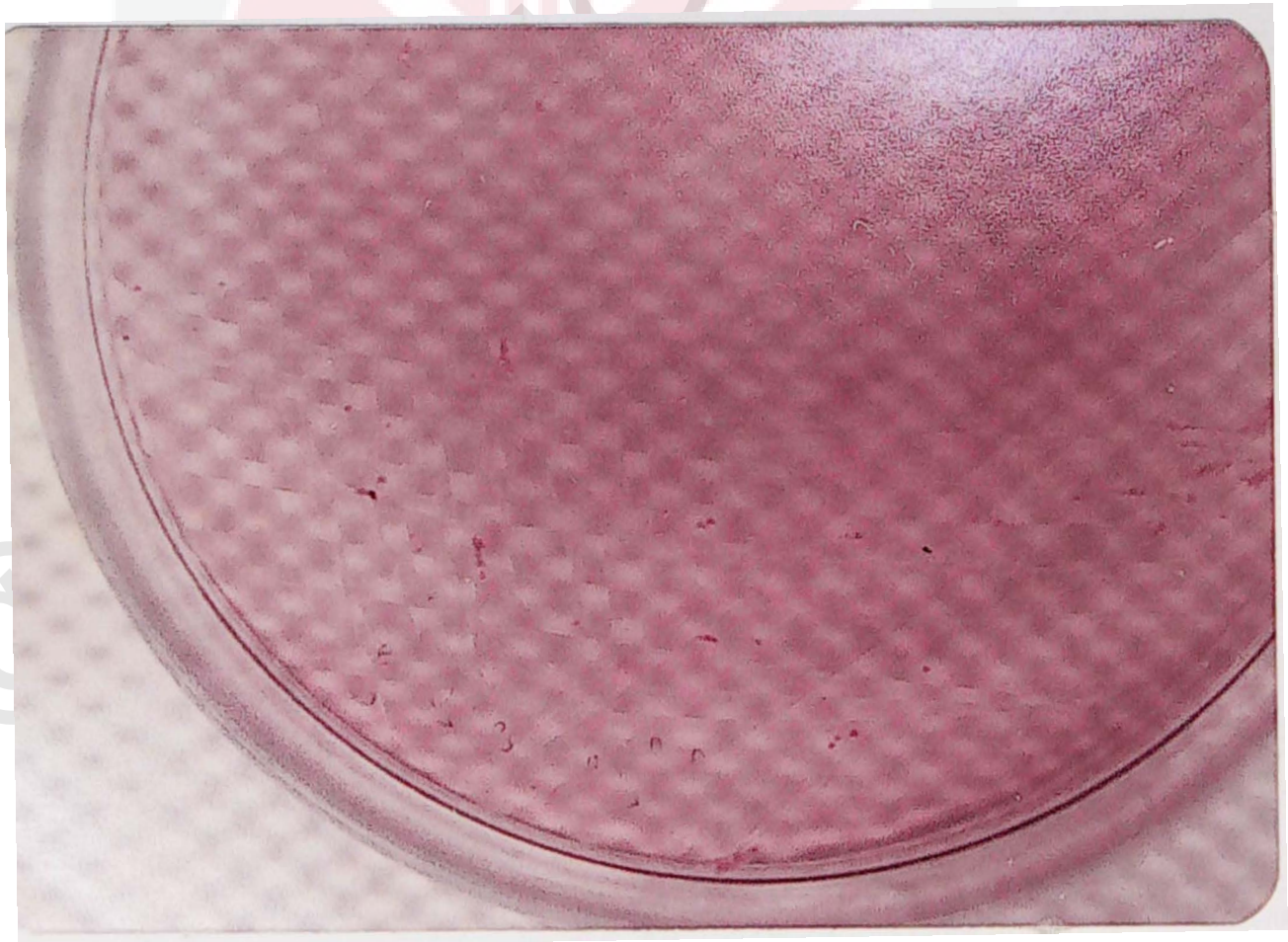


Figure 2: P. haemolytica colonies on MacConkey agar