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Technical Note

Eggshell Apex Abnormalities in a Free-range Hen Farm with Mycoplasma synoviae and Infectious Bronchitis virus in Rio de Janeiro State, Brazil

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ABSTRACT

A farm with 3,000 free-range hens between 24 and 65 weeks of age was investigated. These hens were separated in small flocks of 400 to 700 birds, presenting 10 to 23% egg production reduction. Twenty serum samples were collected during the period of drop in egg production and three weeks later for the investigation of *Mycoplasma synoviae* (MS), *M. gallisepticum* (MG) and Infectious Bronchitis Virus (IBV) antibodies using ELISA. At the time of the second collection, egg production had resumed to normal levels; however, with 10.23% of the eggs showed eggshell abnormalities limited to the apex. Eggshell strength was significantly different between normal and those with eggshell apex abnormalities, but not other egg-quality parameters. ELISA tests showed that MS and IBV titers increased during the evaluated period. MS infection was confirmed by culture and by PCR of tracheal swabs. All samples were negative for MG by ELISA and PCR. Further studies with larger samples to ensure the occurrence of this disease in industrial layer flocks in Brazil are under way.

INTRODUCTION

In 2008, a new abnormality in the eggshell of chicken eggs was identified in The Netherlands (Feberwee *et al*., 2009b), followed by reports of the same abnormality in Italy (Catania *et al*., 2010), Germany (Ranck *et al.*, 2010), and England (Strugnell *et al*., 2011). This egg abnormality is characterized by roughened shell surface, shell thinning, and increased translucency, which leads to an increase in the incidence of cracks and breaks. The abnormalities are confined to the top cone of the egg, up to approximately 2 cm from the apex, and frequently present a very clear demarcation zone. For this reason it was called eggshell apex abnormalities (EAA). These abnormalities were attributed to the infection by *Mycoplasma synoviae* (MS). A MS strain was isolated from the oviduct of hens producing eggs with EAA and yielded the same abnormalities after experimental infection (Feberwee *et al*., 2009b). It was also shown that antibiotic treatment and vaccination against MS reduced the incidence of EAA (Feberwee *et al.*, 2009a; Feberwee *et al.*, 2009b; Catania *et al.*, 2010). Nevertheless, despite these very distinctive characteristics, EAA may not be necessarily pathognomonic of infections by any specific pathogen (Strugnell *et al*., 2011).

This case report describes the occurrence of eggs with EAA in free-range hens positive for MS and Infectious Bronchitis virus (IBV) in Brazil.

MATERIAL AND METHODS

A farm with 3,000 free-range hens between 24 and 65 weeks of age was investigated. These hens were separated in small flocks of

400 to 700 birds. No vaccination program was being done at the time of investigation. The affected flocks showed 10 up to 23% drop in egg production.

After observation of the eggs produced by these flocks, we detected abnormalities compatible with those previously reported by Feberwee *et al*. (2009b). A total of 718 eggs were analyzed for the quantification of abnormal eggs laid, out of which, 46 eggs with EAA and other 60 normal eggs were investigated for egg quality tests and approximate weights. The eggs were analyzed for Haugh units (HU), yolk color, egg weight, eggshell strength, and eggshell thickness measured at the bottom, side and apex.

Twenty blood samples were collected at the time of lowest egg production and five weeks later for the investigation of antibodies against MS, *Mycoplasma gallisepticum* (MG) and IBV by ELISA (IDEXX Laboratories, Westbrook, ME, USA). At the time of the first sampling, 10 tracheal swabs were collected on the farm and five hens were taken to the Poultry Health Laboratory of the School of Veterinary Medicine of Universidade Federal Fluminense, where they were submitted to euthanasia by atlanto-occipital disarticulation followed by necropsy for the collection and swabbing of the trachea and oviduct (magnum and shell gland).

Mycoplasma was cultured using modified agar and broth Frey Medium, incubated at 37°C under microaerophilic conditions, and examined for growth for up to 21 days. PCR was performed according to the procedures described by Lauerman (1998) for MS, using specific primers that amplify a 207 bp amplicon, and by Nascimento *et al*. (2005) for MG, using specific primers that amplify a 481 bp amplicon. DNA extraction was performed using the phenol/chloroform method (Sambrook et al., 1989).

RESULTS AND DISCUSSION

Out of the evaluated eggs from affected flocks, 7.52 % showed EAA (Figure 1). Previous studies reported EAA incidence of 1.3% in an intensive layer farm in Italy (Catania *et al*., 2010), 25% in layers housed on the floor in The Netherlands (Feberwee *et al*., 2009b) and 22.9% in challenged SPF layers (Feberwee *et al*., 2009a).

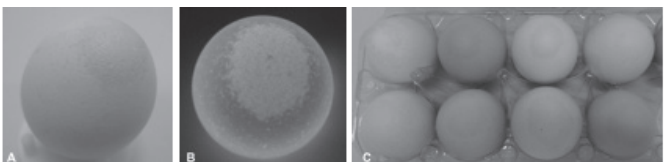

Figure 1 – A. Egg with rough and thin shell limited to the apex. B. Candling of the egg in Figure 1A, noting an increase on translucency at the apex of the shell. C. Eggs with changes compatible to eggshell apex abnormalities (EAA).

No significant egg quality differences were detected between the normal and abnormal eggs, relative to egg weight, Haugh units, yolk color, or eggshell thickness. However, eggshell strength was significantly different ($p<0.0001$) between normal eggs (39.67±7.3N) and abnormal eggs (33.96±7.3N) (Table 1). Ferberwee *et al*. (2009a) compared the eggshell strength and Haugh units of eggs from farms with and without the abnormalities and also found differences in eggshell strength, but not in Haugh units.

ELISA showed an increase in the antibody titers against the evaluated pathogens between the first to the second sample collection, with mean titers (GM) for MS between 216 (CV=68.9%) and 2732 (CV=60%), and GM for IBV between 16 (CV=205.6%) and 4770 (CV=32.5%). The hens probably experienced acute infection of both MS and IBV. No serologic reaction to MG was detected in the sera analyzed. Previous studies showed high prevalence of MS and IBV in Brazil. Buim et al. (2009) found that 55% (16/29) of layer flocks were positive to MS and none to MG by PCR, while Sandri et al. (2008) found 43.5% (20/46) positive to IBV by RT-PCR.

At the time of the second sampling, egg production had resumed to normal; however, the frequency of abnormal eggs increased to 10.23%. After experimental infections, Feberwee *et al*. (2009b) concluded that hens infected with both MS and IBV produced more eggs with EAA than birds infected only with MS.

MS was isolated from 33% (5/15) tracheal samples and not from the reproductive tract samples. Nevertheless, when PCR was used, MS was detected in 73% (11/15) of tracheal samples and 80% (4/5) of reproductive tract samples. None of samples were positive to MG, confirming that hens were infected only with MS.

The presence of abnormal eggshells in free-range hen flocks affected with MS, as confirmed by PCR, culturing and serology, and IBV by serology, with reduced eggshell strength, indicates the possibility of the presence of EAA in Brazil. Further studies with larger samples to ascertain the occurrence of this disease in industrial layer flocks in Brazil are under way.

Table 1 - Means (± Standard Deviation) of egg quality paramenters of normal and eggs with Eggshell Apex Abnormalities (EAA) collected on a free-range hen farm in Rio de Janeiro – Brazil

						Eggshell Thickness (mm)		
Egg group	EW	AH (mm)	UH	YCL	Str (N)	Apex	Side	Bottom
Normal	62.68 (5.0)a	6.25 (1.3)a	76.67 (10.2)a	12.15 (1.2)a	39.67 (7.3)a	0.370 (0.039)a	0.373 (0.035)a	0.350 (0.032)a
EAA	63.89 (4.9)a	6.09 (1.2)a	75.43 (8.3)a	12.22 (0.9)a	33.96 (7.3)b	0.358 (0.037)a	0.375 (0.031)a	0.339 (0.033)a

EW= Egg Weight, AH= Albumen Height; UH= Haugh units; YCL= Yolk color; Str= Eggshell strength; N= Newtons. Means with different letters within the same column are significantly different ($p<0.05$; two-tailed unpaired t test).

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