J. Vet. Malaysia (2004) 16 (1&2): 43-46

ISOLATION OF AEROBIC BACTERIA FROM THE RESPIRATORY TRACT OF LOCAL CHICKENS IN CENTRAL SUDAN

El Jalii, I.M.[1] and ElNasri, M.[2]

[1&2]Faculty of Veterinary Medicine
P. O. Box 32, Khartoum North, Sudan

SUMMARY

A total of 35 apparently healthy chicken of local breeds of both sexes obtained from El-Talbab town in the Central State (Sudan) were examined for aerobic bacterial flora in the respiratory tract. The respiratory tract was divided into four divisions: The nasal cavity, infra-orbital sinuses, trachea and lungs. There were no lesions or exudate indicating respiratory diseases in any of the birds. All the samples from the nasal cavity and infra-orbital sinuses were culture-positive. Swabs from three tracheas (8.57%) and 15 lungs (42.86) were sterile. A total of 302 isolates were recovered from the four divisions and consisted of 271 (89.74%) Gram-positive and 31 (10.26%) Gram-negative organisms. Gram-positive organisms belonged to the genera *Staphylococcus, Micrococcus*, *Corynebacterium* and *Bacillus*. The Gram-negative isolates belonged to the genera *Pseudomonas, Escherichia* and *Yersinia*.

Keywords: aerobic bacteria, respiratory tract, local chickens, central Sudan

INTRODUCTION

The poultry industry has undergone considerable development in the Sudan in recent years. Respiratory diseases is one of the important problems that accompany this industry. Respiratory problems are of complex etiology and multiple infections are frequent. The severity of the disease and lesions are sometimes mainly due to the secondary bacterial invaders. The microbial flora of domestic chickens varied in health and disease. The number and variety of microorganisms increased during acute respiratory diseases. This increase in the bacterial flora may be due to the destruction of the ciliated epithelium of the mucous membrane and the increase in the inflammatory exudates, which could be used as nutrients by bacterial secondary invaders. Some organisms could play a prominent role in the ecosystem of the respiratory tract (Dho and Mouline, 1983).

Micrococci, streptococci, staphylococci, Corynebacterium, *Lactobacillus*, *Bacillus, Vibrio* and *E coli* were isolated from the nasal cavity, sinuses, trachea and lungs of normal chicken (Garg and Sethi, 1971; Dho and Mouline, 1983; Kawaguchi *et al.*,1992; Byrum and Slemons, 1995). Dho and Mouline (1983) found that the flora of the trachea of healthy chickens consisted mainly of Gram-positive organisms. E *coli* was the only Gram-negative organism isolated from the tracheas. On the other hand Kawaguchi *et al.* (1992) reported that micrococci and staphylococci were predominant bacteria in the nasal cavities of normal chicken with three different types of breeding.

In the Sudan, very few attempts were made to isolate bacteria from the respiratory tract of chicken. Eisa and El-Nasri (1985) investigated the aerobic bacterial flora of the respiratory tract of apparently normal exotic White Leghorn breed. The main organisms isolated from sinuses, tracheas, lungs and air-sacs were staphylococci, *E coli*, *Citrobacter*, *Mycoplasma* and *Klebsiella*. Khogali (1970) was able to recover from the respiratory tract of diseased chickens *Haemophilus gallinarum*, E *coli* and *Staphylococcus albus*. *Mycoplasma* has been isolated from respiratory tract of exotic and local chickens (Harbi *et al.*,1982a; Harbi *et al.*, 1982b; El-Amin, 1989).

MATERIALS AND METHODS

Samples

Normal chicken (with no signs of respiratory infection) of local breeds were collected from El-Talbab village in the Central State. The birds were kept in house yards without a particular shelter. Though free-range, they were given some sorghum grains. The samples consisted of 20 females and 15 males which were of different ages ranging from one to 14 months.

Respiratory divisions and sampling

The birds were held in a cage for 24 hours prior to examination and given water only. This was done to prevent contamination of the respiratory tract from soil and dry feed. The birds were then sacrificed by slaughtering and samples were taken from the nasal cavity, infra-orbital sinuses, trachea and lungs. A separate cotton wool swab from nasal cavity, sinuses and trachea and a piece of lung were streaked onto blood agar, nutrient agar, serum agar, pplo agar and MacConkey's agar plates for bacterial growth and incubated aerobically at 37°C up to 7 days. All cultures on solid media were examined with naked eye for growth and colonial morphology and any change in the media. The purified isolates were identified according to the criteria outlined by

Cowan and Steel (1985) as follows: Reaction to the Gram stain, shape of the organism, presence or absence of spores, motility, aerobic growth, the colonial characteristics on the different media and biochemical tests.

RESULTS

No bird showed any exudate or lesions in the respiratory tract. All nasal cavity and infra-orbital sinuses samples were culture-positive while 3 tracheas and 15 lungs were sterile (Table 1)

Table 1: Culture-positive and sterile respiratory divisions

Respiratory division	Number positive	Number sterile	Total
Nasal cavity	35 (100)*	-	35
Infra-orbital sinus	35 (100)	-	35
Trachea	32 (91.43)	3 (8.57)	35
Lung	20 (57.14)	15 (42.86)	35

*Figures in brackets indicate percentage

A total of 302 bacterial isolates were recovered from the four divisions. They consisted of 271 (89.74%) Gram-positive and 31 (10.26%) Gram-negative organisms. Gram-positive organisms recovered were *Staphylococcus epidermidis, Staphylococcus aureus, Micrococcus roseus, Micrococcus luteus, Micrococcus varians, Corynebacterium murium, Bacillus cereus, Bacillus megaterium, Bacillus firmus, Bacillus pumilus, Bacillus pantothenticus*, and *Bacillus brevis*. The Gram-negative isolates were *Pseudomonas diminuta, Pseudomonas putida, Pseudomonas fluorescences, Pseudomonas aeruginosa, Escherichia coil* and *Yersinia enterocolitica*.

The distribution of these organisms among the different respiratory divisions is shown in Tables 2 and 3.

One hundred and forty-six isolates (48.35%) of the total were recovered from the nasal cavities. They included 131 (89.73%) Gram-positive and 15 (10.27%) Gram-negative. Seventy-two isolates (23.84%) of the total were recovered from the infra-orbital sinuses They included 65 (90.28%) Gram-positive and 7 (9.72%) Gram-negative. Fifty-five isolates (18.21%) of the total were recovered from the tracheas. They included 48 (87.27%) Gram-positive and 7 (12.73) Gram-negative. Twenty-nine isolates (9.60%) of the total were recovered from the lungs They included 27 (93.10%) Gram-positive and 2 (6.90) Gram-negative.

DISCUSSION

The present study was carried out to isolate and identify aerobic bacteria from the respiratory tract of healthy chickens of local breeds. This work is the first detailed study on aerobic bacteria in the respiratory tract of healthy local chicken in the Sudan. Satisfactory attempts have not been previously made in the country to investigate the problem despite the fact that some of the bacteria present in the respiratory tract can play an important role in the respiratory diseases as secondary invaders.

The results showed that the recovery rate from all samples was 87.14%. Both Gram-positive and Gram-negative organisms were isolated. The Gram-positive organisms were predominant and consisted of 89.74% of the total isolates while the Gram-negative were 10.26%. This finding supports Kawaguchi *et al.*, (1992) who reported the domination of Gram-positive bacteria in the respiratory tract of normal chickens. The highest recovery rate (100%) during the present work was obtained from the nasal cavity and infra-orbital sinus while the lowest recovery rate (57.14%) was from the lung. The highest isolation rates from the nasal cavity and infra-orbital sinsus obtained during the present study are probably due to the fact that village birds were not kept in cages or in closed pens. They were kept in the open in the backyards where they scratched around a great deal in the soil and refuse heaps in search of food. Dust will rise under such circumstances and particles contaminated with soil organisms will be inhaled and lodged in the nose. This may also account for the high prevalence of *Bacillus* in the nasal cavity as members of this genus are found in the soil. The present results do not support the finding of Garg and Sethi (1971) who could not isolate any bacteria from the tracheas of ten healthy birds. Very few Gram-negative organisms were isolated during the present work and they belonged to the genera *Pseudomonas*, *Escherichia* and *Yersinia*. This confirms earlier work by Byrum and Slemons (1995). The isolation of *Yersinia* from the respiratory tract of chicken does not appear to have been reported previously. The failure of the isolation of *E. coli* from divisions other than the nasal cavity is not susprising. Mahgoub (1986) investigated the prevalence of *E. coli* in various organs of healthy and diseased white Leghorn chicken in the Sudan and isolated the organism from 16 out of 17 lungs of diseased birds but no organisms were recovered from 28 lungs from normal birds. The low recovery rate of *E coli* from the respiratory tract is one of the significant results of the present work because in chickens, *E coli* can aggravate the lesions and symptoms of respiratory disease and virulent *E coli* strains express their pathogenicity in the respiratory tract (Dho and Lafont, 1982). Failure to isolate *Mycoplasma* from the respiratory tract of local chicken is another important result of the present work. The organism was isolated by various workers from local and exotic breeds in the Sudan (Harbi *et al.*,1979; Harbi *et al.*,1982; El-Amin, 1989).

It is concluded that in the light of the results of the present investigation, that local birds kept in backyards in open systems harbored a large number of Gram-positive organisms and very few Gram-negative organisms in the respiratory tract. This is probably due to the environmental factors.

Table 2: Distribution of isolated bacteria genra among different respiratory divisions

	Genus	Nasal cavity	Infra-orbital sinus	Trachea	Lung number of organisms	Total	%
Gram-positive bacteria	*Staphylococcus*	57	32	26	13	128	47.23
	Micrococcus	9	6	3	-	18	6.64
	Bacillus	61	26	19	14	120	44.28
	Corynebacterium	4	1	-	-	5	1.85
	Total	131	65	48	27	271	89.74
Gram-negative bacteria	*Pseudomonas*	12	65	48	27	271	89.74
	Escherichia	2	-	-	-	2	06.45
	Yersinia	1	-	1	-	2	06.45
	Total	15	7	7	2	31	10.26
	Grand Total	146	72	55	29	302	

Table 3: Distribution of isolated bacteria species among different respiratory divisions

	Respiratory division organism	Nasal cavity	Infra-orbital sinues	Trachea	Lung
Gram-positive bacteria	*Staphylococcus epidermidis*	47 (35.88)	27 (41.54)	23 (47.91)	13 (48.15)
	Staphylococcus aureus	10 (7.63)	5 (7.69)	3 (6.25)	-
	Micrococcus varians	4 (3.05)	3 (4.62)	1 (2.08)	-
	Micrococcus roseus	4 (3.05)	2 (3.07)	2 (4.17)	-
	Micrococcus luteus	1 (0.77)	1 (1.54)	-	-
	Corynebacterium murium	4 (3.05)	1 (1.54)	-	-
	Bacillus cereus	24 (18.32)	9 (13.84)	8 (16.67)	7 (25.93)
	Bacillus megaterium	19 (14.50	3 (4.62)	5 (10.42)	1 (3.70)
	Bacillus pantothenticus	(9 (6.87)	7 (10.77)	5 (10.42)	3 (11.11)
	Bacillus firmus	6 (4.58)	6 (9.23)	1 (2.08)	3 (11.11)
	Bacillus brevis	1 (0.77)	1 (1.54)	-	-
	Bacillus pumilus	2 (1.53)	-	-	-
Gram-negative bacteria	*Pseudomonas diminuta*	7 (46.67)	3 (42.86)	3 (42.86)	3 (100)
	Pseudomonas putida	2 (13.33)	4 (57.14)	3 (42.86)	-
	Pseudomonas fluorescences	2 (13.33)	-	-	-
	Pseudomonas aeruginosa	1 (6.67)	-	-	-
	Escherichia coil	2 (13.33)	-	-	-
	Yersinia enterocolitica	1 (6.67)	-	1 (14.28)	-

ACKNOWLEDGEMENTS

I would like to thank the Head and the Technical Staff of the Department of Preventive Medicine and Public Health, Faculty of Veterinary Science, University of Khartoum for help and assistance during the work.

REFERENCES

Byrum, B. R. and Slemons, R. D. (1995). Detection of proteolytic bacteria in the upper respiratory tract flora of poultry. *Avian Diseases* **39**: 622-626.

Cowan, J. S. and Steel, K. J. (1985). Manual of Identification of Medical Bacteria. 2nd. ed. Cambridge University Press, London.

Dho, M. and Lafton, J. P. (1982). *Escherichia coli* colonization of the trachea in poultry: comparison of virulent and avirulent strains in gnotoxenic chickens. *Avian Diseases* **26**:787-797.

Dho, M, and Mouline, C. (1983). Qualitative and quantitative analysis of the bacterial flora of the trachea of healthy chickens. *Ann. Rech. Vet.* **14**:189-194.

Eisa, I. B. and El-Nasri, M. (1985). Unpublished data.

El-Amin, S. M. .M. (1989). Studies on avian *Mycoplasmas* in the Sudan. Ph.D thesis, University of Khartoum.

Garg, D. N. and Sethi, M. S. (1971). Aerobic bacterial flora of the avian respiratory tract. *Journal of Research* **1**: 114 -118.

Harbi, M. S., Mustafa, A. and Salih, M. (1982a). Isolation and identification of *Mycoplasmas* from indigenous chickens in the Sudan. *Sudan Journal of Veterinary Research* **1**: 51.

Harbi, M. S., Mustafa, A., Salih, M. M. and El-Amin, S. M. (1982b). Isolation and identification of *Mycoplasmas* from the respiratory tract of the White Leghorn chicken in the Sudan. *British Veterinary Journal* **138**: 253-257.

Kawaguchi, I., Hayashidani, K., Kaneko, K., Ogawa, M. and Benno, Y. (1992). Bacterial flora of the respiratory tracts with a particular reference to Lacto *Bacillus* species. *Journal of Veterinary Medicine Science* **54**: 261-167.

Khogali, A. R. (1970). Respiratory diseases of the fowl in Sudan. M.V.Sc. Thesis, University of Khartoum.

Mahgoub, M.S. (1986). *Escherichia coli* isolated from the domestic fowl in the Sudan. M.V.Sc. Thesis. University of Khartoum.