Asian Journal of Poultry Science

ISSN 1819-3609

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Asian Journal of Poultry Science

ISSN 1819-3609
DOI: 10.3923/ajpsaj.2021.31.36

Research Article
Seroprevalence Study of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* Infection in Modern Poultry Farms in the Agnibilekrou Region (Ivory Coast)

M.C. Kadja, S. Sourokou Sabi, A. Kouma, Bitsha-K. Kabkia, Y. Kane, R. Bada Alambedji and Y. Kaboret

Inter-State School of Veterinary Sciences and Medicine of Dakar, Senegal

Abstract

Background and Objective: In Côte d'Ivoire, poultry production is an essential link in the animal production system. With the intensification of the poultry sector, numerous pathologies have been observed, including mycoplasmosis. A serological survey was conducted to determine the prevalence of antibodies against avian mycoplasmosis in laying hens on 80 healthy improved poultry farms not vaccinated against avian mycoplasmosis in the Agnibilekrou area of Côte d'Ivoire. **Materials and Methods:** Survey forms were administered and blood samples were taken from 10 randomly selected hens on each farm. A total of 800 sera were collected and analyzed by 2 serological tests: Rapid Agglutination on Blade and semi-quantitative solid-phase ELISA (IMMUNOCOMB) for antibodies against *M. gallisepticum* and *M. synoviae* at the Microbiology Laboratory of EISMV in Dakar. Serum samples were analyzed individually and in pools (80) for ARL and ELISA tests respectively. Respiratory diseases represented 90% of the cases described. No farms used the laboratory for confirmatory diagnosis. **Results:** Laboratory analysis revealed overall seroprevalences of 90.5 and 76.5% for MG and MS, respectively, by the ARL test. By the IMMUNOCOMB ELISA test, seroprevalences of 95 and 76.25% were obtained for MG and MS, respectively. The prevalence rates for MG and MS obtained were high and varied according to geographical areas (A and C higher than B and D). Regardless of the age of the bands (9-90 weeks), the infection rates were greater than 65%. Infection with MG was higher than with MS in those under 40 weeks of age but similar in those over 40 weeks of age. **Conclusion:** This high infection rate requires more epidemiological investigations and the implementation of vaccination and biosecurity protocols, as well as the monitoring of breeding herds.

Key words: *Mycoplasma*, seroprevalence, poultry, gallisepticum, synoviae, farms

Citation: Kadja, M.C., S.S. Sabi, A. Kouma, B.K. Kabkia, Y. Kane, R.B. Alambedji and Y. Kaboret, 2021. Seroprevalence study of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* infection in modern poultry farms in the Agnibilekrou region (Ivory Coast). Asian J. Poult. Sci., 15: 31-36.

Corresponding Author: Mireille Catherine Kadja, Inter-State School of Veterinary Sciences and Medicine of Dakar, Senegal Tel: 00221 77 549 48 07

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The poultry industry reduces poverty by providing enormous opportunities to millions of people in the country. The availability of poultry meat is 3.90 kg per capita in Pakistan, 55 kg per capita in Kuwait, 50 kg per capita in the United States, 12 kg per capita in the world per year and 1 kg 900 per capita for Cote d'Ivoire[1]. There is a large gap in the consumption of poultry meat and eggs, especially in Africa. Indeed, with the intensification of the poultry industry, much pathology is observed[2]. The most frequent are Gumboro disease, Newcastle disease and avian coccidiosis and the most striking in recent decades is the epizootic of Highly Pathogenic Avian Influenza (HPAI) that appeared in Côte d'Ivoire in February, 2006 and has made a resurgence[2].

One of the main problems is the economic losses caused by infectious diseases. The main threats are diseases caused by *Mycoplasma* species[3]. The main pathogenic species of *Mycoplasma* are *Mycoplasma gallisepticum* (*M. gallisepticum*) and *Mycoplasma synoviae* (*M. synoviae*)[4]. *Mycoplasma* causes immense losses in the poultry industry by decreasing egg production, reducing growth and increasing condemnation at slaughter[5,6]. Losses of approximately 10-20% of egg production have been reported in flocks affected by mycoplasmosis[7]. *Mycoplasma* is a free-living, self-replicating bacteria that are known to have the smallest genome and low G+C content of about 23-40%[8]. The cell wall is absent in *Mycoplasma*. The cell membrane of these organisms is incorporated with sterols, which differentiates them from other organisms[3]. Based on 16S rRNA analysis, *Mycoplasma* belongs to the phylum Firmicutes, class Mollicutes and family Mycoplasmataceae. In birds, of the 22 known species of *Mycoplasma*, the four common pathogenic species are *M. gallisepticum*, *M. synoviae*, *M. meleagridis* and *M. iowae*[9]. Of all the avian *Mycoplasma* pathogens, *M. gallisepticum* and *M. synoviae* are important species due to their high prevalence in different types of poultry and *M. gallisepticum* is considered the most pathogenic[4]. *Mycoplasma gallisepticum* causes chronic infections in chickens and is the most virulent of all *Mycoplasma* species[10]. *Mycoplasma gallisepticum* and *M. synoviae* cause respiratory disease in chickens and turkeys.

In Africa and especially in Cote d'Ivoire, there is almost no literature on mycoplasmosis and most researchers rely on existing data from other continents, especially Europe and Asia. The current study would like to fill this gap in information on *Mycoplasma* in Côte d'Ivoire and especially because of the economic losses caused by this disease, to identify the avian *Mycoplasma* present on Ivorian soil and to know their prevalence to help researchers and actors in the poultry sector to prevent the occurrence of this pathology. The general objective of the present study was therefore to conduct a serological survey on avian *Mycoplasma* in Côte d'Ivoire and more particularly in the Agnibilékrou region to better understand the degree of involvement of avian *Mycoplasma* in respiratory diseases.

MATERIALS AND METHODS

Farms: The study was carried out in 80 modern farms of apparently healthy laying hens that had not been vaccinated against mycoplasmosis in the department of Agnibilekrou, northeast of Abidjan, a town bordering Ghana. The distribution of the farms investigated by locality and zone is detailed in Table 1. Laying hens were kept on the floor in open houses. Density, ventilation and hygiene standards were poorly respected.

Survey: The survey took place, from August, 12 to October, 4, 2015, on accessible farms along the main roads leading from Agnibilekrou. The visit to the poultry farms was done spontaneously for some and by appointment for others. The survey was conducted through direct interviews and observations. Consultation of the farmers' monitoring registers also provided important additional information on the survey form.

Blood sampling: On each of the 80 farms investigated, blood samples were taken aseptically from 10 laying hens taken at random from different buildings on the farm. After restraint, approximately 1.5 mL of blood was collected from the wing vein in a tube without anticoagulant and transported in an isothermal box at 4°C to the laboratory. After coagulation, the

Table 1: Distribution of farms by locality according to road axes in the Department of Agnibilékrou

Roads axis	Locations	Number of farms	Subdivision into zones
Agnibilekrou-abengourou axis	Assikasso, Dakoissikro, Kongodja, Tanguela, Nianda	25	A
Agnibilekrou-tanda axis	Yaokro, Akasso, Tankessé	18	B
Agnibilekrou-presso axis	Presso, Nzorekro, Takikro	25	C
Agnibilekrou-yebouakro axis	Assuamé, Yebouakro, Ayenou	12	D
Total	80		

whole blood contained in the dry tubes was centrifuged at 3000 rpm for 15 min. The serum was collected in 1 mL cryotubes, numbered and stored in a freezer at -20°C before being transported later to the microbiology laboratory of EISMV in Dakar for further processing for serological study. A total of 800 blood samples were collected.

Serological analyses: The search for antibodies against the two species of *Mycoplasma*: *M. gallisepticum* and *M. synoviae* was performed by two serological methods (rapid slide agglutination, RSA) using specific antigens from the BIOVAC laboratory and by the semi-quantitative solid-phase ELISA method with Immunocomb Kits) Sera samples were analyzed individually (800 sera) for the ARL test and by the pool (80) for the ELISA test.

For ARL, each serum sample was tested individually on plates with 24 wells. After washing, rinsing and drying the plate, 25 L of serum and 25 L of antigen were added per well, mixed and shaken for two minutes before reading. Positive sera were characterized by the presence of blue-violet agglutinates. The reaction was considered negative if no agglutinates were present within 30 sec of the 2 min shaking period[3].

For the ELISA, pools of sera were made an upper farm, i.e., 80 pools. Thus, using a micropipette, 1 µL of each sample from the same farm was collected and then deposited into a 1 mL Eppendorf tube. The contents of each Eppendorf tube represented the serum pool from one farm. Each tube was identified for analysis by the semi-quantitative solid-phase ELISA for both pathogens (MG and MS). When purple spots appear at the tip of the combs contained in the kit, the intensity of the colour is compared using a ruler present in the kit. The serum is considered positive when the intensity of the colour of the spot is higher than the one identified on the ruler corresponding to the positive control.

RESULTS

Risk factors identified: Certain factors increase the risk of respiratory infections in the farms: the rainy season, high coccidiosis infestation rate, recurrent disease, major factor favouring mycoplasmosis, the presence of other animal species in the farms and buildings housing animals of different ages on the same site. Also, in the vast majority of farms (83.8%), the average distance between buildings is 12.78 m less than the recommended standard of at least 20 m, hence the high risk of propagation of infectious agents by the wind from one building to another, especially when the age groups are different in the same farm.

Dominant pathologies described: According to the farmers met, several poultry diseases occur on the farms with varying frequency in Fig. 1. These farmers base their diagnosis of the various poultry diseases on clinical signs and lesions. In the Agnibilekrou area, respiratory diseases are the most frequent in 90% of cases and generally appear in pullets of an average age of 20 weeks at the time of laying. They are followed by coccidiosis (61.3%), colibacillosis (38.8%), Gumboro disease (8.8%), Marek's disease (6.3%) and other diseases.

Serological results

Overall prevalence: ARL test: 90.5% of sera are positive for MG versus 76.5% for MS in Table 2. IMMUNOCOMB ELISA test: seroprevalence of 95% for MG and 76.25% for MS in Table 3.

Prevalence according to the survey areas: The ELISA test shows the presence of MG and MS infection in all areas. However, the infection rate for both MG and MS was higher in zones A and C than in zones B and D in Fig. 2.

Prevalence by age: Regardless of the age of the animals, the seroprevalence of MG and MS was greater than 65%. On the

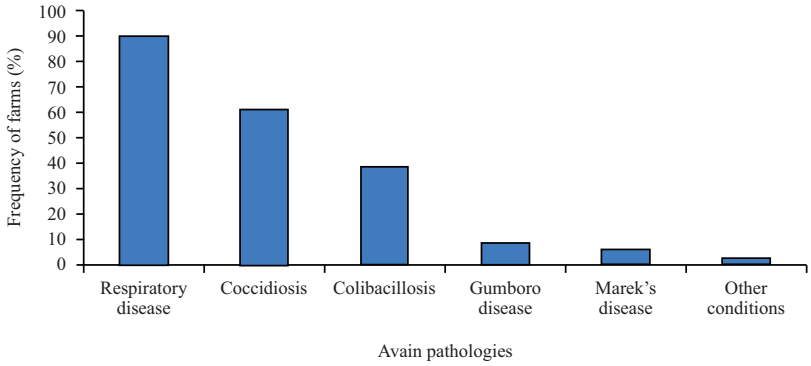

Fig. 1: Frequency of diseases encountered in the laying hen farms visited

Table 2: Overall prevalence of the two *Mycoplasma* species by the ARL test

Mycoplasma	Number of sera tested	Number of positive sera	Percentage
Mycoplasma gallisepticum	800	724	90.5
Mycoplasma Synoviae	800	612	76.5

Table 3: Overall prevalence of the two *Mycoplasma* species by ELISA

Mycoplasma	Number of serum pools tested	Number of positive sera	Percentage
Mycoplasma *gallisepticum*	80	76	95
Mycoplasma synoviae	80	61	76.25

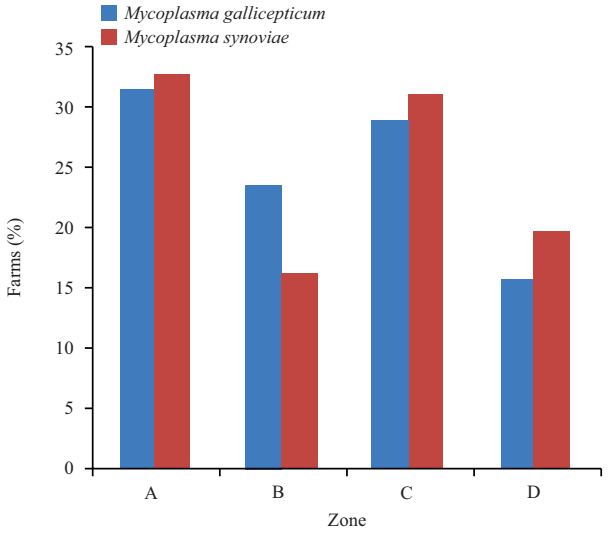

Fig. 2: Prevalence's of MG and MS by study area

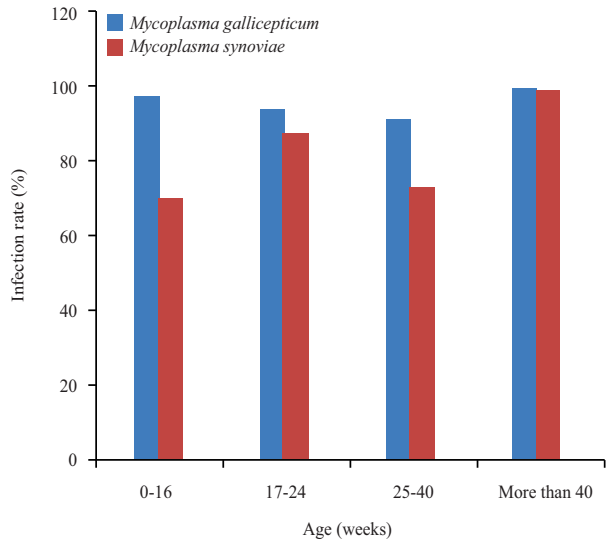

Fig. 3: Prevalence of mycoplasmosis by age

other hand, the MG infection rate was higher than the MS infection rate in laying hens less than 40 weeks of age. In contrast, similar MG and MS infection rates were obtained in those over 40 weeks of age in Fig. 3.

DISCUSSION

Screening for avian *Mycoplasma* infections has been performed using two serological tests. The rapid slide

agglutination test is highly sensitive and inexpensive for the identification of infected flocks but has the potential for false-positive results, especially following bacterial contamination of serum, infection with another *Mycoplasma*, or recent vaccination. ELISA was used as a confirmatory test for ALR because of its high sensitivity and reliability. Seroprevalence was studied individually in all subjects by ARL and confirmed by ELISA from a herd diagnosis. The current technique for determining seroprevalence is different from that used by some authors[11-14]. These authors used PCR. PCR has the distinction of being more specific and the use of PCR is preferred when studying the prevalence of *Mycoplasma* in various bird populations because the species present are often unknown. The main advantages of the PCR test are its ability to identify *Mycoplasma* even when they are no longer viable due to transport.

The infection rates obtained in this survey regardless of the *Mycoplasma* species studied and regardless of the test performed (LRA and ELISA) were very high with a higher prevalence of *M. gallisepticum* infections compared to the results of other studies investigated in Belgium and Western Europe[11,12].

Indeed, few studies[15,16] found a low estimated prevalence of *M. gallisepticum* in and among layer and broiler flocks. These low prevalences may be due to the use of RT-PCR, which is a more sensitive method of detection and explained by the reduction of vertical transmission due to the mandatory surveillance program in breeding flocks in Europe that aims to prevent and control the spread from breeding hens to their offspring and to protect national and international commercial markets. Seroprevalence of *M. gallisepticum* is generally low in poultry categories subject to control and eradication programs, for example in breeding flocks in Europe. In the Netherlands, control and eradication of *M. gallisepticum* began in the mid-1960s.

Current results are similar to Lierz *et al.*[14], who also noted a very high prevalence of *Mycoplasma* in birds of prey.

Although research on the seroprevalence of *M. synoviae* is limited, several studies reported by Wil[17] showed that the prevalence of *M. synoviae* in layers in different parts of the world is very high while published data on the prevalence of *M. synoviae* in other poultry categories are scarce. A recent Dutch study described for the first time the seroprevalence of *M. synoviae* in different categories of commercial poultry and confirmed that it was high, especially in layer flocks where it was 73%. In layer and broiler grandparent flocks, it was 0 and 10%, respectively. In layer and broiler parent flocks, seroprevalence was 25 and 35%, respectively, in broiler and broiler parent flocks, seroprevalence was 6%, in broiler turkeys, seroprevalence was 16%[12]. Until recently, voluntary control and eradication of *M. synoviae* in the Netherlands were limited to grandparents, flocks are culled if infected.

CONCLUSION

The serological study showed the strong involvement of avian mycoplasmosis with *M. gallisepticum* and *M. synoviae* in the respiratory diseases described in the Agnibilekrou area. Regardless of the species of *Mycoplasma* investigated and the test performed (LRA and ELISA), the infection rates obtained were very high, with a higher prevalence of *M. gallisepticum* infections. The control of this disease requires the improvement of biosecurity measures and the implementation of a monitoring and control program for *Mycoplasma* infections in breeding and laying flocks this preliminary study deserves to be deepened by epidemiological investigations on day-old chicks of breeders, to evaluate the importance of vertical transmission in the spread of the infection.

SIGNIFICANCE STATEMENT

This study showed the strong implication of avian mycoplasmosis with *M. gallisepticum* and *M. synoviae* in the respiratory diseases described in the Agnibilékrou region. This may be beneficial to farmers, stakeholders in the poultry industry in Cote d'Ivoire, researchers and the Ivorian government. This study will help researchers to identify avian *Mycoplasma* in the Agnibilekrou region (Cote d'Ivoire), to know their prevalence to better understand the degree of involvement of avian *Mycoplasma* in respiratory diseases.

REFERENCES

1. Ouattara, K., I. Doumbia, A.F. Coulibaly, S.K. Kouakou, J.D. Guessan, A.J. Djaman and A. Coulibaly, 2013. Influence of *Thonningia sanguinea* (THOS) on the productivity of poules pondeuses. Int. J. Biol. Chem. Sci., 6: 1960-1969.
2. Gragnon, B.G., N. Yeo, K.B. M'Bari and Y. Karamoko, 2020. Viral and bacterial pathologies in traditional chicken in the department of Korhogo (Cote d'Ivoire). Agron. Africaine, 32: 403-411.
3. Marois, C., F. Dufour-Gesbert and I. Kempf, 2001. Molecular differentiation of *Mycoplasma gallisepticum* and *Mycoplasma imitans* strains by pulsed-field gel electrophoresis and random amplified polymorphic DNA. J. Vet. Med. Ser. B, 48: 695-703.

4. Umar, S., M.T. Munir, Z.U. Rehman and M.A.A. Shah, 2017. Mycoplasmosis in poultry: Update on diagnosis and preventive measures. World Poult. Sci. J., 73: 17-28.
5. Kleven, S.H., 2008. Control of avian *Mycoplasma* infections in commercial poultry. Avian Dis., 52: 367-374.
6. Ferguson-Noel, N.M. and S.M. Williams, 2015. The efficacy of *Mycoplasma gallisepticum* K-strain live vaccine in broiler and layer chickens. Avian Pathol., 44: 75-80.
7. García, M., N. Ikuta, S. Levisohn and S.H. Kleven, 2005. Evaluation and comparison of various PCR methods for detection of *Mycoplasma gallisepticum* infection in chickens. Avian Dis., 49: 125-132.
8. Nicholas, R.A.J. and R.D. Ayling, 2003. *Mycoplasma bovis*: Disease, diagnosis and control. Res. Vet. Sci., 74: 105-112.
9. Liu, T., M. Garcia, S. Levisohn, D. Yogev and S.H. Kleven, 2001. Molecular variability of the adhesin-encoding gene *pvpA* among *Mycoplasma gallisepticum* strains and its application in diagnosis. J. Clin. Microbiol., 39: 1882-1888.
10. Michiels, T., S. Welby, M. Vanrobaeys, C. Quinet and L. Rouffaer *et al*., 2016. Prevalence ofmycoplasma gallisepticumandmycoplasma synoviaein commercial poultry, racing pigeons and wild birds in Belgium. Avian Pathol., 45: 244-252.
11. Feberwee, A., T.S. de Vries and W.J.M. Landman, 2008. Seroprevalence of *Mycoplasma synoviae* in dutch commercial poultry farms. Avian. Pathol., 37: 629-633.
12. Sumitha, P. and K. Sukumar, 2018. Comparative evaluation of diagnostic assays with clinical signs for detection of *Mycoplasma synoviae* infection in commercial layer chicken. Indian J. Comp. Microbiol. Immunol. Infect. Dis., 39: 7-11.
13. Lierz, M., R. Schmidt and M. Runge, 2002. *Mycoplasma* species isolated from falcons in the middle East. Vet. Record, 151: 92-93.
14. Hafez, H.M., 2005. Governmental regulations and concept behind eradication and control of some important poultry diseases. World Poult. Sci. J., 61: 569-582.
15. Agdelaine, P. and A. Braine, 2010. World and European overview of egg production and consumptionand consumption of eggs. INRA Prod. Anim., 23: 111-122.
16. Landman, W.J.M., 2014. Is *Mycoplasma synoviae* outrunning *Mycoplasma gallisepticum*? A viewpoint from the Netherlands. Avian Pathol., 43: 2-8.