Epidemiological survey to identify eggshell apex abnormalities (EAA) syndrome in commercial layers in France

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Resumen:
Desde el año 2000 se han descrito en todo el mundo cepas de *Mycoplasma synoviae (M. synoviae)* con tropismo en los oviductos, capaces de inducir defectos en el ápice del cascaron de huevo (DAC) y caídas de la postura. Después del primer reporte de prevalencia del síndrome de DAC en Francia que fue realizado en el año 2012, no existe información actualizada.
Entre Mayo del 2015 y Mayo del 2016, Anses e ITAVI realizaron una encuesta epidemiológica. Se visitó 77 granjas que alojaban 96 lotes de gallinas de más de cincuenta y nueve semanas de edad. Las granjas fueron divididas en dos grupos. Un grupo de 40 lotes en sistemas de producción alternativo: huevo campero y huevo biológico (hc,b) y otro grupo de 56 lotes con producción en jaulas mejoradas (jm). La encuesta identifico 6/96 lotes con DAC, con una prevalencia del 7.3% para este estudio. En Bretaña y Baja Normandía se presentaron 4 lotes con DAC de los 69 visitados (5.8 %). La región de Rhône-Alpes presentó la más alta prevalencia con 3 lotes con DAC de las 17 visitadas (16.6 %). La región del Pays de la Loire no presentó reportes de casos con DAC.
Este trabajo demuestra una disminución de la frecuencia de síntomas de DAC en los huevos de gallinas ponedores comerciales. El síndrome se identifica en diferentes regiones muestreadas, en diferentes sistemas de producción y a diferentes edades. El síndrome de huevos DAC aún está presente en los sistemas de producción de huevos en Francia.

Palabras clave: *Mycoplasma synoviae*; DAC; Ponedoras comerciales; Huevos marrones.

Introduction

Mycoplasma spp. are a group of bacteria without cell wall and belonging to the class of *Mollicutes*, order *Mycoplasmatales*, family *Mycoplasmataceae* (4). The main avian pathogenic mycoplasmas, producing various diseases and significant economic losses are *Mycoplasma gallisepticum (M. gallisepticum)* and *Mycoplasma synoviae (M. synoviae)* (1-3). Mycoplasmosis was initially described in turkeys in 1926 and in chickens in 1936 (5). Symptomatology of *M. synoviae* infection can be different according to its tropism; symptoms can pass unnoticed or be subclinical when *M. synoviae* has a respiratory tropism, but *M. synoviae* can also cause synovitis, arthritis, with alteration of indexes of growth, production and hatchability (6, 7).

Endemic *M. synoviae* infection in commercial layer flocks and farms exists because transmission of mycoplasmas may be vertical, through the eggs, or horizontal either by direct contact between clinically affected or unaffected carriers and susceptible birds, or by indirect contact via people, wild animals and insects or contaminated equipment. Such indirect transmission is rather unexpected for wall-less bacteria, which are supposed to be sensitive to osmotic shock, heating or chemical treatments. However, *M. synoviae* may persist on feathers up to 2 or 3 days at room temperature (8) and 10 to 21 days under dry conditions at 20 °C (1). The presence of *M. synoviae* in poultry farms is frequent despite the control measures and biosafety regulations that have been established in the various phases of poultry production, multi-age farms predisposing to the bacteria persistence (7, 9). Once contaminated birds can carry *M. synoviae* for the rest of their life (9).

The diagnosis of *M. synoviae* infection is initially based on epidemiological information, clinical symptoms, analysis of macroscopic lesions, specific serology, isolation, and molecular tests (2). To detect antibodies against *M. synoviae*, several serological tests are used, but due to variations in specificity and sensitivity, these tests are only recommended to analyze flocks. According to the OIE, rapid serum agglutination (RSA), ELISA and inhibition test of hemagglutination (IH) are the most used (12). Monitoring programmes, using serology and polymerase chain reaction (PCR) can afford to keep breeding flocks free of *M. synoviae* (13). Among the different types of poultry production, layers are one of the most affected since the infection in these birds produces eggs with eggshell apex abnormalities (EAA). This alteration in eggs is exacerbated by the association of *M. synoviae* and infectious bronchitis virus (11). From the year 2000 increasing cases of alterations in the quality of the eggshell in commercial layers in different European countries were described (11, 14, 15). In 2009, for the first time, the association between the presence of *M. synoviae* in the oviduct and the production of eggs with EAA was shown, characterized by an alteration of thickness, increase translucency of the shell, cracks and breaks at the apex of eggs (11, 16, 17). Observation of EAA is the only criteria that allows suspected development of the syndrome in commercial hen farms (3).

In 2014, France registered officially 44.8 million of commercial layers producing 960,000 t equivalents of whole egg: 85% of egg production was brown type and the remaining 15% was white. Egg products represented 40% of comsuption and the remaining 60% was consumed as fresh egg, consumption per capita in France was 230 eggs per person (19). In 2015, 2100 commercial layers farms were recordered (20). These farms were mainly located in Bretagne (42%), Pays de la Loire (11%) Nord-Pas-de-Calais and Picardie (11%) and Rhône-Alpes (9%) (23). The industrial egg production in France is made in furnished cages (69%) and alternative systems (31%) (19, 21, 22). Production in alternative systems is subdivided into four types: free-range hens (12%), barn hens (7%), organic type hens (7%) and Label Rouge type hens (5%) (21).

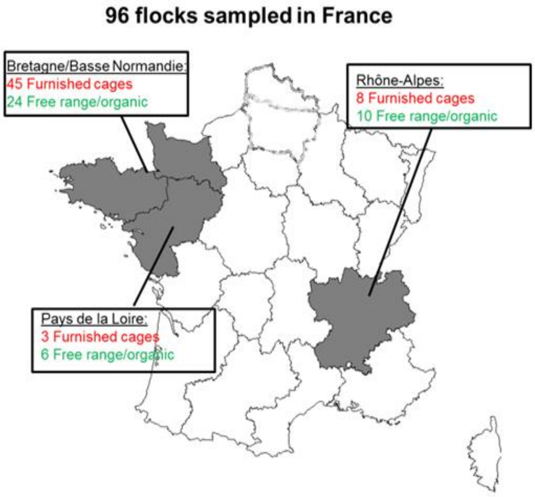
The objective of this work was to obtain data about EAA occurence in commercial layers flocks, by making a 13 months field survey since May 2015.

Materials and methods

Between May 2015 and May 2016, Anses (Agence Nationale de Sécurité Sanitaire de l’Alimentation, de l’Environnement et du Travail) and ITAVI (Institut Technique de l'Aviculture) in relation with other public and private institutions linked to egg layer sector in France, made a single, direct field survey with poultry producers of brown eggs.

During the visit in the farm, the survey performer explained the EAA symptoms to the farmer for their reconigtion in the present flock or in some other past flocks. The survey presentation had an introduction about different symptoms of the EAA syndrome, such as: poor quality of eggshell, increased losses of eggs on the gathering band, increased egg condemnation in the conditioning room, increase working hours in packaging areas because of cleaning of transport bands and eggs before delivery to the market. A questionnaire was filled at each visit, in each farm, with a specific informations gathered. The indepent variable was the presence or absence of EAA in flocks. A flock was considered EAA positive if it their egg production had symptomes such as increased fragility of shells, increasing the number of downgraded eggs (rough appearance of the apex of the shell eggs very top cone fine and very fragile, dirty eggs), increased workload devoted to sorting eggs and cleaning times greater facilities including transport band. The dependent variables were determined by the flock age at the beginning of EAA syndrome; by the percentage of eggs presenting EAA; by the application or not of any vaccine against *M. synoviae* (autogenous or commercial) and by *M. synoviae* monitoring throughout the production life of commercial layer flock.

During the survey 77 farms were visited, hosting 96 flocks, and the questionnaires were recorded with farmers who had brown egg layers of more than 59 weeks of age (9), to ensure that the information collected was after a nearly complete production cycle. For this survey, farms were grouped into two types of production: houses with furnished cages and alternative systems. In total 40 flocks in free range egg production and organic production (fr,o) and 56 flocks with furnished cages (fc) were visited.



These farms were located in main poultry-producing regions, as Bretagne and Basse-Normandie (24 fr,o and 45 fc), Pays de la Loire (6 fr,o and 3 fc) and Rhône-Alpes (10 fr,o and 8 fc) (Figure 1).

The objective of this epidemiological survey was to get updated prevalence of EAA syndrome, to describe EAA syndrome precense among different egg production systems in the country, to identify monitoring test and control tools for EAA symptomes used among commercial layers farms in France.

Figure 1: Sampling map of the EEA survey, by regions

Results and discussion

Seventy-seven brown egg layer farms, including 96 flocks were visited. The results of the survey showed that 7 flocks were positive at the moment of the visit, giving 7.3% prevalence for this sample studied. These results differ with those published by Gautier-Bouchardon in 2012 (17) which reported 2% to 13% of EAA syndrome-positive flocks in France as a result of a survey among layers farms veterinarians. Considering that our survey was based on the identification of symptoms characteristic of the EAA syndrome and that the poultry industry uses control tools such as vaccination and antibiotic therapy, our results suggest the insufficient EAA control among the layer farms. Furthermore this survey exhibited that 16 of the 77 farmers questionned reported previous flocks showing EAA syndrome in their farm during the last five years. This condition suggests that the EAA syndrome presence is variable with time.

In Bretagne which is the greatest egg-producing region in France and in Basse Normandie region, 4/69 furnished cages farms identified EAA presence in current flock (5.8%) and Rhône-Alpes region presented the highest prevalence, with 3/18 (16.6%) EAA positive flocks in alternative farms in the current flock (Table 1).

The results about EAA presence in previous flocks in the farms in the last five years were different between both regions. In Bretagne eleven out fifty-two (21.2%) poultry farmers confirmed the EAA presence in previous flocks in their farms, 8 were furnished cages farms and 3 farm in alternative production systems and Rhône-Alpes region five out of seventeen farmers (29%) visited reported positivity in their farms in previous flocks divided in 4/5 furnished cages and 1 free range farm and organic farms (Table 2).

Table 1 EAA symptoms in courrent flocks (96)

Region	Furnished cages	Free range and organic
Bretagne and Normandie (69)[a]	4	0
Rhone Alpes (18)[a]	0	3
Pays de la Loire (9)[a]	0	0

[a] Visited flocks

Table 2 Farms with previous EAA positive flocks (77)

Region	Furnished cages	Free range and organic
Bretagne and Normandie (52)[b]	8	3
Rhone Alpes (17)[b]	4	1
Pays de la Loire (8)[b]	0	0

[b] Visited farms

Nine flocks in 8 farms were visited in the Pays de la Loire region and none reported EAA (Table 1 and Table 2). The low number of EEA cases identified in current sampled floks, shows difference among different production systems and between the regions of France. These results were in accordance with various studies on EAA syndrome identification in many countries. The EAA syndrome presence variations are probably linked to the diversity of epidemiological, ecological and geographical conditions of the farms, bacterial transmission characteristics through different poultry production systems, types of farm management and biosafety measures, exposure to contaminant vectors, immune status and physiological conditions of birds (7, 9, 10, 18, 23).

Among the 22 EAA positive farms considering farms with current positive flocks and farms with previous positive flocks, 16 farms gave information about age of hens for EAA symptoms onset. In 2 (12.5%) farms with furnished cages, EAA appeared at the beginning of egg production. In 8 (50%) farms, layers had symptoms between 24 and 35 weeks of age, 4 are in fr,o and 4 in fc. The remaining 6 (37.5%) farms reported EAA presence between 40 and 60 weeks of age, 3 are in alternative production systems and 3 in fc. The frequent presence of EAA syndrome starting at 24 weeks of age may be explained by the physiological stress of the production peak, causing an immunosuppressive effect which can facilitate the *M. synoviae* increase and reflect the EAA occurrence. This description is in agreement with the findings published by Moreira *et al*. in 2015 (7).

The vaccination against *M. synoviae* is proposed as a method to control EAA. The live commercial vaccine use was approved since 2011 in Europe. The courrent positive flocks did not use *M. synoviae* vaccination.

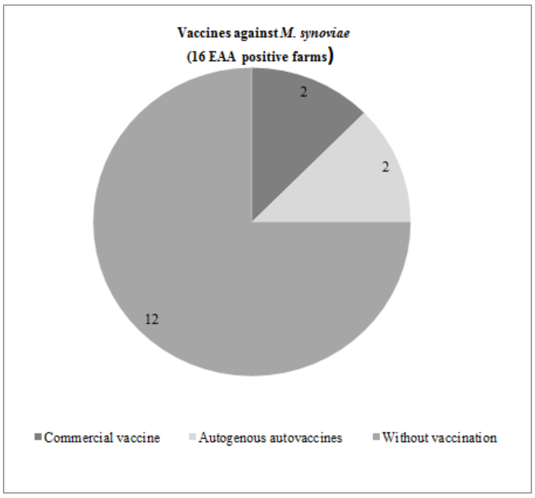


Figure 2. Use of vaccines against *M. synoviae*

Among the positive farms with at least an experience of EAA syndrome, 16 farmers answered about vaccination practice, 12 farmers (75%) did not use vaccines to control *M. synoviae* and 4 (25%) EAA-positive farms had used vaccines as a control tool in previous positive flocks: two farms had used autovaccines prepared with an inactivated *M. synoviae* isolate and the other two farms had used a commercial vaccine (Figure 2). In 2009, Feberwee *et al*. evaluated a commercial vaccine in a laboratory trial, applied to birds challenged with a strain of *M. synoviae*, and concluded that the vaccine could reduce the EAA symptoms but not eliminate *M. synoviae* presence (11). The low number of vaccinated farms did not allow us to evaluate the benefit of the vaccination.

M. synoviae infections are not officially regulated like *M. gallisepticum* infections in Europe (24). However these infections affect poultry production and can have an indirect impact on public health as they are a cause of antibiotic treatments during the egg production period when farmers are looking for reduction of symptoms (3-5, 10, 25).

The laboratory monitoring is performed when there is a suspicions of EAA syndrome in the farm. For this study monitoring and tracking with serological and molecular laboratory tests, such as suggested by the OIE in its Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (12) was conducted in only 11/77 farms (14.2%). Two farmers did not provide information about monitoring. Two farms used PCR tests, only one with positive results for *M. synoviae* and six farms used a serological test (3 were *M. synoviae* positive and 1 was negative, 2 did not give information about the reults), for three other farms, the farmers did not precise the test used.

The observed EAA frequency described in this survey reflects a negative trend of this problem in layer farms. We underline the need for more frequent controls to evaluate the mycoplasmas status of pullets or to identify positive flocks with or without symptoms, to help undertaking and controlling *M. synoviae* infections. This early diagnosis could help to decrease economic losses caused by the infection.

This work shows a decrease of the frequency of EAA symptoms compared to 2012. The syndrome is detected in layers farms from the different regions sampled, in furnished cages and alternative production systems and different production ages. Thus EAA is still present among commercial layer farms in France.

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