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Seroprevalence of *Mycoplasma synoviae* in broiler breeder flock

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Abstract
In this study, the sero-prevalence of *Mycoplasma synoviae* (MS) was carried out in breeder flocks of different age groups. A total of 171 serum samples were collected from 14 broiler parent flocks, out of which 57.80% (99/171) were positive for MS antibodies. The prevalence of MS antibodies was 21.25% (17/80) in breeder flocks of young age (0 to 20 weeks), with a mean titer of 1024.9 and a titer range of 0 to 6432. Similarly, the prevalence of MS antibodies was 90.1% (82/91) in breeder flocks of adult age (21 to 60 weeks), with a mean titer of 4390 and a titer range of 0 to 13511. The study showed that the number of positive sera for MS was higher in birds aged 21 to 60 weeks than in birds of 0 to 20 weeks age and indicated that the seroprevalence of MS increased with the increase age of the birds.

Keywords: Seroprevalence, *Mycoplasma synoviae*, ELISA, titre range

1. Introduction
The rapid growth of the poultry industry has led to the expansion of farms and, thereby, an increase in close proximity which resulted into failure to maintain adequate biosecurity measures. This has resulted in inability to prevent disease outbreaks and implement control measures. The poultry industry is at risk of contagious, infectious, and fatal poultry diseases, such as Mycoplasma, which causes mortality, production losses, and increased culling (Sawale, 2019) [14].
Mycoplasma synoviae causes systemic infections leading to infectious synovitis. Carrier birds and vertical transmission are responsible for spreading the disease on poultry farms. The economic impact of the disease is significant, with high morbidity, reduced feed and egg production efficiency, carcass condemnation, and increased costs of disease eradication procedures such as cleaning, depopulation, medication, and vaccination. (Marois *et al*., 2005 [8]; Khalifa *et al*., 2013 [6]; Moreira *et al*., 2017 [10]). Previous studies found that *M. synoviae* causes air-sacculitis in broilers, leading to an increase in slaughter condemnations (Kleven *et al.*, 1972; Hopkins and Yoder, 1982) [7, 4]. Recent research indicates a significant increase in *Mycoplasma synoviae* (MS) causing infectious synovitis in poultry, particularly in broiler breeder populations in India, particularly in state of Telangana (Sawale, 2019) [14]. The goal of the present study was to use sero-diagnostic assays to know the seroprevalence of *Mycoplasma synoviae* in chickens of various farms of different geographic locations in Maharashtra state.

2. Material and Methods
2.1 History collection
In the present study, the birds showing signs of lameness due to hock joint swelling were selected. Detail history of each farm *viz.* age of birds, flock size, morbidity and mortality were collected.

2.2 Collection of blood
Two mililiter (ML) of bood samples was collected from birds showing signs of lameness for *Mycoplasma synoviae* antibody testing using 5 mL sterile disposable syringes and needles. The samples were transferred into a serum vacutainer and left in a slanting position for 30 minutes. The serum was then separated and stored in 1.5-ml Eppendorf tubes labelled with the farm's name and other details, and kept at -20 °C in a zip-lock bag until further use. Total of 171 blood samples were collected from 14 broiler breeder flock.

2.3 Screening of *Mycoplasma synoviae* (MS) antibody by ELISA

Mycoplasma synoviae (MS) antibody was detected from the serum using the commercially available *Mycoplasma synoviae* (MS) antibody test kit (IDEXX, USA) and performed as per the manufacturer's instructions. The relative intensity of the colour which developed was directly proportional to the quantity of MS antibody in the sample (Figure 1). Absorbance (OD) for each well was measured at 650 nm using an ELISA reader (Biotek). Samples showing SP ratios of > 0.50 or titer greater than 1076 were considered positive for MS antibody.

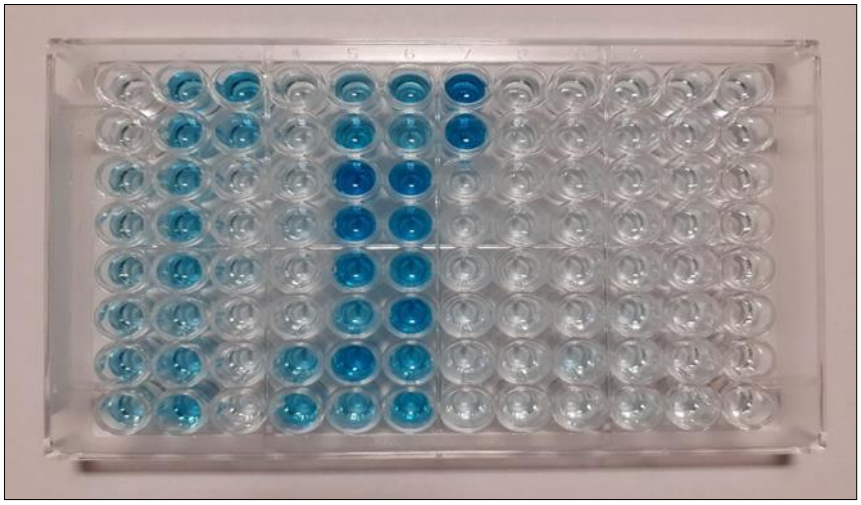

Fig 1: MS serology- Wells in ELISA plate showing no colour (negative) to deep colour development (highly positive)

3. Result and Discussion

The data of individual flock wise mean antibody titer, titer range and coefficient of variation (CV) of the sera samples has been presented in Table 1.

Table 1: Seroprevalence of MS in BP (MS unvaccinated) flocks

S No	Farm code	Age (Wks.)	Total Flock size	Gender	No of sample tested	Per cent positive (positive/total sample)	Titer range	Titer mean	CV
1	F-1	6	15000	F	10	00(00/10)	55-1038	427	61.1
2	F-2	6	1500	M	10	00(00/10)	0-421	219	55.5
3	F-3	11	1500	M	10	10(1/10)	118-1405	537	61.8
4	F-4	11	15000	F	10	00(00/10)	64-534	233	55.1
5	F-5	12.4	1500	M	12	8.33(1/12)	146-1203	527	53.6
6	F-6	17	1500	M	10	00(00/10)	94-1002	794	25
7	F-7	18	11000	M and F	18	83(15/18)	57-6432	2,976	71
8	F-8	35	10000	M and F	19	100(19/19)	1261-6059	3977	35.52
9	F-9	41	11000	M and F	18	100(18/18)	1302-7564	4117	39.73
10	F-10	45	1500	M	10	60(06/10)	0-7074	1962	101.2
11	F-11	49	1500	M	14	100(14/14)	2859-7199	4627	27.0
12	F-12	54	1500	M	10	100(10/10)	3755-12828	7999	36.7
13	F-13	54	16500	F	10	100(10/10)	4145-13511	7063	46.7
14	F-14	58	1500	M	10	50(05/10)	36-7029	2064	104.5

Note: The cutoff point of 1076 and above ELISA titres were considered positive for *Mycoplasma Synoviae*

The serological result carried out by ELISA test showed that 99 out of 171 (57.80%) serum samples were positive for MS antibodies. The age wise sero-prevalence study of MS in breeder flocks revealed 21.25% (17/80) Seroprevalence in breeder flocks of young age (0 to 20 weeks), with a titer range of 0 to 6432 and a mean titer of 1024.9. In contrast, the prevalence of MS antibodies was 90.1% (82/91) in breeder flocks of adult age (21 to 60 weeks), with a titer range of 0 to 13511 and a mean titer of 4390. These findings suggest that adult birds had higher levels of MS antibodies and a wider titer range than younger birds. Moreover, the number of positive sera was greater in birds aged 21 to 60 weeks than in birds aged 0 to 20 weeks, indicating an increase in infection rates with increasing age. The serological data obtained in the present study are in accordance with reports of Seifi and Shirzad (2012) [15], Rajkumar *et al.* (2018) [11], Sawale (2019) [14], Shoaib *et al.* (2019) [16], Yadav *et al.* (2021) [20] and Wei *et al.* (2022) [18] in which they observed Seroprevalence of 47.8%, 52%, 66.36%, 50.13%, 50.32% and 66.53%, respectively. In contrast, lower sero-prevalence of MS was reported by Kapetanov *et al.* (2010) [5], Baksi *et al.* (2016) [2], Michiels *et al.* (2016) [9] and Samojlovic *et al.* (2017) [13] in which they observed Seroprevalence of 36.66%, 41.1%, 26.5% and 40.87%, respectively. However, a higher Seroprevalence of MS was reported by El Ashram *et al.* (2021) [3], Rasool, *et al.* (2017) [12] and Amer *et al.* (2019) [1] in which they observed Seroprevalence of 83.33%, 87.23%, 87.5% and 74%, respectively.

The results indicated that the Seroprevalence and titer range of MS antibodies were increased with increase in age of birds. More number of adults birds were positive for MS antibody than younger age birds. Similar observation of higher

prevalence in older birds was also seen by Seifi and Shirzad (2012) [15], Uddin *et al.* (2016) [17], Baksi *et al.* (2016) [2], Xue *et al.* (2017) [19] and Sawale (2019) [14].

The Seroprevalence of MS antibodies was found both in male and female breeder flocks. Similar finding of higher prevalence weeks than in birds of 0 to 20 weeks age and indicated that the Seroprevalence of MS increased with the increase age of the birds.

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