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DETECTION OF DIFFERENT BACTERIA CAUSING DECREASED HATCHABILITY IN TURKEY EGGS

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SUMMARY

A total of 360 eggs collected from turkey farms, suffered from low fertility and low hatchability, were examined for both Mycoplasma and egg borne bacterial diseases. Fresh fertile eggs revealed the recovery of Mycoplasma meleagridis (50 %) and Salmonella typhimurium (20 %).

From non-fertile eggs, Mycoplasma iowae (50 %), Mycoplasma gallopavonis (25%) were isolated. No bacteria were recovered from the non-fertile eggs. From dead embryos several Mycoplasma species were isolated viz., Mycoplasma iowae (25 %), Mycoplasma meleagridis (25 %) and Mycoplasma gallopavonis (25 %). Mixed infection with coliforms were isolated from all the internal contents of the eggs and dead embryos.

INTRODUCTION

Some bacteria seem to be responsible for decreasing the hatchability of turkey eggs e.g. Mycoplasma species,(M.gallisepticum & M.meleagridis. and M.iowae), Salmonella typhimurium and coliforms (Kleven, 1997).

Mycoplasma was proved to be the cause of one of the most perplexing and economically significant disease of poultry and turkey industry, especially that transovarian transmission via eggs or semen had been proved. (Bradbury 1979 and Sasipreeyajan et al., 1987). Mycoplasma infection in turkeys was described by Dodd (1905) and named "infectious sinusitis" by Dickinson and Hinshow (1938). Meanwhile, Markham and Wong (1952) and VanRoekel and Olesiuk (1953) reported successful cultivation of the organisms from chickens and the turkeys. M. Meleagridis is a specific pathogen for turkeys. It is a the cause of an egg transmitted disease in which the the primary lesion is an air sacculitis in the progeny. Other manifestations include a decreased hatchability, skeletal abnormalities and poor growth performance (Yamamoto and Ghazikhanian, 1997). Economic losses caused by M. meleagridis in turkeys have been associated primarily with egg borne infections.

M. iowae is generally associated with reduced hatchability and embryo mortality in turkeys. It has been shown experimentally to induce mortality in turkey and chicken embryos and mild to moderate air sacculitis and leg abnormalities in chickens and turkeys (Bradbury et al., 1988).

Salmonella is an infectious bacterial disease of poultry, animals and humans. It is the most important egg borne bacterial disease (Snoeyenbos, 1985). Paratyphoid infections in turkey are more common. More than 50 % of paratyphoid infections in turkey flocks are caused by Salmonella typhimurium (Hinshow and McNeil, 1943).

Rigby et al. (1981) found that Salmonella typhimurium was the most common salmonella isolated strain from the imported one-day old turkey in Canada. Many factors help in increasing infection rates of paratyphoid infections of poults. These factors included feed staff, embryonated egg, egg containing carts, rodents, air currents in the hatch, fluff, egg debris in the hatcheries and water (Cox et al., 1990).

Coliform bacilli were isolated from normal appearing yolk (Harry, 1957). Several workers have investigated the incidence of Mycoplasma infections among chicken and turkey flocks in Egypt (EL-Ebeedy, 1973, Abd EL-Rahman, 1985 and 1995). This study aimed to identify the bacterial agents associated with low fertility and hatchability in turkey eggs.

MATERIALS AND METHODS

Samples:

Three hundreds and sixty turkeys' eggs were collected from different farms having a hatchability problems in Kafr-EL-Sheikh Governorate during the period from March to May 1998. The egg samples included 120 fresh eggs, 120 non-fertile eggs and 120 dead embryos.

Mycoplasma isolation and identification:

Each egg was opened at the blunt end and extra-embryonic fluid was swabbed onto Mycoplasma media (Frey et al., 1969) using the method of Hayflick (1965). Mycoplasma isolates were subjected to a digitonin test (Erno and Stipkovits, (1973) followed by biochemical characterization tests including glucose fermentation, arginine deamination and film and spots tests. (Freundt et al, 1979) then to serological identification using growth inhibition test (Clyde, 1964). Antisera were kindly obtained from Professor Dr.N.M. Al-Zeftawi, Mycoplasma Department, Animal health Research Institute, Dokki.

Isolation and identification of isolated bacteria:

Egg wash solution and the internal contents were examined bacteriologically according to Andrews et al. (1978) and Nagaraja et al. (1980). Samples were growing in peptone buffer and Selenite F broth for 24 hours each at 37°C, a loopfull was transferred to blood agar plate, MacConkey agar plate (Oxoide) and Brilliant green agar plate (Difco). The plates were incubated at 37°C for 48 hours. Grown colonies were examined morphologically, picked up and kept on semisolid agar (BBL) for further identification. Biochemical identification was done according to Ewing (1986) and serologically identified after Kauffmann (1972).

RESULTS

Isolation and the identification of Mycoplasma:

Primary isolation of Mycoplasma from 360 eggs revealed the presence of 240 isolates (66.7 %) as shown in Table (1). Fresh eggs gave an incidence 50 % while both non-fertile eggs and dead embryos showed (75%). Digitonin test revealed that the isolates belonged to genus Mycoplasma and no Acholeplasma isolates were detected. Biochemical characterization revealed the presence of three groups. Group (1) comprised (90) strains which were positive for arginine deamination, Group (2) included 90 isolates gave positive results for glucose fermentation and arginine deamination, while group (3) contained 60 isolates positive for glucose fermentation only. The results are summarized in table (2).

Serological typing of Mycoplasma isolates showed the identification of three serovars of Mycoplasma, M.meleagridis was recovered 50% of examined fresh eggs and 25% of dead embryos.

M.iowae was recovered from 50% of non-fertile eggs and 25% of dead embryos. M. gallopavonis was identified from 25% of both non-fertile and dead embryos.

Identification of isolated bacteria

Biochemical identification of suspected Salmonella strains revealed motile organisms urea was not hydrolyzed, simmons citrate and methyl red positive, indole and Vogas Proskauer negative, H2S was produced, glucose, dulcitol, mannitol were fermented while lactose, sucrose and salicin were not fermented.

Serological identification revealed the antigenic structure (1, 4, 5, 12, i, 1,2). Therefore the suspected strains were Salmonella typhimurium.

Bacterial isolates from embryonated turkey eggs were motile, organisms, hydrolyzed urea, simmon citrate and Vogas Proskauer negative, H2S was not produced. Glucose, mannitol, sucrose, dulcitol and lactose were fermented .

These biochemical characteristics revealed that these strains were suspected to be E.coli. Serological identification of E.coli was not done, as the antisera were not available. Therefore Salmonella typhimurium was recorded from 20 % fresh eggs and Escherichia coli was isolated from 100 % of dead embryoes. The isolation results of both Mycoplasma and bacteria from turkey eggs

Table (1) Results of Bacteriological examination of turkey eggs

Type of eggs	Strains	No. examined	No. positive	%
Fresh eggs	*M. meleagridis* • *S. typhimurium*	120	60 24	50 20
Non-fertile eggs	*M. iowae* *M.. gallopavonis*	120	60 30	50 25
Dead turkey embryos (un hatched eggs)	*M. iowae* *M. meleagridis* *M. gallopavonis* * *Coliform*	120	30 30 30 120	25 25 25 100

* Mixed infection with Mycoplasma species.
• *Salmonella typhimurium*

Table (2) Biochemical characterization of Mycoplasma isolates.

Group*	No. examined isolates	Glucose fermentation	Arginine deamination	Film and Spots
1	90	- Ve	+ Ve	- Ve
2	90	+ Ve	+Ve	- Ve
3	60	+ Ve	- Ve	- Ve

* According to Razin and Freundt (1984).
Group (1) Suspected to be *M. meleagridis*
Group (2) Suspected to be *M. iowae*
Group (3) Suspected to be *M. gallopavonis*

Table (3): Serological identification of Salmonella species

Salmonella	Results
Poly group I	+ Ve
Poly group II	- Ve
Poly group III	- Ve
Somatic group B	+ Ve
Somatic group D	- Ve
Somatic Factor	
Factor (0) 1	+ Ve
Factor (0) 4	+ Ve
Factor (5) 5	+ Ve
Factor (12) 12	+ Ve
Poly flagellar (H)	+ Ve
Factor (H) I	+ Ve
Factor (H) (1,2)	+Ve

- Ve negative, + Ve positive
Therefore the antigenic structure is 1,4, 5, 12, i, 1, 2.

were shown in tables 1, 2, and 3.

DISCUSSION

Embryonated eggs play an important role in transmission of egg borne bacterial diseases such as salmonella infections, coliforms and Mycoplasma (Harry, 1957 and Kleven 1997).

Pathogenic Mycoplasma affecting turkeys are M. meleagridis, M. iowae, M. gallisepticum, M. synoviae (Abd EL-Rahman, 1995). M iowae (MI) is generally associated with reduced hatchability and embryo mortality in turkeys and induces mild to moderate air sacculitis and leg abnormalities under experimental conditions (Kleven and Baxter-Jones 1997). Embryo death is common in experimental infection of chicken and turkey eggs (Trampel and Goll, 1994).

M. meleagridis, M. gallisepticum and M synoviae are transmitted through the eggs by transovarian infection (Hofstad, 1974; Glisson and Kleven, 1985). While for M meleagridis and M iowae infection may spread venereally through insemination of infected semen (Yamamoto, 1967 and 1978 and Bradbury and Ideris, 1982). Bacterial infections of eggs may occur through shell penetration of Salmonella typhimurium from the faecal material to inside the egg (Williams and Dillard, 1969 and Baker et al., 1980, a). The inhibitory effect of egg albumin on salmonella organisms was reported by Baker et al. 1980, b. The present investigations dealt with examining several turkey farms suffered from low fertility and low hatchability and were subjected to bacterial and mycoplasmal studies. 360 eggs were collected from 3 different groups including fresh, non-fertile and dead embryo eggs. Examination of fresh and dead embryo eggs revealed the recovery of both Mycoplasma and bacteria, while from non-fertile eggs Mycoplasma was recovered only. Biochemical and serological typing revealed the presence of *M. meleagridis* and Salmonella typhimurium from fresh eggs (50 and 20% respectively). This agrees with the results obtained by (Reis and Yamamoto, 1971) who observed localization of M. meleagridis and M. gallisepticum in intestine of turkey embryos and proposed that this could give rise to a genital infection early in life.

In this study, Salmonella typhimurium was isolated from fresh eggs in rate 20 %. This result was similar to those recorded by Gordon and Tucker (1965) who recovered salmonella organisms from eggs, parents and offsprings and also by Baily et al. (1994) who found Salmonella organisms from 17 % of egg shell samples as well as by Cason et al. (1994) isolated Salmonella typhimurium from the intestinal tract of baby chicks hatched from experimentally infected eggs.

Examination of non-fertile eggs showed the occurrence of both M. iowae and M. gallopavonis (50 and 25 % respectively). This agrees with EL-Ebeedy and Stipkovits, 1975, Mc-Clenaghan et al., 1981; Rhoades, 1981, Bradbury and McCarthy, 1983 and Fatma Moustafa, 1994) who reported the mortality of M.iowae infection in turkey embryos, death and abnormalities in chick embryo.

Salmonella organisms couldn't be isolated from non-fertile eggs and this disagree with Cox et al. (1990) who isolated 12 salmonella serotypes from egg fragments collected three broiler hatcheries.

Examination of dead embryo eggs (unhatched eggs) revealed the presence of M.iowae, M. meleagridis and M. gallopavonis in equal percentage of 25% while coliforms were recovered as a mixed infection of all the samples. The recovery of M.iowae is in agreement with those obtained by (Trampel and Goll, 1994) who mentioned that it is a poultry pathogen whose disease characteristics have been largely determined by experimental infections rather than by the observation of natural field cases. Embryo death is common in experimentally infected chicken and turkey eggs with M.iowae can be widely invasive in embryos and young poultry, but it has a predilection for the intestinal tract of turkey embryo (Mirsalimi et al., 1989).

M.gallopavonis (MGP) a common isolates from domestic and wild turkeys which has been reported to cause air sacculitis and reduced hatchability in turkeys (Sharp et al., 1991). M. gallopavonis were isolated from wild turkeys by Luttrell et al. (1992).

Coliforms were isolated from all egg samples with dead embryos at the rate of 100 % and this agree with Harry (1957) who isolated pathogenic coliform bacilli from 70 % of yolk sacs of dead embryos. Siccardi (1966) noticed that ten E.coli organisms of serotype O 1 a : k 1: H 7 caused 100 % mortalities in embryos. From this study, transmission of coliforms vertically causing high embryonic mortalities in the incubator or in the first week. It is concluded that M.meleagridis, M.iowae and M. gallopavonis are the most pathogenic organisms for turkeys and must be taken more importance for elimination of the disease.

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