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BACTERIOLOGICAL STUDIES ON THE CAUSATIVE AGENTS OF LOW HATCHABILITY AND INFERTILITY OF QUAIL EGGS IN ASSIUT GOVERNORATE

(With 4 tables)

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دراسات بكتريولوجية عن أسباب قلة فقس وعدم خصوبة بيض السمان فى محافظة أسيوط

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لاستبيان المسببات البكتيرية لمشكلة انخفاض معدل الفقس فى مزرعة السمان بكلية الزراعة بمحافظة أسيوط تم جمع عدد ١١٥٢ بيضة على ستة دفعات وبالفحص اتضح ان منها ٢٠٦ يحتوى على أجنة ميتة (بيض كابس) ، ٨٢ كانت غير مخصبة ولقد تبين من الدراسة أن ٨٤ عترة بكتيرية قد تم عزلها منها ٥٧ عترة بكتيرية من بيض السمان الكابس والمتبقي منها وعددها ٢٧ عترة بكتيرية عزلت من البيض الغير مخصب, ولقد تم تصنيف الميكروبات المعزولة وكانت عترات الميكروب القولونى وعددها ١٣ عترة هى السائدة بنسبة ٤٨.٨٨% ، ٤.٣٧ % فى البيض الغير المخصب والكابس على التوالى (أمكن تصنيف ٩ عترات منها سيرولوجيا)، كما امكن عزل ٧ عترات من ميكروبات السالمونيلا والتى صنفت سيرولوجيا الى ٥ عترات للسالمونيلا تيفوميوريم ، ٢ عترة للسالمونيلا انترايتيدس، هذا وقد أعطت المحاولات المبذولة لعزل الميكوبلازما نتائج سلبية. وفى هذه الدراسة أيضا تم اختبار حساسية ١٣ معزولة من الميكروب القولونى و٧ معزولات من ميكروب السالمونيلا ضد تسعة أنواع من المضادات الميكروبية ولقد وجد مدى الاستجابة الشديدة لكل من الفلومكوين والدانوفلوكساسين فى السالمونيلا تيفوميوريم ، السالمونيلا انترايتيدس والميكروب القولونى الممرض بينما عترات الميكروب القولونى التى لم يتم تصنيفها اتضح أنها حساسة للجنتاميسين والدانوفلوكساسين والانروفلوكساسين بفاعلية ١٠٠% ، ٧٥% ، ٧٥% على الترتيب. توصى الدراسة بالعمل على تنظيف البيض المعد للتفريخ بالغسل ثم بالتطهير وكذلك غرف التفريخ لما لهذه العملية من أهمية في تقليل نسبة عدم الفقس لاجنة السمان.

SUMMARY

A total of 1152 clean ans spoilage quail eggs were collected on six batches from different hatcheries of quail farm belonging to Faculty of agriculture located in Assiut Governorate. 206 of examined eggs were dead in-shell embryos and 82 were infertile eggs. The samples were examined bacteriologically for detection the actual bacterial causes of decreased hatchability problem in this farm. The obtained results pointed out that a total of 84 strains were isolated. 57 out of 84 strains were isolated from dead in-shell embryos, while the remaining bacterial isolates (27) from infertile quail eggs. All the bacterial isolates were identified morphologically, culturally, biochemically and serologically for *E. coli* and *Salmonella* microorganisms. *E. coli* isolates (13) were the most prevalent organism isolated from infertile eggs and dead in-shell embryos with an incidence of 4.88% and 4.37% respectively (only 9 isolates could be typed serologically). 7 isolates of Salmonella were recovered in this study and identified serologically into *Salmonella typhimurium* (5 strains) and *Salmonella enteritidis* (2 strains). Trials for mycoplasma isolation gave negative results. In this study, 13 *E. coli* and 7 *Salmonella* organisms were tested for their sensitivity to 9 antimicrobial agents and the results revealed that *S.typhimurium, S.enteritidis* and Enteropathogenic *E. coli* proved to be highly sensitive to Flumequine and Danofloxacin. While untypable *E. coli* strains, were highly sensitive to Gentamycin, Danofloxacin and Enrofloxacin with an activity of 100%, 75% and 75% respectively. Cleaning and disinfection of eggs for hatching as well as the hatcheries are recommended to decrease hatching losses.

Key Words: Bacteriological studies-Quail

INTRODUCTION

Hatchability is the most essential measure for the reproductive efficiency of domestic birds, depending on several factors either environmental, management or due to infectious agents. Bacterial contamination appeared to be one of the infectious agents having tremendous effect on the survival of embryos and final hachability rate. (Sokkar et al., 1985 and Ibrahim et al., 1998).

Microorganisms may enter eggs by two routes. The first route involves invasion from the exterior via the shell (Varnam and Evans, 1991), while the second route is by trans-ovarian infection during the development of the egg (Mayes and Takeballi, 1983).

Antibiotics are used therapeutically and for prophylaxis in intensive domestic birds farming. However, strains of bacteria resistant to antibiotics emerge even under controled use of antibiotics (Claud et al., 1985 and Helm et al., 1999).

Drastic drop in hatchability rate 25% was recorded among the breeding quail flocks at Faculty of Agriculture quail farm. The effective antimicrobial agents required for egg or breeding quail treatment shoud be selected and tested carefully to ensure better results and to avoid the use of ineffective antimicrobials and therefore, this work was planned to determine the most common bacterial agents which may be responsible for low early and late hatchability beside infertility in quail farm at Assiut Governorate in addition to, test the available antimicrobial agents against *Salmonella* and *E. coli* strains isolated in this work

MATERIAL and METHODS

A) Birds and collection of samples:

A breeder quail farm belonging to Faculty of Agriculture, Assiut University, Assiut Governorate suffered from reduced hatchability (25%). A total of 1152 clean and spoilage eggs were collected on six batches from different hatcheries from the quail farm of the Faculty of Agriculture located in Assiut Governorate were used for this investigation. 288 quail eggs did not hatch. The outside of the egg shells were cleaned and disinfected with tincture iodine 2% for five minutes to get rid of surface contamination and then all eggs were opened. 206 of examined eggs were dead in-shell embryos and 82 were infertile eggs (Table,1). Bacterial isolation was done from liver, yolk sac and lung in case of dead in-shell embryos. On the other hand, from egg yolk in case of infertile eggs.

Table, 1: Percentage of infertile eggs and dead in-shell embryos among examined quail eggs collected on six batches

No. of Batches	No. of eggs Collected	No. of eggs which did not hatch				Total	
		Infertile egg		Dead in shell embryos			
		No.	%	No.	%	No.	%
1	208	13	6.25	61	29.33	74	35.58
2	192	11	5.73	35	18.23	46	23.96
3	142	16	11.27	28	19.72	44	30.99
4	286	22	7.69	26	9.09	48	16.78
5	192	8	4.17	15	7.81	23	11.98
6	132	12	9.09	41	31.66	53	40.15
Total	1152	82	7.12	206	17.88	288	25.00

B) Isolation of bacterial agents:

i) Isolation of Mycoplasma.

Cotton swabs were used to swab the samples of both infertile eggs and dead in-shell embryos. The swabs were aseptically inoculated into tubes containing 5 ml of Mycoplasma broth " brain heart infusion broth, 20% fresh horse serum, 5% yeast extract, 2% Thallium acetate and 1000 IU/ml Penicillin G sodium", at 37°C for 3 days, then subcultured on Mycoplasma agar plates "Mycoplasma broth + 20 g./Lit. agar", in moist candel jar under low oxygen tension at 37°C. After 3 days incubation the plates were examined microscopically for the appearance of characteristic fried egg colonies (Sabry, 1968).

ii) Isolation of microorganisms other than Mycoplasma.

Culturing was done on Selenite "F" broth. Selenite cultures from which samples were incubated at 37°C for 18 hours. Approximatelly three loopfuls of broth were then streaked on three specific selected solid media (MacConkey, brilliant green and S&S agar plates) and incubated over night at 37°C. In the same time, Culturing was also done on tryptose broth which incubated at 37°C for 24 hours. loopfuls from tryptose broth were cultivated on plates of: Nutrient agar; Crystal violet blood agar; 10% blood agar; Enterococcus selective differential agar; Mannitol salt agar and pseudomonas selective agar (Cetrimide agar) media. The inoculated plates were incubated at 37°C for 24 hours, while pseudomonas selective agar plates were incubated at 25°C for 24 hours. The isolates were grouped according to their cellular morphology and motility using Gram staining reaction and semisolid agar stabbing respectively. Different colonies were picked up into nutrient agar slants

for further purification and identification according to the methods of Koneman et al., (1994) and Quinn et al., (1994).

iii) Serotyping of the isolates.

Isolates that produced biochemical reaction simulating *Salmonella* were subjected to serological identification as described by Edward and Ewing (1972) and the instruction of the manufacturer laboratory (Anon, 1975). The final decission of typing was made according to the scheme of Kauffmann (1972). While serological identification of the isolates that produced biochemical reaction simulating *E. coli* was carried out after their purification by determination of the group antigens using slide agglutination test, against the *E. coli* antisera obtained commercially from AG, Marburg, Germany and following the instruction of the manufactures. On the other hand, other organisms either Gram -ve or Gram +ve were identified only by biochemical tests as their respective immune sera were not available.

C) Antimicrobial susceptibility testing:

All *Salmonella* and *E. coli* isolates obtained in this study were tested for antimicrobial susceptibility by disc diffusion method as described by Lin et al., (1993), using nine antimicrobial agents.

RESULTS

The results are tabulated in Tables 2,3 and 4

DISCUSSION

The hatchability rate depends on many factors; bacterial contamination is considered to be the one having termendous effect on the survival of embryos and final hatchability rate. The lack or even complete absence of hygienic measures, unsanitary conditions of egg collection, unsuitable storage and hatching process are the main causes of the bacterial infections. Such infections lead to early embryonic deaths, lowering hatchability and infertility of quail eggs. The present work was planned to throw some lights on the possible bacterial agents which may be incriminated in lowering hatchability beside infertility in breeder quail farm. The present study revealed the isolation of *E. coli* and *Citrobacter* spp. (4.88%) for each; *Pseudomonas*, *Streptococci*, *Staphylococci* and *Enterobacter* species (3.66%) for each; *Salmonella*, *Klebsiella* and *Shigella* species (2.44%) for each and *Proteus* spp.

(1.22%) from infertile eggs. While the bacteriological examination of dead in-shell embryos revealed the detection of the following organisms: *E. coli* spp. (4.37%), *Citrobacter* and *Staphylococci* species (3.40%) for each; *Pseudomonas* and *Proteus* species (2.91%) for each; *Salmonella, Streptococci* and *Enterobacter* spp. (2.43%) for each; *Klebsiella* spp. (1.94%) and *Shigella* spp. (1.46%) (Table,2). In our present study we could not isolate any of *Mycoplasma* spp. either from infertile eggs or dead in-shell embryos, while on opposite side, Hamouda (1996) could recover *Mycoplasma* species with an incidence percentages of 6% and 10% from infertile quail eggs and dead-in shell quail embryos respectively and it could be identified serologically into *Mycoplasma gallisepticum*.

As far as we know, little available literatures dealing with the bacterial agents incriminated in reduced hatchability of quail embryos, and therefore, it was hard to discuss the aforementioned results but generally several authors in Egypt and allover the world reported on the microbial agents isolation from embryonic mortalities of other species of birds. In Egypt, Azzam (1998) concluded that aerobic bacterial agents associated with drop infertility and hatchability in poultry are *E. coli, Pseudomonas, Klebsiella, Proteus, Staphylopcocci* spp. and low incidence of *Salmonella*. Furthermore, Venkanagauda et al., (1996) in India could recovered many isolates from early died chicks, those genera were *E. coli* (56.8%), proteus (7.5%), *Salmonella* (6.8%), *Pseudomonas* (6%), *Klebsiella* (3.7%), *Enterobacter* and *Citrobacter* in addition to *Staphylococci* spp. (5.3%). These findings agree to great extent with our findings with some incidence variations.

Comparing the results presented in this work with the information derived from the previous findings of Venkanagauda et al., (1996) and Azzam (1998), one can easily conclude that although only quail eggs are discussed here, it is assumed that the microbiology of the eggs of other domestic poultry will be similar.

The results given in table (2) reveal that *E. coli* was the most prevalent bacteria, the incidence of *E. coli* was (4.88%) and (4.37%) of the examined infertile eggs and dead in-shell embryos. 13 isolates of *E. coli* were recovered in this study, the majority of E. coli strains (9/13) [69.23%] serologically examined in this study only belonged to five serotypes namely, O_{126}:K_{71} "B16"; O_{125}:K_{70} "B15"; O_{124}: B17; O_{114}:K_{90} and O_{86}:K_{61} "B7". However 4/13 (30.77%) of the tested strains remained untypable with available antisera (Table, 3). Reddy and Koteeswaran

(1994) found that 5 serotypes of *E. coli* (O_{19}; O_{60}; O_{68}; O_{101} and O_{109}) were pathogenic for 7 days quail chicks and the infection was acute with rapid death and few lesions and they suggested that quails could play an important role in the spread of *E. coli* on fowls where they are in contact. contaminations, as it is a normal inhabitant of the intestinal tract. This organism can grow and penetrate the shell contaminating the contents (Mayes and Takeballi, 1983). Under certain conditions *E. coli* may migrate upwards from the cloaca or be carried up when the oviduct undergoes violent antiperistatic contraction (Rommanoff and Rommanoff, 1949). Moreover, Mubarak et al. (1998) isolated *E. coli* from the ovaries and oviducts of laying hens at the incidence rate of 2% and 3% respectively. Therefore, we suggest that *E. coli* organisms are egg-transmitted organisms which can invade the yolk in the ovary. This is may explain that vertical transmission of *E. coli* is through genital tract of breeding quails.

Regarding the isolation of *Salmonella*, it was isolated from infertile eggs and dead in-shell quail embryos at the incidence rate of 2.44% and 2.43% respectively (Table, 2). These organisms were identified serologically as *S.typhimurium* which isolated two times (7.41%) and three times (5.26%) from examined infertile eggs and dead in-shell quail embryos and *S. enteritidis* recovered two times (0.97%) from dead in-shell embryos (Table, 3). These findings substantiate what has been reported by Yang (1992) who isolated *S. enteritidis* from the albumen of 7, yolk of 15, shell of 13 and shell membrane of 15 of 164 quails eggs and concluded that *S.enteritidis* phage type 4 is invasive for Japanese quails and the infected eggs laid by *S.enteritidis* infected quails are probably the result of transovarian infection. There is, however, abundant evidence that *Salmonella* spp. pass from the alimentary canal via the blood to the ovaries (Gordan and Tucker, 1965).

The results given in table (2) show that one out 82 and 6 out 206 of infertile eggs and dead in-shell quail embryos cotained *Proteus* spp. with an incidence of 1.22% and 2.91% respectively. The high mortality rate in quail embryos might be attributed to septicemic shock due to the toxic effect associated with the lipopolysaccharide fraction of the *Proteus* organisms (Wilson and Miles, 1975). Furthermore, Sah et al., (1982) found that a 24-hour broth of *Proteus mirabilis* killed albino mice and quail chicks within 48 hours of intraperitoneal inoculation.

Currently, *Klebsiella* spp. were isolated from infertile eggs and dead in-shell quail embryos with an incidence of 2.44% and 1.94% respectively (Table,2). Adverse effects of *Klebsiella* organisms on the reproductive tract of laying chickens were reported by some investigators (El-Atreby, 1982 and Mahalingam et al., 1988). *Citrobacter freundii* and *Shigella* spp. were recovered from examined samples "infertile eggs and dead in-shell quail embryos" at varying percentages (Table 2&3). These findings agree to a certain extent with those reported by several authors (Sadek et al., 1991; Ibraheem and Ahlum, 1997 and Ibrahim et al., 1998). It has been definetely established that such contaminants enter the eggs after they have been laid and that pentration rate is accelerated when the shells are stained with contaminated material while the eggs are still warm (Jull, 1984).

Pseudomonas spp.; *Streptococci* spp. and *Staphylococci* species were recovered from infertile eggs and dead in shell embryos at varying percentages ranging from 2.91% to 3.66% (Table,2). These findings agree to a certain extent, with those reported by several authors (sadek et al., 1991; Ibraheem and Ahlum, 1997; Azzam 1998 and Ibrahim et al., 1998), they reported on isolation of the same species of microorganisms from infertile eggs and dead embryos of turkey, chicken and duck.

The aforementioned results either of the current study or those demonstrated in the available literatures declare that the microbial etiology incriminated in reduced hatchability in quails may be closely resembling those of other domestic birds.

The antibiogram study conductd on *S.typhimurium* (Table, 4) showed that Flumequine was the most effective antibiotic against *S.typhimurium* isolated from infertile eggs and dead in-shell quail embryos at a rate of 100% while these isolates afforded a significantly high degree of sensitivity against Danofloxacin, Enrofoxacin and Neomycin at a rate of 80%, 60% and 60% respectively. These findings agree to a certain extent with those reported by Stefanov et al., (1986); Shahata et al., (1990) and Ibraheem and Ahlum (1997). On the other hand a high degree of resistant (80-100%) *S.typhimurium* isolates were detected against four out of 9 tested antibiotics (Table,4). This finding agrees to a certain extent with that reported by Threlfall et al., (1994); Threlfall et al., (1996) and Helm et al., (1999) who indicated that *S.typhimurium* strains were highly resistant to many antibiotics such as ampicillin, chloramphenicol, streptomycin, sulfonamides, tetracycline, erythromycin and clindamycin. Furthermore, Helm et al., (1999) isolated

multiple drug-resistant *S.typhimurium* in three separate hunting preserve bobwhite quail outbreak.

Regarding to antimicrobial susceptibility of *S.enteritidis* our results revealed that Flumequine and Danofloxacin were the most effective ones followed by Neomycin, Gentamycin and Enrofloxacin. Finally Trimthoprim "Sulfamthoxazol", Colistin sulphate, Ampicillin and Tetracycline did not affect any of the isolated strains indicating that the organism acquired resistance possibly due to the treatment given. Nearly similar results were reported by Cicek and Kovarik (1994) who found that *S.enteritidis* strains were highly resistant to Tetracycline, Ampicillin and Colistin sulphate. Moreover, different *Salmonella* serovars resistance to Neomycin, Ampicillin, Sulfamethoxazol and Tetracyclines were reported by Pope et al., (1995).

The results of *in-vitro* susceptibility of the enteropathogenic *E. coli* strains to antibacterial agents showed resistance to Colistin sulphate in 88.89%, to Tetracycline in 77.78%, to Neomycin in 66.67%, to Flumequine in 55.56% and to Enrofloxacin in 33.33% of the nine strains examined. While all and 77.78% of strains proved to be sensitive to Danofloxacin, Gentamycin and Trimethoprim "sulfamthoxazol" respectively. These findings agree to a certain extent with those reported by Ibraheem and Ahlum (1997) who found that all *E. coli* strains isolated from dead in-shell chicken embryos and baby chicks were varied from highly to moderate sensitive to Danofloxacin, Gentamycin, Enrofloxacin and Nalidixic acid. On the other hand, it disagreed with that reported by Masalmeh et al., (1994) who found that 90 *E. coli* isolates showed resistance to Ampicillin in 46%, to Tetracycline in 10%, to Sulfonamides in 5% and to Kanamycin in 4%. While Sayed et al., (1998) on studying the antimicrobial sensitivity of *E.coli* isolated from duck unhatched eggs, who found several antimicrobial resistance; Colistin resistance (18.2%), Nalidixic acid resistance (54.5%) and 100% resistance for both Amoxycilline and Sulfademidine.

Concerning untypable *E. coli* strains, our results showed that 50% or more of tested strains were sensitive to Danofloxacin, Enrofloxacin, Gentamycin, Flumequine and Trimthoprim "Sulfamthoxazol". To some extent our findings were in agreement with the results obtained by Raemdonck et al., (1992) who found that Danofloxacin, Lincomycin prevent the growth of 90% of the *E. coli* isolates.

From the results achieved, one can easily conclude that mutiple-drug resistant *S.typhimurium*, *S.enteritidis* and *E. coli* strains were recovered from unhatched quail embryos and infertile eggs. This is may be due to the rampant use of antibiotics in treatment and as feed additives leading to appearance of resistant bacteria to those drugs at different times and at different places (Moharona et al., 1993). Moreover, the continous drug application well increase the minimum inhibitory concentration (MIC) range of the same drug category (Kobe et al., 1995) and consequently, appearance of resistant bacteria to those drugs and related antimicrobials (Sayed et al., 1998). Such resistant mutants are of very important value in explanation of drug treatment failure. Finally we concluded that the *in-vitro* sensitivity of isolated strains of *Salmonella* and *E. coli* are of highly significance to choice the effective drugs for controling of such economic problem. Furthermore in this work, variation in resistance pattern among *Salmonella* and *E. coli* isolates of the same serovars may referred to obtaining the quail's eggs at different six batches.

In conclusion, the information given by the achieved results revealed that several microoganisms were incriminated in reduced hatchability of quail embryos and therefore, periodical egg collection, pre-incubation fumigation, early and late fumigation of incubated eggs in addition to cleaning and disinfection of incubators, hatcharies and equipments may be of value in solving such problem. Furthermore, this risk can be avoided by the best sanitary measures and planned programms for eradication of diseases from parent flocks. Realizing that the contents of newly laid eggs from a healthy quail, are usually sterile, and that the rate of contamination of produced eggs depend mainly on the hygienic measures adopted in the farm or during handling, thus proper farm hygiene, handling and storage are necessary for obtaining eggs of good quality and fit for hatching purposes.

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Table 2: Incidence of bacterial isolates recovered from both infertile eggs and dead-in-shell quail embryos.

Type of microorgainsms	Infertile eggs		Dead in shell embryos		Total	
	Rate	%	Rate	%	Rate	%
Salmonella spp.	2/82	2.44	5/206	2.43	7/288	2.43
E. coli spp.	4/82	4.89	9/206	4.37	13/288	4.51
Pseudomonas spp.	3/82	3.66	6/206	2.91	9/288	3.13
Proteus spp.	1/82	1.22	6/206	2.91	7/288	2.43
Klebsiella spp.	2/82	2.44	4/206	1.94	6/288	2.08
Citrobacter spp.	4/82	4.89	7/206	3.40	11/288	3.82
Streptococci spp.	3/82	3.66	5/206	2.43	8/288	2.78
Staphylococci spp.	3/82	3.66	7/206	3.40	10/288	3.47
Shigella spp.	2/82	2.44	3/206	1.46	5/288	1.74
Enterobacter spp.	3/82	3.66	5/206	2.43	8/288	2.78
Mycoplasma spp.	-	0.00	-	0.00	-	0.00
Total	27/82	32.93	57/206	27.67	84/288	29.17

Table 3: Bacterial identification, incidence and frequency of the bacterial agents isolated from the infertile eggs and dead-in-shell quail embryos.

Isolates	Infertile eggs			Dead-in-shell quail embryos		
	Incidence		Frequency*	Incidence		Frequency
	No. (82)	%	%	No. (206)	%	%
Salmonella typhimurium	2	2.44	7.41	3	1.46	5.26
Salmonella enteritidis	-	0.00	0.00	2	0.97	3.51
E. coli (O_{126}:K_{71} "B16")	1	1.22	3.70	2	0.97	3.51
E. coli(O_{125}:K_{70} "B15")	-	0.00	0.00	2	0.97	3.51
E. coli(O_{124}:B17)	-	0.00	0.00	2	0.97	3.51
E. coli(O_{114}:K90)	-	0.00	0.00	1	0.49	1.75
E. coli (O_{86}:K 61 "B7")	-	0.00	0.00	1	0.49	1.75
Unidentified *E. coli*	3	3.66	11.11	1	0.49	1.75
Pseudomonas aeruginosa	1	1.22	3.70	4	1.94	7.02
Pseudomonas fluoresence	2	2.44	7.41	2	0.97	3.51
Proteus vulgaris	-	0.00	0.00	4	1.94	7.02
Proteus mirabilis	1	1.22	3.70	1	0.49	1.75

Table 3: Continued

Isolates	Infertile eggs			Dead-in-shell quail embryos		
	Incidence		Frequency*	Incidence		Frequency
	No. (82)	%	%	No. (206)	%	%
Proteus rettegeri	-	0.00	0.00	1	0.49	1.75
Klebsiella pneumoniae	1	1.22	3.70	3	1.46	5.26
Klebsiella oxytoca	1	1.22	3.70	1	0.49	1.75
Citrobacter freundii	4	4.88	14.81	7	3.40	12.28
Streptococcus faecalis	3	3.66	11.11	5	2.43	8.77
Staphylococcus aureus	2	2.44	7.41	5	2.43	8.77
Staphylococcus epidermidis	1	1.22	3.70	2	0.97	3.51
Shigella species	2	2.44	7.41	3	1.46	5.26
Enterobacter cloacae	3	3.66	11.11	5	2.43	8.77
Mycoplasma species	-	0.00	0.00	-	0.00	0.00
Total isolates	27	32.93		57	27.67	

* Frequency = positive isolates/total isolates

Table 4: Antimicrobial *in-vitro* sensitivity testing of *Salmonella* and *E. coli* strains recovered from infertile eggs and dead in-shell quail embryos

Content/disc	*Salmonella typhimurium*				*Salmonella enteritidis*				Enteropathogenic *E. coli*				untypable *E. coli*			
	No. of strains (5)				No. of strains (2)				No. of strains (9)				No. of strains (4)			
	S	%	R	%	S	%	R	%	S	%	R	%	S	%	R	%
Neomycin (30 µg)	3	60	2	40	1	50	1	50	3	33.33	6	66.67	1	25	3	75
Trimetoprim"Sulfamthoxazol" (1.25 µg + 23.75µg)	-	0.00	5	100	-	0.00	2	100	7	77.78	2	22.22	2	50	2	50
Ampicillin (10µg)	-	0.00	5	100	-	0.00	2	100	4	44.44	5	55.56	1	25	3	75
Colistin sulphate (10µg)	1	20	4	80	-	0.00	2	100	1	11.11	8	88.89	-	0.00	4	100
Flumequine (30µg)	5	100	-	0.00	2	100	-	0.00	4	44.44	5	55.56	2	50	2	50
Tetracycline (30µg)	1	20	4	80	-	0.00	2	100	2	22.22	7	77.78	1	25	3	75
Gentamycin (10µg)	2	40	3	60	1	50	1	50	7	77.78	2	22.22	4	100	-	0.00
Danofloxacin (5µg)	4	80	1	20	2	100	-	0.00	9	100	-	0.00	3	75	1	25
Enrofloxacin (10µg)	3	60	2	40	1	50	1	50	6	66.67	3	33.33	3	75	1	25

S : sensitive R : resistant

Sensitive : Zone diameter is at least 12 mm of 6 mm disc

Rsistant : Inhibitory zone not more than 10 mm (2 mm on each side) Cruickshank et al., (1980)