Research in: Agricultural & Veterinary Sciences
Vol.3, No.1, 2019, pp.36-42

SEROPREVALENCE OF *Mycoplasma gallisepticum* IN NON VACCINATED BROILER FLOCKS IN ABBOTTABAD KHYBERPAKHTUNKHWA, PAKISTAN

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Abstract. *Mycoplasma gallisepticum* (*MG*) is the contributory agent of chronic respiratory disease (CRD) in the commercial poultry world including Pakistan. The breathing ailments in the broiler flocks are upsetting the poultry economics. The study was designed to investigate seroprevalence of *Mycoplasam gallisepticum* (MG) during the period of May to September 2017. The infected birds showed different clinical signs i.e. coughing, sneezing, rales and exudate from nostrils and eyes. Blood samples (*n=360*) were collected from selected areas of Abbottabad, Khyberpakhtunkhwa *viz;* Qalanderabad, Abbottabad central and Havellian due to its rich broiler population. Serum Plate Agglutination Test (SPAT) and Enzyme Linked Immunosorbent Assay (ELISA) were used to determine the prevalence of MG. Serum samples (*n=57*) were found positive through SPAT (15.8%) and samples (*n=29*) were found positive through ELISA (8.5%). The age of bird affected the occurance of *Mycoplasma gallisepticum* infection in broilers. Infection was more prevalent between the age of 20-30 days *Viz;* 29.1% and 23.6% through SPAT & ELISA respectively.The prevalence was lowest in first three weeks of age. However, infection prevailed throughout the flock rearing at various levels. Climatic conditions affected the occurance of *Mycoplasama gallisepticum* infection. A high prevalence of 23.6% & 29.1% through ELISA & SPAT respectively was observed in the month of August (Table 2). Infection was comparitively lower in the months where humidity level was low i.e. May June and July due to less frequent rain patterns. The prevalence of infection was highest in qalandrabad area followed by Abbottabad central and Havelian area. The ELISA protocols were found more specific and SPAT was more sensitive test for *Mycoplasama gallisepticum* antibodies.

Keywords: *Mycoplasam gallisepticum, Serum, Plate Agglutination, ELISA, Vaccination*.

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Received: *22 March 2019;* ***Accepted:*** *1 April 2019;* ***Published:*** *4 April 2019.*

1. Introduction

Commercial poultry is considered to be second largest growing industry in Pakistan, started in early 1960s showed rapid development within ten years and play a major role to fulfill the demand of protein in the country. Poultry industry is the most vital and well-organized sector in Pakistan that contribute 26.8%, 5.76% and 1.26% respectively to the total meet production, agriculture sector and overall Gross Domestic Product (GDP) (Nayyar *et al.*, 2014). Over 2.5 million broiler is annually reared in Hazara region, in addition to 04 million broilers breeder stock, 0.5 million commercial layers and 3.00 million rural poultry (Ayaz *et al.*, 2010).

Infectious diseases are among the main factors that affects poultry industry. In Pakistan, poultry industry faces a number of disease challenges which cause huge economical losses due to high rate of mortality, morbidity and decreased production egg (Alexander, 2000). Among these, Avian Mycoplasmosis is one of the important disease addressing the economic concerns.

Avian Mycoplasmosis was first time isolated in turkey in 1926 and in chicken in 1936 (Nascimento *et al*., 2005). MG is an avian pathogen which not only causes complications of respiratory infection but results in heavy mortality and morbidity and huge economic loss to the farmers (Levishon, 2000; Nascimento *et al*., 2005). Occasionally it also causes keratoconjunctivitis, salpingitis, arthritis and fatal encephalopathy (Lysnyansky *et al.,* 2005). Managemental stress results in severity of its infection (Papazisi *et al*., 2002). Its transmission is possible through discharges from live birds and fomites. It is also transmitted by both vertical and horizontal ways (Sarkar *et al*., 2005). Infected birds remain asymptomatic for a days or month under stress condition, so incubation period of pathogens may also vary. Occurrence of infection higher in larger flocks as compared to small flocks. (Islam *et al*., 2011; Mukhtar *et al.,* 2012).

Usually the vaccinations for Mycoplasma gallisepticum is performed in breeder stock but out breaks are concurrently occurring. Therefore, it was necessary to evaluate the actual prevalence of this disease. Therefore, the current study was designed to know about its sero-prevelance of *Mycoplasma gallisepticum* in District Abbottabad of Hazara Region in order to devise a proper strategy to combat its harmful impacts on socio-economic condition of farmers and the country.

The aims of the current study were to determine the serological status of *Mycoplasama gallisepticum* in broiler population and also to determine the sensitivity and specificity of diagnostic tests (ELISA and SPAT).

2. Material and Methods

This study was conducted at the Veterinary Research and Disease Investigation Center (VR&DIC) Abbottabad.

Area and Season of study

Three main areas of Abbottabad (Abbottabad central, Havelian and Qalanderabad) having rich broiler bird population were selected. Sampling was performed during the months of May-September.

Grouping of Birds

Birds were divided into five age groups *viz;* Group A =1-10, Group B=11-20 days, Group C=21 to 30 days, Group D=31 to 40 days and group E= 41 to 50 days. Serum samples (*n=360*) were collected from broiler birds in study areas. 120 samples were collected from each area.

Sample collection

An aliquot of 1ml blood was collected in Gel Vaccutainers randomly from wing of broiler birds using disposable syringe. The blood sample is Blood sample was transported in a cold chain to the microbiology/ ELISA Lab of VRDIC Abbottabad. The

serum was separated in centrifuge at 3,000 rpm for 5 min to have clear serum for further analyses.

ELISA Technique:

Indirect ELISA technique was used to detect MG infection. In indirect ELISA sample antigen is sandwich between antigen coated plat and is a two step ELISA which involves the binding processes of primary antibody and labeled secondary antibody. The primary antibody is incubated with the antigen followed by incubation with secondary antibody. The addition of enzymes substrate chromogen-regent changes the color of the wells. The change in color is directly proportional to the amount of the bounded antibody, means more antibody present in the sample stronger the color development is the wells.

Procedure

The 96-welled polystyrene MG antigen coated plates were used. Before starting the procedure, serum, plates and other solution were kept at room temperature and labeled. 100µl of negative & Positive controls were added into labeled wells. 1µl of sample was diluted with 500µl diluent. Diluted samples were added in the appropriate wells, cover plate with lid and kept at 37^{o}C in incubator for 30 minutes. After that, contents of wells were washed 3-5 times with 350µl of distilled water. Invert plate and tap firmly on absorbent paper until no humidity was seen. 100µl of highly purified immunoglobulin G (IgG) was used as conjugate in wells and plates were covered aluminum foil and placed in incubator for 30 minutes at 37^{o}C. Unbounded conjugates were washed away after incubation. 100µl of substrate was added in all wells, covered and incubated at 37^{o}C for 15 minutes. After it, 0.16M sulfuric acid was added as stop solution. Optical Density (OD-value) was determined using ELISA reader at 650nm.

Serum Plate Agglutination Test:

Serum plate agglutination test was performed according to the OIE manual (2008). Collected sera specimen and MG antigen (Lilli test *MG* RSA antigen, manufactured by Charles River Laboratories Inc Wilmington) were used. 40µl antigen & 40µl serum were mixed on a glass plate with the help of micropipette. Formation of definite clumps showed positive while no clumps as negative reaction.

Statistical Analyses

The recorded data was arranged in Microsoft Excel Sheet and was analyzed.

3. Results

In the present study, blood samples (*n=360*) of broiler birds were analyzed to determine the prevalence of the *Mycoplasma gallisepticum* infection and to obtain the more sensitive and specific diagnostic protocol. Twenty Nine (29) samples (8.05%) were found positive through ELISA while through SPAT, fifty seven (57) samples (15%) were positive.

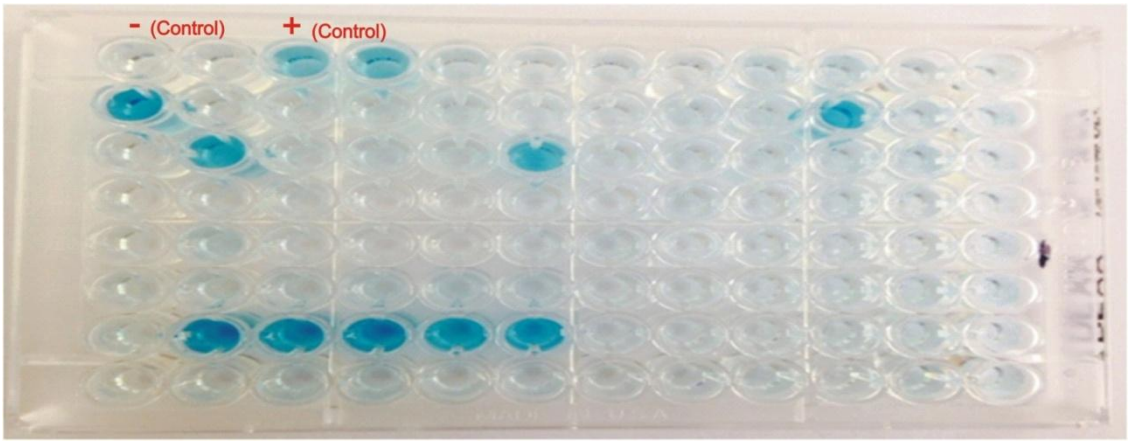

Figure 1. Showing Positive (Blue) and Negative (clear) serum samples for *Mycoplasma gallisepticum* antibodies through IDEXX ELISA kit

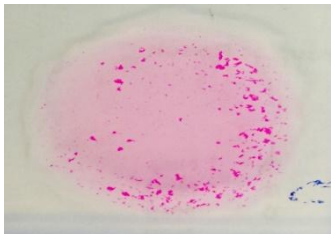

Figure 2. (+ive Sample)

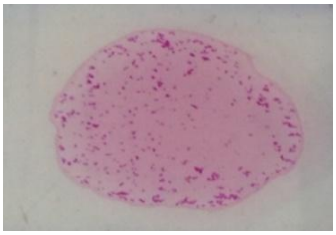

Figure 3. (+ive Control)

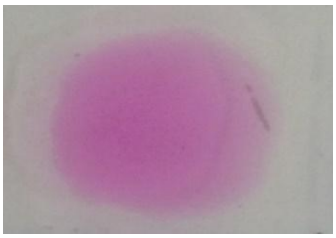

Figure 4. (-ive Control)

In above figures the results of SPAT is elaborated for *Mycoplasma gallisepticum* Antibodies.

Table 1. Effect of age on antibody titre of *Mycoplasma gallisepticum* in broilers

Group	Age of birds (days)	Number of samples analysed	ELISA (+)	SPAT (+)	ELISA(%)	SPAT(%)
A	1-10	40	0	4	0%	10%
B	11-20	68	4	11	5.8%	16.1%
C	21-30	117	13	25	11.1%	21.3%
D	31-40	85	9	10	10.5%	11.7%
E	41-50	50	3	7	6%	14%
TOTAL		360	29	57	8.05%	15.8%

The age of bird affected the occurrence of *Mycoplasma gallisepticum* infection in broilers. It was observed that the infection was more prevalent between the age of 20-30 days (Table 1). The prevalence was lowest in first three weeks of age. However, infection prevailed throughout the flock rearing at various levels.

Table 2. Effect of Season on occurance of *Mycoplasama gallisepticum* in Broilers

	ELISA(+)	**ELISA(-)**	**ELISA % for Positive**	**SPAT (+)**	**SPAT (-)**	**SPAT % for Positive**
May	0	72	0%	4	68	5.5%
June	3	69	4.1%	7	65	9.7%
July	4	67	5.5%	10	62	9.7%
August	17	56	23.6%	21	51	29.1%
September	5	67	6.9%	15	57	20.8%
Total	29	331	8.05%	57	303	15.8%

Climatic conditions affected the occurrence of *Mycoplasama gallisepticum* infection. A high prevalence of 23.6% & 29.1% through ELISA & SPAT respectively was observed in the month of August (Table 2). Infection was comparatively lower in the months where humidity level was low i.e. May June and July due to less frequent rain patterns.

Prevalence of Mycoplasma gallisepticum infection in areas of study

Out of 120 analyzed serum samples from Qalandrabad area; thirteen *(n=13)* samples (10.8%) through ELISA & twenty one *(n=21)* samples (29.1%) through SPAT were positive for *Mycoplasma gallisepticum* antibodies.

Table 3. Area wise prevalence of *Mycoplasma gallisepticum* in Broiler birds

Area	Protocol	Total samples analysed	No. of samples positive	Percentage of samples positive
Qalandrabad	ELISA	120	13	10.8%
	SPAT		21	29.1%
Abbottabad	ELISA	120	9	7.5%
	SPAT		19	26.3%
Havelian	ELISA	120	7	5.8
	SPAT		17	23.6

In Abbottabad central; out of 120 analyzed serum samples; nine *(n=09)* samples (7.5%) through ELISA & Ninteen *(n=19)* samples (26.3%) through SPAT were sero-positive for *Mycoplasma gallisepticum* antibodies. The overall prevalence was slightly lower than Qalandrabad area.

The Serology of serum samples *(n=120)* collected from Havelian region showed that only seven *(n=07)* samples (5.8%) through ELISA & seventeen *(n=17)* samples (23.6%) through SPAT were sero-positive for *Mycoplasma gallisepticum* antibodies. It was also noticed that prevalence was lower than both Qalandrabad and Abbottabad areas.

4. Discussion

In the current study, seroprevalence of *Mycoplasma gallisepticum* in three main areas of District Abbottabad (Hazara Region) Khyberpakhtunkhwa Pakistan were broadly studied. A total 360 serum samples were systematically analyzed at Veterinary Research & Disease Investigation Center Abbottabad. Serum samples (*n=360*) were collected from broiler birds in study areas. Birds were divided into five age groups *viz;* Group A =1-10, Group B=11-20 days, Group C=21 to 30 days, Group D=31 to 40 days and group E= 41 to 50 days.

The prevalence of *Mycoplasma gallisepticum* varied with age of birds. High prevalence (21.3%) was seen in group C (20 to 30 days) which supports the results of Hassan *et al*. (2014) on broilers in Faisalabad. However, these results are contrary to the findings of Saba *et al*. (2013) which shows a high prevalence during 4-7 weeks. Similarly, and the studies of Heleili *et al.* 2012 showed more prevalence in broilers at the age of 30-39 days.

It was also observed that the number of positive samples was very low which might be due to previous antibiotic therapy in parent flocks. These results supports the study of Hassan *et al*., (2014) who suggested that it is difficult to identify and isolate *Mycoplasma gallisepticum* infection between the first two weeks of age, because the previous injectable antibiotics therapy which can temporarily suppressed the bacterium. Seasonal variation also affects the occurrence of avian mycoplasmosis. Heleili *et al*. (2011) studied that the occurrence of avian mycoplasmosis was 91.13% in summer. Moreover, abrupt change in climatic conditions can also expose the birds to this disease. The environmental conditions in the study area are moderate however, changes were observed in the month of August due to severe & unexpected rain. So, high prevalence (23.6% by ELISA, 29.1% through SPAT) were recorded in the month of August which is supported by the studies of Abbas *et al*. (2015) who reported that the time period from April to June seemed to be safer for broiler flocks and low incidence of disease was observed.

Comparison between protocols (ELISA and SPAT) used for diagnosis of *Mycoplasma gallisepticum* was also investigated and it was found that the SPAT was found more sensitive (15.8%) than ELISA (8.05%). However, SPAT test is only limited to initial stage of infection detecting IgM antibodies while ELISA was found more specific and excludes the false positive samples through detecting IgG antibodies as also presented in the studies of Ahmad *et al*. (2008).

5. Conclusion

The current study concluded that *Mycoplasma gallisepticum* infection is prevalent in broiler flocks in various areas Abbottabad/Hazara Region which invites the researchers to address the actual causes of its prevalence to reduce economic losses to the poor farmers of the area.

Elisa protocol is considered more specific than SPAT however, it can be used for intial screening of the samples.

Recommendations

Losses can be reduced through proper and timely screening of flocks. Likewise, birds transportation should be monitored through screening for MG. Proper vaccination schedules should be followed for parent stocks and contractors selling infected birds should be banned. An early diagnosis and treatment is recommended where vaccination is not feasible.

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