ISSN (Online): 2091-2609
DOI Prefix: 10.3126/ijasbt

International Journal of Applied Sciences and Biotechnology (IJASBT)

Research Article

Seroprevalence of Mycoplasmosis in Poultry of Bhaktapur

Doj Raj Khanal[1], Eliza Ranjit[1], Sanjeev Kumar Adhikari[1], Bimal Ghemosu[2], Meera Prajapati[1] and Swoyam Prakash Shrestha[1]

[1]Animal Health Research Division, NARC, Khumaltar, Lalitpur, Nepal
[2]Himalayan College of Agricultural Sciences and Technology (HICAST), Kathmandu, Nepal

Abstract

Mycoplasmas are amongst the avian pathogens that causes chronic respiratory and joint diseases incurring a huge economic loss to poultry industry of Nepal. Among different species of Mycoplasmas, we investigate *Mycoplasma gallisepticum-synoviae* from the serum samples of the poultry using Enzyme Linked Immuno Sorbent Assay (ELISA) and dot-ELISA test of ImmunoComb. These tests rely on the antibodies present in the serum samples which binds to the pre-coated antigen in the ELISA/developing plates. A total of 92 sera samples were collected, of which 62 were from broiler and 30 were from layers. Of the 92 samples taken from different farms of Bhaktapur in early 2017, thirty (32.6%) were found positive on standard or routine ELISA and fifty-four (58.7%) on dot-ELISA. It further indicates high rate of infections in broiler (42 on dot-ELISA and 24 on standard ELISA) than in layers (12 in dot-ELISA and 6 on standard ELISA) to *M. gallisepticum* and *M. synoviae*. The present result indicates higher efficacy of dot-ELISA over standard one as the latter could not efficiently detect the infections as compared to the dot-ELISA. Furthermore, this finding poses an alarming threat to the commercial poultry industries of Nepal where there is lack of effective biosecurity practices and preventive vaccination policy.

Keywords: Mycoplasma; gallisepticum-synoviae; ELISA; ImmunoComb

Introduction

Mycoplasma gallisepticum (MG) is a causative agent of chronic respiratory disease (CRD) in chickens and infectious sinusitis in turkeys (Ley, 2008). MG is leading the most significant economic losses in large commercial operations as a result of decreased hatchability and egg production, reduced quality of day-old chicks, reduced growth rate, increased costs of eradication procedures involving site cleaning and depopulation, increased costs of medication and vaccination (Khalda *et al.*, 2013). MG is a persistent, highly transmissible microorganism causing mycoplasmosis, which is characterized by rales, coughing, nasal discharge, sinusitis, and severe air sac lesions (Evans *et al.*, 2005). The MG infection is associated with mortality, carcass condemnation, reduced egg production, hatchability, feed efficiency and weight gain. Transmission in MG infection can be horizontal, from bird to bird, and vertical, from dam to offspring through the eggs (Nascimento *et al.*, 2005). Some factors such as age, size of flock, location, etc., may have effect on severity of this disease (Seifi *et al.*, 2012).

MG can be diagnosed based on morphological and cultural characteristics, biochemical and serological properties

Cited this article as:

D.R. Khanal et al. (2018) Int. J. Appl. Sci. Biotechnol. Vol 6(1): 23-26. DOI: 10.3126/ijasbt.v6i1.19103

[1]***Corresponding author**
Doj Raj Khanal,
Animal Health Research Division, NARC, Khumaltar, Lalitpur, Nepal
Email: drkhanal7@gmail.com

Peer reviewed under authority of IJASBT
© 2018 International Journal of Applied Sciences and Biotechnology

This is an open access article & it is licensed under a Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/)

(Edward, 1960). Serodiagnosis is considered to be effective and reliable methods for determining the exposure of birds to various infectious agents (Alam *et al.,* 2012). Serologic tests commonly used are seroagglutination reaction (SAR), ELISA and hemagglutination inhibition (HI), which can be followed by isolation and identification, and of recently by PCR. However, each method is limited by sensitivity and specificity. Nevertheless, diagnosis by culture is not effective for this disease because the pathogen has many strains (Qasem *et al.*, 2015). Though SAR is used as the screening test, ELISA has been proved to have good sensitivity and more specificity compared to SAR. HI has high specificity but the disadvantages are low sensitivity and non-availability at commercial scale (Kleven, 1998). More recently, ELISA with high sensitivity and specificity has become available (Sun *et al.*, 2014). Still further, dot-ELISA, a solid phase immunoassay based test has emerged as a relatively cheaper, reproducible and cost-effective test than standard ELISA. It requires a minimum equipment, is rapid, easy and user-friendly test having high specificity and sensitivity. The aim of the present study was to determine the seroprevalence of MG in boiler and layer chickens and also to compare the efficacy of standard ELISA versus dot -ELISA test in terms of detecting MG antibody titres.

Material and Methods

Study Site

Study was conducted at Animal Health Research Division (AHRD) in Nepal Agricultural Research Council (NARC) in six months period from January 2017 to June 2017.

Sampling of Farms and Birds

One **c**ommercial layer and broiler farm each suspected of mycoplasmosis from Bhaktapur district were selected purposely for sampling. A total of 92 sera were collected randomly, of which 62 were from broiler and 30 from layers.

Blood Collection and Serum Preparation

Two ml of blood was collected aseptically from wing vein using 3 ml sterile disposable syringe and needle. The syringe with blood was kept undisturbed at room temperature in a standing position for 5 hours to prevent haemolysis of blood. After clotting of blood inside the syringe, serum fluid portion of blood was harvested. The harvested sera were transferred to serum vial and were kept in a cool box before shipping to the deep freeze storage. Then serum samples were shipped to AHRD by keeping serum vial in thermo cool box along with frozen ice packs for the detection of *MG* infection.

Serology

Enzyme Linked Immunosorbent Assay (ELISA)

Sera were screened with Synbiotics commercial test kit (Synbiotics Corporation, USA), following the manufacturer's instructions. Briefly, diluted sera were added onto a MG antigen-coated plate, incubated, washed, and peroxidise labelled antibody (conjugated antibody) was added. After incubation, the plate was again washed before adding a substrate, and adding the stop solution. The plate was read in ELISA reader. The optical density of the negative, positive controls, and samples were calculated and interpreted according to the manufacturers' recommendation. According to the interpretation of Synbiotics ELISA, titre levels 0, and levels equal or higher than 744 were positive, respectively.

Solid Phase Immune Assay (ImmunoCombTest)

Solid phase immunoassay (ImmunoComb) was carried out following manufacturer's instructions. Briefly, sera samples (5 µl each) from chicken were deposited into separate wells in Row A of the developing plate. Positive Control and Negative Control samples supplied in the kit were deposited (5 µl) in separate wells in Row A. Comb was inserted into sample wells and incubated for 10 minutes so that antibodies from samples bound to the antigens on the comb's teeth. After piercing the cover of wells in Row B with the tweezers, comb was gently shaken off onto tissues to remove excess liquid and inserted into wells of Row B for 2 minutes. Same procedure was followed for the remaining rows at the end of each step. Non-bound antibodies were washed off in the second row. The comb was transferred into Row C and incubated for 10 minutes followed by placing in Row D for 2 minutes, Row E for 2 minutes and Row F for 10 minutes for colour development. After the comb has completed the cycle for Row F, it was transferred back to Row E for 2 minutes for colour fixation.

Reading Results Using the Comb Scale

When the comb was completely dried, it was aligned with the calibrated colour CombScale. The tone of the purple-grey on the CombScale that most closely matched the Positive Control spot was found to calibrate the Positive Control (C+) serum for setting cut-off points. The most suitable colour spots of test samples were chosen and read in the yellow windows.

Statistical Analysis

The data were entered and analysed using descriptive statistics, frequency distribution and hypothesis testing was used to determine the significance of associations between categorical variables. In all tests, confidence interval of 95% and significance level of 0.05 were considered. The results were computed by using the computer Statistical Package for the Social Sciences (SPSS -16).Graphical representation and tabulation was done in MS excel- 2007.

Result and Discussions

When ninety-two sera were tested separately by dot and standard ELISAs, the former was able to detect more positive cases (58.69%) than the later (32.6%) as shown in Table 1. In the absence of vaccination practice against Mycoplasmosis, MG antibody titres were found more

among broilers when compared to layers (Table 2) possibly due to highly sensitive nature of broilers to infections compared to layers. Higher cases of mycoplasma infection in broilers in the current investigation indicate a dire need for the adoption of good biosecurity practices for preventing such infections and thereby reducing the economic losses in the commercial poultry industries of Nepal.

When the reliability of serodiagnosis of mycoplasmal infection in terms of detecting MG antibodies by dot and Standard ELISAs was assessed, the former was more reliable and easy to adopt in the field condition. Twenty-six cases that were positive on dot ELISA were turned negative on the standard ELISA whereas only two cases negative to the dot-ELISA was found positive to standard ELISA. This comparative study indicated higher sensitivity of dot-ELISA over standard ELISA and is significantly different as evident on Chi-square test ($p<0.00001$) (Table 3).

Table 1: Seroprevalence of MG antibodies by dot-ELISA and Standard ELISA

Test Methods	Positive cases	Percentage (%)
Dot ELISA (N=92)	54	58.69
Standard ELISA (N=92)	30	32.6

Table 2: Comparison of different test methods for detecting MG antibodies in layers (n=30) and broilers (n=62)

Test Methods	**MG positive layers**	**MG positive broilers**	**Total positive cases**
Dot ELISA	12 (40%)	42 (67.75%)	54
Standard ELISA	6 (20%)	24 (38.71%)	30

Table 3: Comparison of Standard ELISA with dot-ELISA

Standard ELISA	**Dot ELISA**		**Total**	**P-value**
	Positive cases	Negative cases		
Positive cases	28	2	30	0.00001
Negative cases	26	36	62	
Total	54	38	92	

Conclusion

Higher seroprevalence of mycoplasmosis detected among the poultry in Bhaktapur district of Kathmandu valley with 32.6% positivity on standard ELISA versus and 58.7% on dot-ELISA is giving alarming signal to the poultry industry. Timely detection, treatment and effective biosecurity measures are needed to safeguard and enhance the poultry productivity.

Acknowledgement

We would like to thank the staffs of AHRD for their support for successfully accomplishment this research work.

References

Evans JD, Leigh SA, Branton SL, Collier SD, Pharr GT and Bearson SM (2005) Mycoplasma gallisepticum: Current and developing means to control the avian pathogen. *J Appl Poult Res* **14**: 757-763. DOI: 10.1093/japr/14.4.757

Khalifa KA, Sidahmed AE, Badwi M, Mohamed AM (2013) Isolation and Molecular Characterization of Mycoplasma gallisepticum and Mycoplasma synoviae in Chickens in Sudan. *Journal of veterinary medicine* **Article ID 208026:** 4 pages. DOI: 10.1155/2013/208026

Kleven SH (1998) Mycoplasmosis. In: Swayne DE, Glisson JR, Jackwood MW, Pearsonand JE and Reed WM (Eds.) *A Laboratory Manual for the Isolation and Identification of*

Avian Pathogens. 4th Edn., American Association of Avian Pathologists, Kennett Square, pp: 74-80.

Ley DH (2008) Mycoplasma Gallisepticum Infection. In: Fadly AM, Gilson JR, McDougald LR, Nolan LK and Swayne DE (Eds.) *Disease of Poultry*. 12th Eds., Iowa State University Press, Ames, Iowa, pp: 807-834.

Nascimento ER, Pereira VLA, Nascimento MGF, and Barreto ML (2005) Avian Mycoplasmosis Update. *Brazilian Journal of Poultry Science* **7**(1): 1 – 9. DOI: 10.1590/S1516-635X2005000100001

Qasem JA, Al-Mouqati SA, Al-Ali EM, and Ben-Haji A (2015) Application of Molecular and Serological Methods for Rapid Detection of Mycoplasma gallisepticum Infection (Avian mycoplasmosis. *Pak J Biol Sci* **18**(2): 81-87. DOI: 10.3923/pjbs.2015.81.87

Seifi S and Shirzad MR (2012) Seroprevalence and Risk Factors of Mycoplasma gallisepticum Infection in Iranian Broiler Breeder Farm. *International Journal Animal and Veterinary Advance* **4**(1): 45-48.