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Prevalence of Egg Shell Apex Abnormalities in Commercial Layer Chicken of Namakkal Region of Tamil Nadu

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ABSTRACT

Egg shell plays a major role in determining the quality of commercial layer chicken table eggs. Many factors are involved in causing abnormalities of egg shells, particularly in the apex part of eggs. The present investigation was aimed to study the prevalence of Mycoplasma associated eggshell apex abnormalities (EAA) in commercial layer chicken of Namakkal poultry zone of Tamil Nadu. Flock history and clinical samples from live and dead birds were collected from 24 flocks belongs to 14 different farms with the history of showing egg shell abnormalities. Collected clinical samples were subjected for detection of *Mycoplasma gallisepticum* (Mg) and *Mycoplasma synoviae* (Ms) by culture and polymerase chain reaction. Among the 24 flocks Ms and Ms + Mg infection were observed in 16.6 and 12.5 per cent of flocks respectively. In EAA affected flocks 0.65 to 1.35 per cent of eggs showed shell defects at the apex (pointed end). The incidence of the condition was increased in large flocks (ie., above 10,000) under peak production of laying from 25 to 40 wk of age and winter seasons.Egg production drop and mortality were ranged from 2.0 to 7.7 and 0.05 to 0.35 per cent respectively. Four out of 14 farms experienced EAA like defects in their previous flocks. Antimycoplasmal drugs were given periodically however periodic mycoplasmalmonitoring was not carried out. The study indicates the prevalence of mycoplasma associated EAA in commercial layer flocks is increasing and should be controlled by proper monitoring and enhancing biosecurity measures.

HIGHLIGHTS

- EAA is most commonly noticed in 25 to 45 weeks of age with 0.66 to 1.35 % of produced eggs revealed these defects.
- EAA affected flocks showed 2.0 to 7.7 % drop in production and 0.05 to 0.35 % mortality without any clinical signs.
- Among the *Mycoplasma* Spp., *M. synoviae* associated egg shell quality problem is increasing in commercial layer chicken.

Keywords: Eggshell apex abnormalities, Prevalence, Mycoplasma and Influencing factors

Egg shell plays a crucial role in protecting the contents of the egg from the microbial and physical environment and in controlling the exchange of water and gases. Incidence of inferior shell quality remains as one of the major causes of economic loss to egg producers throughout the world. Total eggs loss due to poor egg shell quality, prior to reaching their final destination from the point of lay was 5 to 10 per cent in India (Harikrishnan and Mohan, 2018). Egg shell mineralization and quality in layer chicken is affected by genetic, environment, nutrition and health status of birds. Among the diseases, infectious bronchitis (IB), Newcastle disease (ND), avian influenza (AI), egg drop syndrome (EDS) and avian mycoplasmosis affect the shell quality (Roberts, 2004).

Among the avian mycoplasmosis *Mycoplasma synoviae* is considered the second most important mycoplasma affecting chickens. *Mycoplasma synoviae* in turkeys and chickens causes synovitis and subclinical respiratory infection (Kleven, 2008). Pathogenic spectrum of *M.*

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synoviae increased further after the demonstration of causal relationship with eggshell apex abnormalities (Feberwee *et al*., 2009a). The EAA affected eggs shows altered shell surface (roughening), shell thinning, increased translucency and cracks and breaks. Economic losses associated with EAA includes the loss of eggs due to breakage of soft-shell eggs, increased number of downgraded eggs and increased labour costs due to the selection of eggs with EAA and cleaning of the facilities due to broken eggs (Feberwee *et al*., 2009b).

Srinivasan *et al*. (2014) reported oviduct changes associated with avian mycoplasmosis in commercial layer flocks of Namakkal district of Tamil Nadu with seropositivity of 100 and 91.10 per cent for *Mycoplasma gallisepticum* and *Mycoplasma synoviae* respectively by indirect ELISA. However, very little information is available on prevalence of eggshell apex abnormality in commercial layer flocksin India. Keeping this in view, the present investigation was conducted to assess the prevalence of mycoplasma associated EAA in layer chicken in Namakkal poultry zone of Tamil Nadu.

MATERIALS AND METHODS

The study was conducted in 24 layer flocks from 14 different commercial farms, located in and around Namakkal District of Tamil Nadu from April 2016 to March 2017. Birds in all investigated flocks had been vaccinated according to the standard vaccination schedule including Marek's disease, Newcastle disease, infectious bursal disease, fowl pox, infectious bronchitis and infectious coryza.

During visit to the farm EAA symptoms were explained to the farmer for recognition in the present flock or in some of their past flocks. A flock was considered EAA positive if their egg production had symptoms such as increased fragility of shells, increasing the number of downgraded eggs (rough appearance of the apex of the eggs shelland very fragile eggs).

Data on the flock size, strain, age at which EAA symptoms begun, type of management, vaccination and antimicrobial treatment, mycoplasma monitoring programme during the production cycle of commercial layer flock, morbidity, mortality and previously observed pathologies were collected from the investigated flocks at the time of visit.

Six choanal cleft swabs were collected randomly from live birds of each flock. Necropsy was conducted on dead birds from the EAA investigated flocks. Pooled samples of trachea, air sac, and lung swabs were collected aseptically. The outer surface of the oviduct was seared with a hot scalpel blade; an incision was made with a sterile scalpel and sterile cotton swabs used to swab both the isthmus and the uterus.Swabs taken from the live and dead birds were used for general bacteriology and mycoplasma culture.

Mycoplasma culture was performed as previously described (Marois *et al.,* 2000). The swabs were placed into 2ml modified Frey's medium and transported to the laboratory then agitated on a vortex mixer for 30 sec and then swabs discorded. Broth medium was incubated under microaerophilic condition with 90 % relative humidity at 37 ºC until the phenol red indicator changed from red to yellow (4 – 5 days).

Molecular detection of Mycoplasma gallisepticum and Mycoplasma synoviae

The genomic DNA was extracted from enriched broth culture which showed colour change using DNA extraction kit (Qiagen) according to the manufacturer instructions. PCR for detectionof Mg and Ms was performed separately on extracted genomic DNA (OIE, 2008). The forward primer for identification of *M. gallisepticum* was 5' GAGCTAATCTGTAAAG TTGGTC '3 and the reverse primer was 5 'GCTTCCTTGCGGTTAG CAAC '3. These primers amplified a 185 base pair fragment of the 16S ribosomal RNA gene of the *M. gallisepticum.* For the *M. synoviae* the forward primer was 5'GAGAAGCAA AATAGTGATATCA '3 and the reverse primer was 5'CAGTCGTCTCC GAAGTTAACAA '3 were used. These primers amplified a 215 base pair fragment of the 16S ribosomal RNA gene of *M. synoviae.*

The DNA amplification was performed in the thermal cycler with initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 54°C for 30 sec and 1 min at 72°C with a final extension cycle of 10 min at 72°C. Electrophoresis was performed on 1.5 % agarose gel. Negative controls (containing water instead of genomic DNA) were included in the PCR. A flock was considered Mycoplasma infected if at least any one of the swab yielded the amplifications of either *M.gallisepticum* or *M. synoviae*.

RESULTS AND DISCUSSION

Good eggshell quality is the primary concern of poultry industry and economic viability of the egg industry. If the eggshell has defects on its surface, it is prone to damage, dehydration, and microbial infection. Many factors have been identified as having direct or indirect effects on egg shell quality including strain of chicken, nutrition, management and disease (Roberts, 2004). The eggs examined from 24 commercial poultry flocks from 14 different farms showed shell defect at the apex (pointed end), which was thin, soft and easily broken with a sharp zone of demarcation from the rest of the shell, which appeared normal. These findings were consistent with the previous reports describing EAA (Faberwee *et al*., 2009a).

In the present study, samples collected from 7 out of 24 flocks showed colour change (red to yellow) in Frey's mycoplasma broth, which were subjected to PCR for confirmation by specific amplification of 16S rRNA gene (185 bp for Mg and 215bp for Ms). The results obtained by PCR were in agreement with early workers (OIE, 2008) who used a primer pair complementary to 16S rRNA gene designated for the detection of Mg and Ms at 185 bp and 215 bp respectively. Among the 7 flocks 4 (16.6 %) were positive for *MS* alone and 3 (12.5%) were positive for both Ms and Mg. The results demonstrated overall avian mycoplasma prevalence in EAA affected flocks was 29.1%. These findings concurred with earlier studies that found 27 % (Tomar *et al*., 2017) and 33 % (Rajkumar *et al.,* 2018) positivity for avian mycoplasma in Indian poultry. Since vaccination against mycoplasma is not currently carried out in commercial layer flocks of Namakkal poultry zone, cultural and molecular confirmation indicate natural infection.

In the investigated flocks, eggs with EAA ranged from 0.66 to 1.35% (Table 1) which was in agreement with Catania *et al.* (2010) since they also reported EAA incidence

Table 1: Details of flocks affected with egg shell apex abnormalities

Flock No.	Age (wk)	Flock size	Strain	Per cent of egg production			No. of EAA eggs	Per cent of EAA
				Expected	Actual			
					No	%		
1	28	24,000	Strain III	94.8	21,360	89.0	140	0.66
2	31	22,000	Strain III	95.2	20,700	92	170	0.82
3	49	20,000	Strain I	92.9	17,600	88	180	1.02
4	34	16,000	Strain II	95.5	14,560	91	125	0.86
5	34	19,000	Strain I	96.6	17,670	93	140	0.79
6	36	7,000	Strain II	95.5	6,300	90	85	1.35
7	29	10,000	Strain I	89.6	8,600	86	80	0.93
8	35	11,000	Strain I	96.4	10,010	91	135	1.35
9	43	8,000	Strain I	94.7	6,960	87	75	1.08
10	36	25,000	Strain I	96.2	22,250	89	195	0.88
11	72	12,200	Strain I	85.3	9,760	80	70	0.72
12	33	15,000	Strain I	96.8	13,650	91	170	1.25
13	29	14,000	Strain III	95.0	13,020	93	100	0.77
14	65	18,500	Strain I	87.7	18,500	83	125	0.68
15	31	10,000	Strain I	97.0	9,300	93	110	1.18
16	41	22,000	Strain I	95.2	19,800	90	180	0.91
17	26	16,000	Strain III	94.0	14,560	91	120	0.82
18	36	12,500	Strain II	95.4	11,250	90	75	0.67
19	31	17,000	Strain I	97.0	15,640	92	200	1.28
20	28	10,000	Strain II	95.6	9,200	92	70	0.76
21	33	12,000	Strain III	95.2	10,920	91	75	0.69
22	27	9,000	Strain II	96.2	8,280	92	80	0.97
23	35	16,000	Strain I	96.4	14,720	92	190	1.29
24	26	11,000	Strain II	96.1	10,175	92.5	75	0.74

of 1.3% in an intensive layer farm in Italy, however Faberwee *et al.* (2009b) reported 25% in layers housed on the floor in Netherlands and 22.9% in challenged SPF layers (Feberwee *et al*., 2009a). The low prevalence in the present study might be due to a pure Mycoplasmal infection and absence other factors such as IBV which contributed strongly to a higher number of abnormal eggs (up to 25%) in the earlier studies.

It is not clear how *M. synoviae* affects the normal eggshell calcification process or why the defect is confined to a distinct zone at the apex of the egg. *M. synoviae* may affect the composition and concentration of eggshell matrix proteins in the uterine fluid, which are needed for the regulation of the growth of calcite during eggshell calcification (Gautron *et al*., 1997; Hinke *et al*., 1999 and 2003). *M. synoviae* may also affect ciliary motility in the oviduct, which could lead to changes in the uterine fluid content affecting the deposition of calcium carbonate crystals (Dominquez-Vera *et al*., 2000). Preferential colonization of a specific area of the uterus might also offer an explanation for the localization of the eggshell defects.

The flock size may also affect the prevalence of Mycoplasma by promoting its circulation between birds. Mycoplasma associated egg shell apex abnormalities was noticed in flock size above 10,000 birds (100 %) and absent in flock size below 10,000 birds (Table 1). Highest infection rate in larger flocks might be correlated with poor management and bio-security measures in addition to horizontal transmission of the organisms (Dufour-Gesbert *et al*., 2006).

Comparing the frequency of mycoplasma associated EAA in different strains (Table 1), results showed that highest incidence in strain 1 (71.4%) comparing to strain 2 (14.3%) and strain 3 (14.3%). This might be due to highest placement of strain 1 (60 to 70%) in Namakkal poultry zone. Among the various age groups of flocks examined 25 to 40 wk showed highest (75%) incidence of EAA (Table 1). Well maintained and disease free commercial layer flocks reach its peak production at 28th wk (97 per cent) and it will be maintained at above 90 per cent up to 65 wk. It was opined that stress of peak egg production might cause an immunosuppressive effect (Srinivasan *et al*., 2012) which can facilitate the mycoplasmal infection and might induced EAA in commercial layer chicken.

Birds in the egg shell apex abnormality affected flocks were apparently normal and no evidence of clinical disease was observed. One of the main characteristics of mycoplasmosis, especially the one caused by Ms, is an asymptomatic manifestation, although it induces damages to the infected host's health, and may even cause suppression of their immune system (Stipkovits and Kempf, 1996).

Mortality, egg production drop and seasonal incidence of EAA in commercial layer chicken were presented in Table 2. Mortality in the affected flocks was ranged from 0.05% to 0.35%. Similar findings have been reported by earlier workers (Srinivasan *et al*., 2014). Egg shell apex abnormalities affected flocks showed a production drop varied from 2.0 to 7.7 per cent from the expected production as prescribed by the breeder. Santos *et al*, (2014) observed 10 to 23 % drop in egg production in free range chicken and Sumitha *et al*. (2017) reported 10 to 20% drop in 60 to 70 wk old flocks affected with EAA.

In the present investigation, highest incidences of EAA was recorded in the months of October and November (2 each) followed by August, September and December (each One). Similar results were published earlier (Srinivasan *et al*., 2014). Prevalence of rain, cold weather and sudden changes in temperature in the above said months favour the growth and transmission of mycoplasma.

Persistence of egg shell apex abnormalities in the affected flock varied from 9 to 41 days. Present investigation revealed 04 out of the 14 farm revealed EAA like defect in their previous flocks. It has also been reported that infection was more frequent in multiage farm (Kleven, 2008). In our study all the mycoplasma positive flocks were maintained in multiage production complexes within a restricted geographical area which facilitated persistence of infection in above said flocks and transmission to subsequent flocks by horizontal transmission (Stipkovits and Kempf, 1996; Kleven, 2008).

Investigated flocks were periodically treated with antimycoplasmal agents such as tiamulin and oxytetracyclin through feed as recommended by the poultry health consultant. Although medication can reduce the clinical signs and economic impact of mycoplasma infection, it cannot completely eliminate mycoplasmal infection (Kelvin, 2008) and birds become persistently infected with mycoplasma and remains carrier for life. This can be

Table 2: Mortality, Egg production drop and seasonal incidence of Egg shell apex abnormalities in commercial layer chicken

Flock No.	Age of the flock when EAA detected (Wk)	Flock strength	Total no. of birds died	Mortality %	Per cent of egg production drop	Per cent of EAA affected eggs	Month of outbreak
1	28	24,000	—	—	5.8	0.66	April
2	28	10,000	—	—	3.6	0.76	April
3	31	22,000	—	—	3.2	0.82	May
4	33	12,000	—	—	4.2	0.69	May
5	49	20,000	15	0.05	4.9	1.02	June
6	41	22,000	24	0.11	5.2	0.91	June
7	34	16,000	—	—	4.5	0.86	July
8	34	19,000	—	—	3.6	0.79	July
9	29	10,000	09	0.09	3.6	0.93	August
10	43	8,000	20	0.25	7.7	1.08	September
11	36	7,000	25	0.35	5.5	1.35	October
12	31	17,000	30	0.18	5.0	1.28	October
13	72	12,200	—	—	5.3	0.72	October
14	33	15,000	41	0.27	5.8	1.25	October
15	35	11,000	26	0.24	5.4	1.35	November
16	65	18,500	—	—	4.7	0.68	November
17	35	16,000	27	0.17	4.2	1.29	November
18	31	10,000	22	0.22	4.0	1.18	December
19	27	9,000	11	0.12	4.2	0.97	December
20	36	25,000	—	—	7.2	0.88	January
21	26	16,000	—	—	3.0	0.82	January
22	36	12,500	—	—	5.4	0.67	February
23	26	11,000	—	—	3.6	0.74	February
24	29	14,000	—	—	2.0	0.77	March

one of the reasons for inability to eradicate mycoplasma in commercial layer flocks that stay a long period in rearing and production sites. In Namakkal poultry zone, commercial layer flocks were not routinely monitored for avian mycoplasmosis during their production cycle. A consistently applied monitoring system is essential for prevention of mycoplasma infection.

CONCLUSION

The present study revealed 0.66 to 1.35 per cent of eggs produced showed egg shell apex abnormalities (EAA) in commercial layer chicken. Among the mycoplasma positive flocks *M. synoviae* in four and Ms and Mg as a concurrent infection in 3 flocks were detected by PCR. EAA was most commonly noticed in the age group of 25 to 40 wk (75%) of age. EAA affected flocks showed 2.0 to 7.7 per cent drop in production and 0.05 to 0.35 per cent mortality without any clinical signs. Hence poultry health keepers should be aware of the EAA-like presentations and consider the syndrome in the differential diagnosis of eggshell quality problems in layer flocks. The early diagnosis could help to decrease economic losses caused by these infection.These findings underline the increasing importance of *M. synoviae* in commercial layer chicken despite the absence of clinical signs in infected birds.

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