ISOLATION OF BACTERIA AND MYCOPLASMA FROM DEAD CHICK EMBRYOS

BY

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Introduction

Chick embryos are one of the means of disease transmission. Some pathogens are known to be transmitted transovarian, (Jordan, 1979) and embryonated eggs and their fibroblast are widely used as vehicles for the propagation of microorganisms and for the preparation of biological products. Failure of large number of fertile eggs to hatch causes great worry to poultry breeders. This study include two big farms, Kuku and Sudanese-Kuwaiti farms, where the average hatchability is 55% and 44% respectively. The percentage of embryos dead in shell in Kuku farm is 20-30%. To include or exclude infection in this problem is of great importance. Studies on the microorganisms of chick embryos are negligible. Most of the work done was concentrated on isolation of Mycoplasma from the respiratory tract of domestic fowl, (Harbi **et al.**, 1979; and Hussni, 1979).

The importance of embryonated eggs necessitate the study of microorganisms carried by these embryos.

Materials and methods

Fertile eggs that were incubated for 18 days were discarded due to their failure to hatch were collected from Kuku, Sudanese-Kuwaiti, Elobeid and Atbara poultry farms. The shell was removed at the airsac after the shell being cleaned with alcohol. Sterile forceps were used for this purpose. Using a sterile pasteur pippette some of the allantoic fluid was transfered to inoculate nutrient broth, selenite broth and blood agar (Oxoid). Inoculated blood agar and nutrient broth were incubated at 37°c. for a week before they were considered negative. Selenite broth cultures were subcultured onto MacConkey agar or desoxycholate citrate agar (Oxoid) after 48 hours of incubation at 43°c. Subcultures from nutrient broth to blood agar were made when the primary blood agar plates showed no growth. Isolated cultures were identified according to Cowan and Steel (1974).

Allantoic fluids were also cultured for **M.gallisepticum, M.gallinarum** and **M.iners** in mycoplasma broth and agar (Oxoid); for **M.synoviae** (C) medium was used after the addition of nicotinamide adinine dinucleotide (NAD) and cystein-Hcl; and for T-mycoplasma Gourlay's medium containing 20% horse serum and 3g/litre yeast extract was used. Incubation was carried out at 37 c for 3—5 days. Isolated cultures were tested for reversion on a medium without antibiotics.

Colonies having the typical fried egg appearance were identified by growth inhibition test (Erno and Slipkovits, 1973). The isolates were tested for senst ivity to digitonin, a property characteristic of sterol dependant mycoplasma (Freundt **et al.**, 1973). Phosphotase activity, fermentation of glucose, hydrolysis of urea and arginine and reduction of 2,3,5 triphenyl tertazolium chloride. Identification of the species was carried out by paper disc technique (Clyde, 1964).

Control:

Fertile eggs (18- days old) that were supposed to hatch were treated the same way as the tested eggs.

Results

The bacterial spp. isolated and identified are shown on tables I,II,III, and IV. From some samples more than one isolate were obtained. Isolates from the control eggs are shown on table V.

13 digitonin positive isolates were identified as **M.gallinarum,** table VI, All the isolates had the characteristic of type strain PG16 (Edward and Freundt, 1956). They failed to hydrolyse arginine, urea or ferment glucose and sorbitol , but they reduced tetrazolium. Growth was strongly inhibited by the anti-sera to type strain. A total of 5.77% of the samples tested were positive for **M.gallinarum,** but no other mycoplasma species were isolated.

Bacteria isolated from chick embryos infected with **M.gallinarum** are shown on table VII.

Discussion

Many types of microorganisims were isolated from the allantoic fluids tested. The cause of death in chick embryos that showed no bacterial or mycoplasmal growth may be attributed to other factors or the infective agent has disintegrated after the death of the embryo.

Physiological factors and enviromental conditions may cause death to chick embryos. Egg shape and air cell position during incubation of the egg influence the frequency of death of embryos; malposition of embryos results also in death, (Benoff & Renden 1980). Eggs with specific gravities lower than 1.080 have the highest embryonic mortalities and the lowest hatchability (McDaniel **et al** , 1979). Embryonic mortality may also be due to food difficiencies' Scott and Krook (1972) reported that vitamin difficiency causes poor hatchability. Some of the bacteria isolated from chick embryos may be present as a result of shell penetration. Williams and Dillard, (1968), Sauter **et al.** (1979) reported that presence of salmonella in chick embryos was due to shell penetration.

The isolation of **E.coli** from dead chick embryos was reported by Gross, 1972 who found that **E.coli** Killed 13 days old chick embryos following allantoic inoculation. He also found that yolk sac of embryos is the focus of infection and many embryos died before hatching particularly late in incubation. The eggs from which **E.coli** strains were isolated were layed by healthy hens. This agrees with Savov (1966) who found that 0.5-6 % of eggs from normal hens contained **E.coli.** Pseudomonas aeroginosa was isolated from one case in this study. The isolation of such varieties of microorganisms stimulate further studies on their pathogenicity to chick embryos.

Mycoplasma gallinarum was the only Mycoplasma species isolated in the present study. Many authors considered this microorganism as non-pathogenic for chickens (Yoder & Hofstad, 1978 & Jordan, 1979). On the other hand Salim, (1979) reported that this organism is pathogenic to chick embryos.Jordan (1979) reported, that it could be pathogenic when associated with other avian pathogens. Although **M.gallinarum** was the only species isolated but it is possible that other known pathogenic mycoplasmas such as M.gallisepticum and **M.synoviae** were present. The presence of such pathogenic organisms could be masked by the rapid growth of **M.gallinarum** or other isolated bacteria. Transovarian transmission is important in some diseases. The isolation of **M.gallinarum** from embryonatd eggs draw the attention to this transmission; however, Harbi **et al.,** (1979) reported that the respiratory tract infection of chicken by mycoplasma may not be transovarian. Prevelance of mycoplasma infection (5.77 %) reveals the role played by **M.gallinarum** in high mortality rate in chick embryos.

Contaminated incubators, poor sanitation and food defficiencies each may be a cause of dead embryos as well as an important factor in pathogen dissmination. It is clear that chick embryos harbour large number of microorganisms. Control measures help in decreasing the number of microorganisms as well as lowering the mortality rate. Studies of pathogenicity of isolates of the present study is of great importance.

Summary

Isolation of bacteria and Mycoplasma was carried out from dead chick-embryos in Kuku, Elobeid, Atbara and Sudanese-Kuwaiti farms. Different bacterial species and **M.gallinarum** were isolated from the allantoic fluid. Staphylococcus epidermidis, proteus mirabitis, Streptococcus avium or Micrococcus roseus were isolated from embryos infested with **M.gallinarum.**

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Table I

Showing bacteria isolated from Sudanese-Kuwaiti poultry farm.

No. tested	No. negative	No. positive	% + ve	Isolates
88	47	41	46.6	5 Streptococcus avium 2 Choromobacterium violaceum 3 Salmonella spp. 3 Micrococcus roseus 2 Entrobacter aerogenes 15 Staphylococcus epidermidis 4 Escherichia coli 1 Citrobacter Roseri 1 Hafnia alvei 1 Streptococcus faecalis 1 Micrococcus varius 1 Aerococcus viridans 1 Proteus mirabilis 3 Citrobacter koseri 1 Erwinia herbicola 1 Klebsiella oxytoca 1 Proteus inconstans

Table II

Showing bacteria isolated from Kuku poultry farm

No. tested	No. negative	No. positive	% +ve	Isolates
140	110	30	21.4	1 Erwinia herbicola 2 Streptococcus faecalis 1 Klebsiella aergenes 1 Klebsiella ozaenae 4 Staphylococcus epidermidis 3 Aerococcus hydrophila 2 Esc herichia coli 1 Cardiobacterium hominis 1 streptococcus salivarius 3 Chromobacterium violaceum 2 Bacillus brevis 1 Pseudomonas aerogenosa 1 Mirococcus luteus

Table III

Showing bacteria isolated from Elobeid poultry farm.

No. tested	No. negative	No. positive	% +ve	Isolates
40	12	28	70	2 Enterobacter cloacae 6 Proteus mirabilis 10 Escherichia coli 1 Proteus vulgaris 1 Enterobacter aerogenes 1 Citrobacter freundii 1 Klebsiella oxytoca 1 Salmonella spp. 2 Corynebacterium hoffmanii 1 Yersinia enterocolitica 1 Bacillus coagulans 1 Alcaligenes faecalis 1 Staphylococcus epidermidis

Table IV

Showing bacteria isolated from Atbara poultry farm

No. Tested	No. negative	No. positive	% + ve	Isolates
8	2	6	75	3. Staphylococus epidermidis 1. Proteus mirabilis 1. Micrococcus roseus 1. Micrococcus varians

Table V

Showing bacteria and Mycoplasma isolated from the control

Source	No. tested	No. Negative	No. positive	% + ve	Bacteria isolated	Mycoplasma isolated
S-Kuwaiti farm	8	5	3	37.5	1. Staphylococcus eqidermidis 2. Micrococcus luteus	none
Kuku farm	9	6	3	33.3	2. Staphylococcus epidermidis 1. Alcaligenes faecalis	none

Table : VI

Showing Mycoplasma gallinarum isolated from different farms

Farm	No. tested	No. negative	No. positive	% +ve
S-Kuwaiti	111	105	6	5.44
Kuku	66	63	3	4.5
Elobeid	40	37	3	4.5
Atbara	8	7	1	12.5
Total	225	212	13	5.77

Table VII

Showing samples from which bacteria were isolated together with **Mycoplasma gallinarum.**

Farm	Serial No. of sample	Bacteria isolated
S-Kuwaiti	146	no bacteria were isolated
	159	Staphylococcus epidermidis
	173	no bacteria were isolated
	187	no bacteria were isolated
	203	no bacteria were isolated
	214	Micrococcus roseus
Kuku	45	no bacteria were isolated
	79	no bacteria were isolated
	87	no bacteria were isolated
Elobeid	244	Proteus mirabilis
	247	Proteus mirabilis
	252	Streptococcus avium
Atbara	258	Micrococcus roseus

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