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RESEARCH ARTICLE

Risk factors and incidence of Mycoplasma synoviae infection in broiler breeder farms in Morocco.

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Abstract

Mycoplasma synoviae (MS) is an important avian pathogen of poultry production, and a worldwide emergent infection, causing respiratory tract infection and infectious synovitis.

The aim of the present study is to determine the incidence of MS infection in broiler breeder farms in Morocco, and analyze the relationship between risk factors and infection. The study was based on direct diagnosis using Real time PCR, and evaluation of risk factors such as: age of flocks, age of farms, season, zone of sampling, and biosecurity measures.

In fact, MS infection was associated significantly with the age (P<0,001). The prevalence was highest (50%) in autumn, (40%) in winter and lowest (25%) in spring. MS infection was significantly associated with age of farms at 8th week of age of flocks, and no significant difference was noted at 16th week.The prevalence was recorded highest 50% at 8th week, 75% at 16th week in farm situated in zone with high number of sites of poultry production above 300 farms. This same trend was observed in farm with lack of biosecurity and hygiene measures.The results showed that MS infection and transmission was significantly associated with age of flocks, age of farms and highest without significant relationship with season, zone of sampling, and biosecurity measures.

Introduction

Mycoplasma synoviae (MS) is an important avian pathogen, most frequently causing a subclinical respiratory tract infection, which can progress to respiratory disease or to infectious synovitis (IS) (Kleven, 2003).MS infection is responsible for significant economic impact in intensive production, due to decreased egg production, decreased growth and hatchability rates, and inefficient food conversion (Marois et *al.*, 2002). It is considered one of the most frequent pathogenic avian Mycoplasmas, because of its worldwide emergence (Browning et *al.*, 2000).As the vertical transmission plays a major role in spread of MS in poultry; many countries are invested to control MS infections by monitoring the breeder farms. Although, the risk of vertical transmission was minimized by culling *M. synoviae*-infected grandparent flocks, horizontal transmission is expected to be the most important transmission route for infected rearing breeder flocks (Seifi & Shirzad, 2012). Indeed, control of mycoplasma infections by vaccination was limited because only a few vaccines are available (Bouchardon et al., 2002).Control of these infections by chemotherapy was also limited because of the increasing of resistance to antibiotics, and because of the persistence of infection, it is necessary to improve biosecurity measures and minimize the risk factors related to

MS infections in complement of these controls. In fact, some factors such as: age, size of flock, region, season, etc… may effect on severity of MS infection(Seifi & Shirzad, 2012).
The aim of the present study was to determine the prevalence of *Mycoplasma synoviae* in broiler breeders flocks in Morocco and to identify and analyze the risk factors such: Age, size of flock, region, season, biosecurity measures…

Materials and methods:

Samples: The study was conducted from October 2011 to April 2013. Eleven broiler breeder flocks located in different regions in Morocco were followed for this study.
First, "20 to 60" one day old chicks imported from Europe were sampled, before being placed in farms. Then, every selected farm was visited at the age of 8, 16, 32 and 56 weeks, to collect 60 tracheal swabs. The sampling of the chickens was carried out on a single randomly selected house of each farm. During this survey, all birds were apparently healthy. Samples were transported under cold conditions and stocked at (- 20°C) until analysis with PCR in the Laboratory of Avian Pathology Unit at the Agronomy and veterinary Institute Hassan II in Rabat, Morocco.
Molecular Biology Analysis: Real time PCR was conducted in this study to avoid the lack of specificity and sensitivity of serological assays.
DNA from five pooled tracheal swabs was manually extracted using the Pure Link® Genomic DNA Mini Kit (Life technologies™, Foster City, CA) according to manufacturer's instructions. The Real time PCR reactions were conducted on 7500 Fast Real Time PCR system (Life Technologies™, Foster City, CA) using VetMaxTM – Plus qPCR master mix with TaqMan Probe and primers designed by Life technologies™(Life Technologies™, Foster city, CA) according to the manufacture's protocol. The Data analysis was performed using the 7500 Software V2.0.6.
Risk factors:A survey was performed, in fact, Flock size, age and localization farms, number of poultry farms in sampled region, conditions of hygiene and biosecurity measures were recorded on every visit.(Table.3)
Statistical analysis: Results were analyzed using Epi.info V 6.0 (Chi square test and odds ratio).

Results:

Results showed that the age of detection of MS infection by PCR is variable and the prevalence increased significantly (P<0,05) with the increaseof age (Table.1). In fact, all samples of one day old chicks were negative to MS by Real time PCR, but at 8^{th} week of age 4 farms became positive and the infection rate situated between 8, 3% and 33,3%. At the 16^{th} week, the number of positive farms has increased to reach 7 farms, with a variable rate of infection varying between 8, 3% and 100% (Table.1), and at 32^{nd} week of age 9 farms became positives. At the end of this survey (56^{th} week), all farms were positive by PCR with rates of infection exceeded 90%. (Table.2)
At the 8^{th} week of age, the prevalence was highest in autumn and winter (50 and 40%) and lowest (25%) in summer. The same prevalence was noted in flocks with 15.000 -30.000 population and above 30.000 (40%) (OR=1,33).The prevalence of mycoplasmosis in new farms (up to 10 years) was significantly higher 100% than old farms with only 12,5%, (P<0,05).
Farms localized in area with high number of poultry farms (Above 300 farms) were most infected by MS (50%, OR=2,5). Indeed, the prevalence was highest in farms near to others one by less than 3 Km (42,9%, OR=2,25), and situated on accessible road (50%, OR=4).Lack in measures of biosecurity seems influence MS infection, in fact, the highest prevalence was seen in farms with neglected hygiene, and with access of others wild birds such as sparrows to house (66,7%, OR=6 and 60%, OR=7,5) respectively.(Table.3)
Relationships between risk factor and MS infection had changed at 16 weeks of age (Table.4).In fact, the prevalence was highest in flock with 15.000- 30.000 population (80%, OR=4). No significant difference was seen in new farms (Up to 10 years)(75%) and old one (Above 10 years) (57,1%). Localization of farms seems to have an important role for developing MS infection, actually, the highest prevalence was noted in farms in close to others poultry farms (less than 3 km) (83,4%, OR=7,5) and situated on accessible road (100%). Contact with poultry and others wild birds (sparrow) can predispose to MS infection (OR=4)

Discussion:

M. synoviae can be transmitted vertically and horizontally (Stipkovits and Kempf, 1996), but horizontal transmission is expected to be the most important transmission route for infected rearing breeder flocks. In fact, results showed that all one day old chicks were negatives to MS, and the infection increase with age, similar report was demonstrated by (Seifi & Shirzad, 2012) who reported highest MS prevalence in age above 60 weeks of age (47,8%), and by Feberwee et *al,*. (2008), who noted a prevalence of 60% at 51 weeks of age.

In this present study, seasonal variation for prevalence of MS infection was noted, high prevalence in autumn and winter 50% and 40% respectively, similar results were reported in other different geographical conditions(Heleili et *al*,.2011, Sikder et *al*,. 2005 and Hossain et *al*,. 2010, Seifi & Shirzad, 2012). It might be due to the influence of cold weather in these different countries and occurrence of respiratory pathogens in cold season or more resistance of MS in cold environment.

The influence of flocks size was observed, actually, PCR showed the highest prevalence in large scale flocks (Above 15.000), the same results were found by Dufor-Gesbert et *al*,.2006 and Seifi & Shirzad, 2012.

The impact of sampled region was noted at 8th and 16th week of age, in fact the prevalence of MS was higher in region with important density of poultry production this results is in agreement with Hagan's study (2004), which demonstrated that MS infection is more frequent on farms with several houses. We have also noted that the sites situated in close in the other farms and on accessible road (Table.3 and 4) are most infected by MS, it might be due to the indirect transmission by spread of infection from other farms and vehicles.

The results showed that MS infection is significantly higher in news farms especially at 8^{th} week of age with a prevalence of 100%, this finding could be explained by the lack of experience of these owners' farms.

This study confirmed the role of hygiene in transmission and persistence of MS infection; the highest prevalence was noted in farms with low hygiene and with lower biosecurity standards, but without a significant statistical relationship. In fact, the hygiene play an important role to minimize the introduction of pathogens in farms but it must be accompanied by others biosecurity measures.

The MS infection was associated with presence or access of others birds (sparrow) into the farms, due to direct transmission from birds, this result need more investigation to determine and confirm that sparrows play an important role as carriers.

This study showed the presence and the importance of *M. Synoviae* infection in broiler breeder farms in Morocco and the role of horizontal transmission. Many risk factors were also implicated to increase MS infection in broiler breeder farms.

Table.1: Evolution of MS infection with the age

Risk factor	Level of risk factor	Positive flocks (%)	Significance of difference (p value)
Age (week)	One day old to 8week	36,4	
	8 – 16	63,3	**0,001**
	16 – 32	81,8	
	32 - 56	100	

Table.2: Prevalence (%) of MS infection per farms revealed by PCR.

farm	One day old	8th week	16 th week	32nd week	56 th week
1	0	0	41,7	100	100
2	0	0	0	8,3	8,3
3	0	33,3	16,7	25	100
4	0	0	16,7	91,7	100
5	0	0	0	100	100
6	0	0	8,3	8,3	100
7	0	8,3	100	100	100
8	0	0	0	100	100
9	0	8,3	8,3	0	91,7
10	0	0	0	91,7	90
11	0	25	8,3	0	100

Table.3: Relationships between *Mycoplasma synoviae* infection and various risk factors at 8 week of age.

Risk factor	Level of risk factor	Positive flocks (%)	Odds ratio	Significance of difference (p value)
Season	Autumn	50	2	
	Winter	40	1,33	0,9
	Spring	25		
Flock size	Up to 15.000	0		
	15.000 – 30.000	40	1,33	0,5
	Above 30.000	40		
Age of farms (years)	Up to 10	100	--	0,001
	Above 10	12,5		
Number of poultry farms per region	Up to 200	33,3		
	200 - 300	25		0,9
	Above 300	50	2,5	
Distance between selected farm and others poultry farms	Up to 3 km	42,9	2,25	0,5
	Above 3 km	25		
Accessible road	yes	50	4	0,3
	no	20		
Hygiene	High - intermediate	25		0,2
	low	66,7	6	
Access of the others birds	yes	60	7,5	0,1
	no	16,7		

Table.4: Relationships between *Mycoplasma synoviae* infection and various risk factors at 16 week of age.

Risk factor	Level of risk factor	Positive flocks (%)	Odds ratio	Significance of difference (p value)
Flock size	Up to 15.000	0		
	15.000 – 30.000	80	4	0,2
	Above 30.000	60	2,25	
Age of farms (years)	Up to 10	75	2,25	0,5
	Above 10	57,1		
Number of poultry farms per region	Up to 200	66,7		
	200 - 300	50		0,9
	Above 300	75	2,25	
Distance between selected farm and others poultry farms	Up to 3 km	83,4	7,5	0,1
	Above 3 km	40		
Accessible road	yes	100	--	0,05
	no	33,4		
Hygiene	High - intermediate	71,4		0,9
	low	66,7	--	
Access of the others birds	yes	80	4	0,3
	no	50		

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