Incidence of Avian Mycoplasmosis in the region of Batna, Eastern Algeria

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Abstract

Avian mycoplasmosis is infectious and contagious disease which affects chicken and turkey as well as many other species with many economics losses. The absence of data on avian mycoplasmosis in Algeria and the importance of the poultry breeding in Batna encouraged us to undertake the prevalence of the most pathogenic mycoplasmas in broiler and layer chickens in this area, *Mycoplasma gallisepticum* (MG). 143 Mycoplasmas were isolate from 237 samples, at a rate of 60.33%. MG was isolate at a rate of 21.67% (2.09% in layer hens and 19.58% in broiler chickens). The serological screening using of breedings showed a sensitivity of 83.10%. This study shows that mycoplasmosis and in particular MG infection, represent a serious problem in chickens in Algeria in the absence of hygiene conditions and vaccination especially.

Key words: *Mycoplasma gallisepticum*, Growth inhibition test, Seroprevalence, Broiler chickens, laying hens, Mycoplasmosis, Avian Disease.

Introduction

Avian mycoplasmosis is caused by several pathogenic mycoplasmas of which *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) are the most important. MG causes chronic respiratory disease of domestic poultry especially in the management stresses and/or other respiratory pathogens. Disease is characterized by coryza conjunctivitis sneezing and sinusitis particularly in turkeys. It can result in loss of production and downgrading of meat-type birds and loss of egg production. MS may cause respiratory disease synovitis or may result in a silent infection. MG and MS strains vary in infectivity and virulence and infections may sometimes be unapparent (Bradbury, 2001; Ley, 2003; OIE, 2008).

The frequent occurrence of unspecified clinical signs of respiratory disease among poultry flocks in Batna region and the lack of data on the role of mycoplasmas in these diseases had encouraged us to undertake this investigation.

The aim of this study was to evaluate the prevalence of MG infection in broilers and laying hens in the region of Batna (Eastern Algeria) using the rapid slide agglutination test and the isolation and identification method. Among 237 analyzed samples MG was isolate at a rate of 21.67% of which 2.09% in laying hens and 19.58%in broilers.

Material and Methods

Our study was conducted between October 2008 and September 2010 in 23 flocks divided as follows: laying hens (n = 11), broiler chickens (n = 13). These farms were divided into 10 districts of the wilaya of Batna. Birds collected during this investigation were apparently healthy or with respiratory lesions of lung, air sacs, or trachea. The number of poultry per farm varied between 3000 and 5000. Broilers were reared in the soil over a straw litters. Laying hens were caged. It should be noted that the density standards, ventilation and hygiene were exceptionally observed.

Sampling: 148 of blood samples were taken as follows:

69 blood samples from laying hens

79 blood samples from broiler-chickens.

After coagulation during 2 hours at room temperature, the serum is centrifuged at 1500 tr/min for 15 min and inactivated at 56°C for 30 min. then the serum is diluted to 1/5 to reduce non specific and cross-reactions between MG and MS.

All serum are put in sterile aliquots and maintained at 4°C until use within the following 48 hours.

Bacteriology: For the bacteriological diagnostic, 237 samples of tracheas, lungs and air sacs were taken from recently dead birds or those dead frozen until use (Evans et al., 2009).

Table-1. Serological and bacteriological results of *Mycoplasma gallisepticum* infection

Chicken	Serology	Bacteriology
Laying hens	56 /69 (81.15%)	3/87 (2.09%)
Broiler chickens	67/79 (84.81%)	28/150 (19.58%)
Average	83.10%	13.08%

Culture medium of mycoplasmas: Liquid and solid Frey`s medium was used for the isolation of mycoplasmas (Frey et al., 1968). It consist of PPLO broth base (2.1%) and PPLO agar (1%) (Difco Laboratories).

The media base was enriched with 15% horse serum; 1%of glucose; and 10% yeast extract. Contamination of media was prevented by adding 1% thallium acetate and 0.5% penicillin G potassium.

Serology: The detection of antibodies against MG was achieved by the rapid slide agglutination method.MG antigen was kindly supplied by Dr Bouchardon.AG, the laboratoire national de pathologie aviaire (LNPA) of Ploufragan France. The serum quality was checked using a negative SPF avian serum, a positive serum against *Mycoplasma gallisepticum* Mg15.

25µl of serum and 25µl of antigen are put on a glass slide previously washed, rinsed and dried. The antigen and serum are mixed at room temperature of the reaction is carried out according to Stanley et al., 2001.

Mycoplasma isolation: The reference strain of MG used is MG ATTC 15302 produced in rabbit and kindly supplied by the LNPA, France.

About 0.5 g of tissues (tracheas, lungs and air sacs) are cut in small pieces and ground in a sand bath containing 5 ml of mycoplasma medium.

0.2 ml of this suspension is inoculated in Frey`s liquid medium. When a color change is observed in the liquid medium, another inoculation of a fresh liquid medium is perform.

Later on an inoculation of Frey`s solid medium is carried out after a color changing had occurred in the liquid medium. If no color changing has occurred in the liquid medium a subculture is done on a solid medium because it is well known that mycoplasmas are very slow-growing microorganisms (Kleven, 2008). If Mycoplasma growth is noted on solid medium, agar blocks containing colonies are transferred in tubes of liquid medium for mycoplasmas cloning (Stipkovits et al., 1975).

Positive cultures were characterized by Dienes staining, biochemical and growth inhibition tests using MG antiserum produced in rabbit kindly supplied by Dr Bouchardon.A.G.

Results

Serology: Among 148 tested sera, 123 were positive representing a rate of infection of 83.10% showing the strong spread of MG. However, the rate of infection was slightly higher in broiler-chickens (84.81%) than in laying hens (81.15%) (Table I).

The highest prevalence (100%) of MG infection was found in the present study in El Madher, Ain yagout and Boumia (TableII) Seasonal variation of prevalence of MG infection was observed in the present study. The prevalence was higher (91.13%) in winter season and lower (73.91%) in summer season (Table III).

According to the age, the highest prevalence of MG infection was 86.95% in chicks whereas lowest prevalence was 82.35% in adult (Table IV).

Bacteriology: 143 mycoplasmas strains were isolated from 237 organ samples representing a positivity rate of 60.33%.

Table-2. Seroprevalence of *Mycoplasma gallisepticum* according to the study area

Study area	Number of samples	Number of positive sera	Rate of SPA %
El Madher	6	6	100
Tazoult	16	15	93.75
Arris	7	5	71.42
Ain Touta	40	25	62.5
Ain Yagout	6	6	100
Oued Chaaba	32	30	93.75
Djerma	10	8	80
Merouana	7	6	85.71
Boumia	13	13	100
Batna	11	9	81.81
Total	148	123	-

Table-3. Seasonal seroprevalence of *Mycoplasma gallisepticum* infection

Season	Tested sera	Positive sera	Negative sera	Rate of positive sera
winter	79	51	18	73.91%
summer	69	72	7	91.13%

Table-4. Seroprevalence of *Mycoplasma gallisepticum* in chickens according to age

Age	Number of samples	Positive cases	Negative cases
Chicks	46	40 (86.95%)	06
Adults	102	84 (82.35%)	18

31 strains were identified as MG (21.67%). The MG distribution was as follows: 3 in laying hens and 28 in broiler-chickens representing 2.09% and 19.58% respectively (Table I).

It should be stated that all flocks either laying hens or broiler-chickens harbor MG.

Discussion

The serological test in this study, for instance the rapid serum agglutination (RSA) may lack specificity and/or sensitivity. Therefore, its use is strongly recommended for monitoring flocks rather than for testing individual birds. For this reason we had tried to establish the test sensitivity and specificity under our laboratory conditions by using fresh sera. The latter had been decomplemented in order to ovoid cross reactions between MG and MS and diluted to 1/5. The evaluation of the RSA test was validated using known positive and negative control sera. In our study the size of tested samples is similar to that reported by several others ranging between 10 and 30 birds (Boucetta et al., 1997; Kermorgant, 1998 and Sabir, 2003).

This study has revealed a very high prevalence of *Mycoplasma gallisepticum* infection either in laying hens or in broiler-chickens in Batna region. Our results are higher than those of similar studies.

For instance, Thai et al. (2009) and Papanikolaou (2002) found a rate of infection of 37.83% and 21% respectively. However, our findings are slightly in accordance with those of Wunderwald et al. (2002), Sarkar et al. (2005) and Hossain et al. (2007) with a rate of infection of 75%, 58.90% and 55.13% respectively.

Concerning broiler-chickens, 84.81% of the flocks are MG infected (Table I). This value is greatly higher than results obtained by Baruta et al. (2001) and Aimeur et al. (2010) (1.25%, 30%). However, our results are in accordance with those obtained by orajaka et al. (2002) and mainly those of Evans et al. (2009) with a positivity rate of 64.9% and 100% respectively.

For laying hens, in a study Nascimento et al. (2005) found a rate of infection by MG of about 90%. A similar study conducted in Bangladesh showed that the rate of infection varies from 45.1% to 66.5% (Hossain et al., 2010; Barua et al., 2006). These results are higher than those in the present study.

This study has revealed a sharp influence of season on the incidence of avian infection by MG (Table III). Indeed, rate of infection of 90% has been noted during winter, compared to that of 73.91% obtained during summer. These results are in accordance with those of Sikder et al. (2005), Sarkar et al. (2005) and Hossain et al. (2010).

The effect of age on the occurrence of mycoplasmal infection is revealed in the study with a rate of infection of 69.9% and 48.7% in adult and young birds respectively (Table II).

In the present study, MG has been isolated at a rate of 21.67% (Table V). This rate is higher than to those found by Helail, 1998 (11.89%).

The results of mycoplasma isolation from different organs showed that air sacs are the main site of multiplication of this microorganism from dead birds (90%). Similar result was also obtained by Helail, 1998 (36.29%) and Shaker, 2005 (92%). Lungs are the second site of isolation (71.42%) followed by tracheas (62.93%).

MG isolation rate from tracheas (62.96%) is higher than to those obtained by Paakpinyo and Sasipreeyajan, 2007; Feberwee et al., 2005 and Gharaibeh and Al Roussan, 2008 (44.66%, 33% and 31.6% respectively).

Results obtained in our study revealed that the isolation rate of MG from the lungs is higher than in others studies (shaker, 2005; Helail, 1998).

The discordance observed between serology and isolation may be attributed to different factors such as the existence of tissular inhibitors of mycoplasma growth (Boussetta et al., 1997), the use of dry swabs reducing the viability of microorganisms (Zain et al., 1995), the absence of localization of mycoplasmas

Table-5. Isolation of *Mycoplasma gallisepticum* from different type of sampled

Organsamples	samples	Positive digitonin	Rate of mycoplasmas	Rate of MG
Tracheas	100	27	27%	62.96%
Lungs	64	7	10.94%	71.42%
Air sacs	73	10	13.70%	90%

especially MG in some sites after initial infection (Takase et al., 2000; Kempf et al., 1998), the difficulty of isolating mycoplasmas after some antibiotics treatment (Aimeur et al., 2010).

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