

# Meta-analysis on the prevalence of Mycoplasmosis in poultry of India and the World

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## Research Article

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# Abstract

Mycoplasmosis, is one of the important disease of poultry industry causing huge economic loss. In the present study, the prevalence of *Mycoplasma gallisepticum* (MG) and *Mycoplasma Synoviae* (MS) in poultry from India and the World was estimated using meta-analysis from the studies during 2010–2020. The meta-analysis of studies from the world showed that overall pooled prevalence estimates for MS (42.52% CI: 33.16–52.16) is more than MG (39.31% CI: 25.76–53.74). In India also, the pooled estimate for prevalence of MS (29.34% CI: 14.40-46.93) is more than MG (25.97% CI: 19.41–33.11). However, the prevalence of both MG & MS is comparatively less in India than other parts of world. Pooled estimate through serological techniques was more for both MG & MS than other diagnostic methods and ELISA was the most reported diagnostic method followed by other methods including serum plate agglutination assay (SPA), PCR and isolation etc. Zone wise prevalence estimate showed that south zone has comparatively higher prevalence (37.37%) for both MG & MS than other zones in India. Continent wise analysis revealed that African continent has more prevalence for both MG (50.09 % CI: 31.75–68.42) and MS (44.79 % CI: 23.26–67.37) followed by others. Overall, the current study indicates higher prevalence of Mycoplasmosis worldwide including India and other countries emphasizing the importance of strict implementation of biosecurity measures and vaccinations.

## Introduction

Mycoplasmosis is an economically important disease of poultry industry worldwide including India. More than 20 *Mycoplasma* spp. of the class Mollicutes are known to infect avian hosts among them *Mycoplasma gallisepticum*-MG and *Mycoplasma Synoviae*-MS are the major pathogens of chicken and are considered economically significant (Ferguson and Noormohammadi 2013). *Mycoplasma meleagridis* and *M. iowae* are limited only to turkeys. MG is known to cause chronic respiratory disease (CRD) in chickens and infectious sinusitis in turkeys (Osman et al. 2009). MS causes infectious synovitis primarily affecting the synovial membranes of joints. In recent times MS infection is more often associated with airsacculitis, egg peritonitis, eggshell abnormalities etc., (Feberwee et al. 2009). Mycoplasmosis cause huge economic loss due to mortality, drop in egg production, poor hatchability, poor FCR, reduced growth rate and carcass condemnation in both broilers and layers (Ferguson-Noel and Williams 2015). Further, secondary bacterial infections, cost involved in vaccination, medication, screening of the flock and depopulation etc., are the additional burden for the poultry producer.

In addition, MG and MS are widespread in many avian species and reported in backyard poultry and in numerous wild bird species (Sawicka et al. 2020; Ayala et al. 2020). Often co-infection and multiple infection with MG/MS complicates the situation. Due to their transmission both by vertical and horizontal mode and persistent infection in the host, mycoplasmosis became quickly endemic in many parts of the world including India. In addition, backyard poultry and wild bird species become infected and remain as reservoir or carrier for these pathogens posing threat to commercial poultry. High intensity poultry rearing areas multi-age flocks and close contact with backyard poultry & wild birds are the major risk factors for

mycoplasmosis. Owing to their immune evasion strategies, antigenic variations in surface lipoproteins and persistent infection status makes their control and eradication programs more difficult (Kleven 2008).

Meta-analyses are helpful in better understanding the disease status and in prioritizing the research focus for the effective control. It has been increasingly used as a tool in improving animal productivity in recent times. In the present study we systematically reviewed the publications available on the prevalence of MG & MS worldwide with emphasis on India during recent decade 2010-20. We performed meta-analysis to obtain the pooled prevalence for India and worldwide.

## **Materials And Methods**

### **Information sources and search**

A systematic search was conducted in three electronic search engines including 'PubMed', 'Google Scholar' and 'Biomed' databases for recent decade from 2010 to 2020. The search terms 'prevalence', 'Mycoplasma', '*Mycoplasma gallisepticum*', '*Mycoplasma Synoviae*', and 'chicken' were used in combinations for systematic electronic searches. Articles were extracted individually by two authors to avoid any bias. All the searches, search strategy, inclusion criteria, data extraction and analysis were performed according to the 'Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol recommended by the Cochrane library (Page and Moher 2017). The reviewed articles from a recent study (Yadav et al. 2021) were also screened for inclusion.

### **Inclusion Criteria**

The inclusion criteria were as follows: (i) Study design- Cross-sectional studies (ii) Sampling procedure- random sampling (iii) timeframe- 2010 to 2020 (iv) language- English (v) Full article available for data extraction (vi) Diagnostic method and sample data mentioned (vii) Published in peer-reviewed journal (viii) studies only on chicken. Retrospective studies, field outbreak studies, experimental infection studies, case reports and review articles were excluded. Studies that investigated other poultry species like turkey, duck, goose, and other migratory birds were also excluded.

### **Data extraction**

A template was prepared to collect and organize the information from selected publications. Data including author name, year of publication, state/country, total sample size, positives, a diagnostic method used, disease investigated (MG/MS) were extracted manually from selected publications and entered in a Microsoft Excel sheet (2016, Microsoft Corporation, WA). Two data sets were prepared for studies from India and worldwide for both MG and MS. All the concerns about the inclusion of a study were resolved by discussion until consensus upon any disagreement.

### **Meta-analysis**

We conducted meta-analyses in R studio of R open-source software (Version 3.4.3). Meta-analysis was performed for the prevalence of MG and MS in chicken in India and worldwide. The outcome was measured and reported as pooled prevalence with point and 95% confidence intervals. The inbuilt software packages 'Metafor', 'MetaProp' and 'Meta R' were used for the analysis. A random effect model was used to calculate the pooled prevalence in the data using the package. DerSimonian and Laird random effect model, Jackson method and Freeman-Tukey double arcsine transformation methods were used for deriving the pooled prevalence at 95% confidence interval (CI) (DerSimonian and Laird 1986). Data was stratified for zone-wise and diagnostic method wise for included studies in India. For worldwide data, continent-wise and diagnostic method stratification was done, and subsequent sub-group meta-analyses were performed. Cochran's Q-statistics,  $I^2$  and P values were computed to measure the heterogeneity among the studies. Between the study variance was assessed by  $\tau^2$ . The effective sizes and weighted average of individual studies were given in forest plots.

## Results

Flow chart representing the selection and inclusion of studies retrieved from public sources is given in Fig.1.

### Meta-analysis of Mycoplasma prevalence from India

A total of 24 publications from India were selected based on the inclusion criteria and subjected for meta-analysis. Out of 24 studies, 11 and 6 exclusively reported on MG and MS, respectively. 6 studies reported the prevalence of both MG and MS (Table 1). Data from 17 and 13 studies were extracted separately for MG and MS respectively for meta-analysis. MG & MS prevalence from India was reported through only three diagnostic methods viz., SPA and ELISA for serology and culture and PCR for pathogen/antigen detection, respectively. 14, and 3 out of 17 studies reported the MG prevalence through single and dual diagnostic methods, respectively. For MS prevalence, 9, 3, and 1 out 13 studies reported the prevalence through single, dual, and multiple diagnostic methods. The most frequent serological test reported was ELISA (7/17 & 5/13 for MG & MS, respectively) followed by SPA. Among the antigen detection methods PCR (9/17 & 8/13 for MG & MS, respectively) was the most reported in the studies followed by culture techniques (2/17 & 3/13 for MG & MS, respectively).

The meta-analysis showed that overall pooled prevalence estimates for MS (29.34% CI: 14.40-46.93) is more than MG (25.97% CI: 19.41-33.11). The  $I^2$ , Q and P value are presented in Table 1. The zone-wise and diagnostic method wise pooled prevalence of MG & MS through subgroup meta-analysis are presented in Table 1. Zone wise prevalence estimate showed that south zone has comparatively higher prevalence (37.37%) for both MG & MS than other zones. Pooled estimate through serological techniques especially ELISA was more for both MG & MS than other diagnostic methods. The forest plots depicting the pooled prevalence estimates for MG and MS are presented in fig. 2 & 3, respectively.

### Meta-analysis of Mycoplasma prevalence from the world

A total of 36 publications from worldwide were selected based on the inclusion criteria and subjected for meta-analysis. Out of 36 studies, 13 and 5 exclusively reported on MG and MS, respectively. 18 studies reported the prevalence of both MG and MS (Table 2). Data from 30 and 23 studies were extracted separately for MG and MS respectively, for meta-analysis. Studies from India were excluded for better comparison. 22 and 7 out of 30 studies reported the MG prevalence through single and dual diagnostic methods, respectively. One study reported MG prevalence by three diagnostic methods. For MS prevalence, 19, 3, and 1 out of 23 studies reported the prevalence through single, dual, and multiple diagnostic methods. The most frequent serological test reported for MG prevalence was ELISA for MG followed by PCR and SPA (each 10/30 studies). For MS, PCR was the most frequently reported method (9/23) followed by ELISA (8/23) and SPA (7/23).

The meta-analysis showed that overall pooled prevalence estimates for MS (42.52% CI: 33.16-52.16) is more than MG (39.31% CI: 25.76-53.74). The  $I^2$ , Q and P value are presented in Table 2. The continent-wise and diagnostic method wise pooled prevalence of MG & MS through subgroup meta-analysis are presented in Table 2. Continent wise analysis revealed that African continent has more prevalence for both MG and MS followed by others. Pooled estimate through serological techniques was more for both MG & MS than other diagnostic methods. The forest plots depicting the pooled prevalence estimates for MG and MS are presented in fig. 4 & 5, respectively.

## Discussion

Mycoplasmosis has become endemic in many parts of world including India and impacts the poultry production. It also causes huge economic loss by causing mortality, drop in production apart from the cost incurred in vaccination, treatment, and biosecurity measures (Ferguson and Noormohammadi 2013). Meta-analysis provides pooled estimate of prevalence of disease despite the variation in data. We performed meta-analysis on the extracted data from systematically reviewed publications on the prevalence of *Mycoplasma gallisepticum* (MG) & *Mycoplasma Synoviae* (MS) in India and worldwide to obtain the pooled prevalence.

Serological assays mainly, ELISA is the most reported method for prevalence studies for both MG & MS in the current analysis. Generally, serological assays including serum plate agglutination (SPA), enzyme linked immunosorbent assay (ELISA) and Hemagglutination inhibition (HI) are often used for screening of MG/MS infections in commercial poultry. SPA a commonly employed serological screening procedure, is a quick, economical, and sufficiently sensitive method. Nevertheless, it detects IgM indicating any recent infection and lacks specificity due to cross-reaction (Kleven 2008). On the other hand, HI and ELISA detect IgY and IgG from post-infection. Hence, prevalence estimates of MG & MS by these three serological techniques varies. In general, serological method of detection is more compared to antigen detection as the antibodies are detected for longer period. ELISA is the most employed assay in prevalence studies in recent times owing to the increased availability of commercial kits and better access to laboratories. The studies on MG & MS steadily increased over the years during the decade owing to the diagnostic facilities and awareness.

Prevalence rates of MG & MS in India and world were comparatively lesser in antigen detection methods than serology. Serological assays detect both past and present infections whereas antigen detect methods identifies only current infections. Culture method is generally considered as gold-standard for the confirmatory diagnosis of *Mycoplasma* infections. Nevertheless, the isolation of *Mycoplasma* through culture techniques has limitations. Slow growth in selective media and overgrowth with fastidious *Mycoplasma* species are the major issues limiting their use in definite diagnosis (Umar et al. 2017). Moreover, it also needs subsequent PCR for *Mycoplasma* species identity and confirmation. Among the antigen detection techniques, PCR is rapid, sensitive, and effective method of detection either from direct clinical samples or culture. In addition, they are also valuable in epidemiological investigation about the origin and relationship among the strains. Certain latest antigen detection techniques such as loop mediated isothermal amplification (LAMP) and lateral flow assay (LFA) are recently developed for quick testing of *Mycoplasma* infection. Their scope in routine screening is yet to be explored.

MG is one of the best examples of bacterial pathogen spillover to wild birds. MG spilled over to house finches causing MG epidemic in North America and subsequently became endemic in house finches and wild turkey (Ley et al. 2016; Sawicka et al. 2020). Potential spillback of MG from house finches to poultry has also been demonstrated indicating risk for commercial poultry (Pflaum et al. 2017). A recent systematic review showed that MG was present in 56 wild bird species emphasizing the ubiquitous nature of this pathogen (Sawicka et al. 2020). In addition, MG and MS were also commonly detected in backyard chickens and is considered as an important reservoir of different MG/MS strains (Junior et al. 2017). Although only 7 out of 36 studies from world reported the prevalence in backyard poultry, their significance in acting as a link between domestic chicken and wild birds for the spread of *Mycoplasma* warrants more research focus. High heterogeneity (85–99%) in the present analysis arise due to high diversity among the studies including study design, varying sample size, different diagnostic methods used etc.

The prevalence estimates for both MG & MS was comparative higher in south zone than other zones. The south zone includes states that contribute nearly 60% of poultry population. High intensity of commercial poultry may attribute to this higher prevalence facilitating the spread of *Mycoplasma* among the poultry population. Under reporting from certain zones such as East and north-east zones coincide with sparse commercial poultry population and limited access to research institutes and diagnostic facilities. In India, the prevalence of MG and MS vary in different states and ranges between 10–55% and 2–52%, respectively (Prajapati et al. 2018). However, in the present study, the pooled prevalence of both MG & MS was less than the worldwide countries indicating better situation in India.

African continent had higher pooled prevalence of both MG and MS followed by other continents. Lower prevalence in American continent indicates extensive use of vaccination, biosecurity, and other measures. However, the higher prevalence in North America is in conformity to earlier reports. Higher occurrence of MG in wild birds in more than 38 species may be one of the attributing factors for the wider prevalence in domestic poultry (Sawicka et al. 2020). Less number of studies from countries of Europe and Oceania continents indicates either better control or underreporting and the less emphasis given on these

diseases. It is interesting to note that MS is more prevalent than MG contrast to the perception that MG is more problematic and cause more economic loss. It may be because the disease presentation by MS is mostly unnoticed and complex than understood.

Currently, live attenuated, inactivated / killed, and recombinant vaccines are available for protection against mycoplasmosis in poultry (Kleven 2008). Killed vaccines are reported to be effective in controlling the vertical transmission and drop in egg production in breeders (Ferguson-Noel et al. 2012). However, killed vaccines have limitation in inducing cell mediated immunity that plays a role in controlling the intracellular pathogen. Live vaccine strains such as F, 6/85, ts-11 & K for MG and MS-H & MS1 for MS are available worldwide and widely used (Kleven et al. 2008; Umar et al. 2017). Although, the live vaccines competitively exclude the virulent field Mycoplasma strains and colonizes in the upper respiratory tract and persistently induce immune response in the flocks; these vaccines sometimes induce mild to severe reactions and gets eliminated by using certain group of antibiotics for other ailments (Ferguson-Noel et al. 2020). Moreover, they effectively colonize only in Mycoplasma free stocks. Mostly live vaccines and sometimes killed vaccines are used in India restricted only to breeder flocks in the last few years. Recombinant vaccines such as Fowl pox as vector are of promising future alternate candidates and their efficacy in commercial poultry are yet to be explored (Leigh et al. 2013). Macrolide antibiotics including tiamulin, tylosin, tylvalosin and tilmicosin are generally used in effective control and treatment of Mycoplasma infections (Morrow et al. 2020). However, cost and development of resistance are the limiting factors in their use (Nhung et al. 2017). Biosecurity measures, screening, maintain Mycoplasma free stocks, in combination with effective vaccination program are the promising strategy for the control of Mycoplasma infection in poultry stocks.

The prevalence estimate from the current study shall be viewed with little caution, as it does not provide the estimate for the association of risk factors with the disease prevalence. Various factors such as poultry density, age, production systems etc. play a pivotal role in the outcome of the Mycoplasma spread and infection. The overall prevalence data on mycoplasmosis in poultry will help in devising strategies for effective biosecurity measures, vaccination policies and treatment procedures by the various stakeholders including policy makers, government agencies and poultry farmers for improving the poultry production and to minimize the economic loss.

## Declarations

**Author contribution** TRK conceived and designed the work. EP and YN collected the published papers and extracted data. TRK analyzed data and wrote the manuscript and SH critically reviewed the manuscript. All authors read and approved the manuscript

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**Conflict of Interest** The authors declare that they have no conflict of interest.

**Data availability** The data underlying the results presented in the study are available from the published articles given in supplementary files

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## Tables

**Table 1** Pooled prevalence of *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) in chicken of India

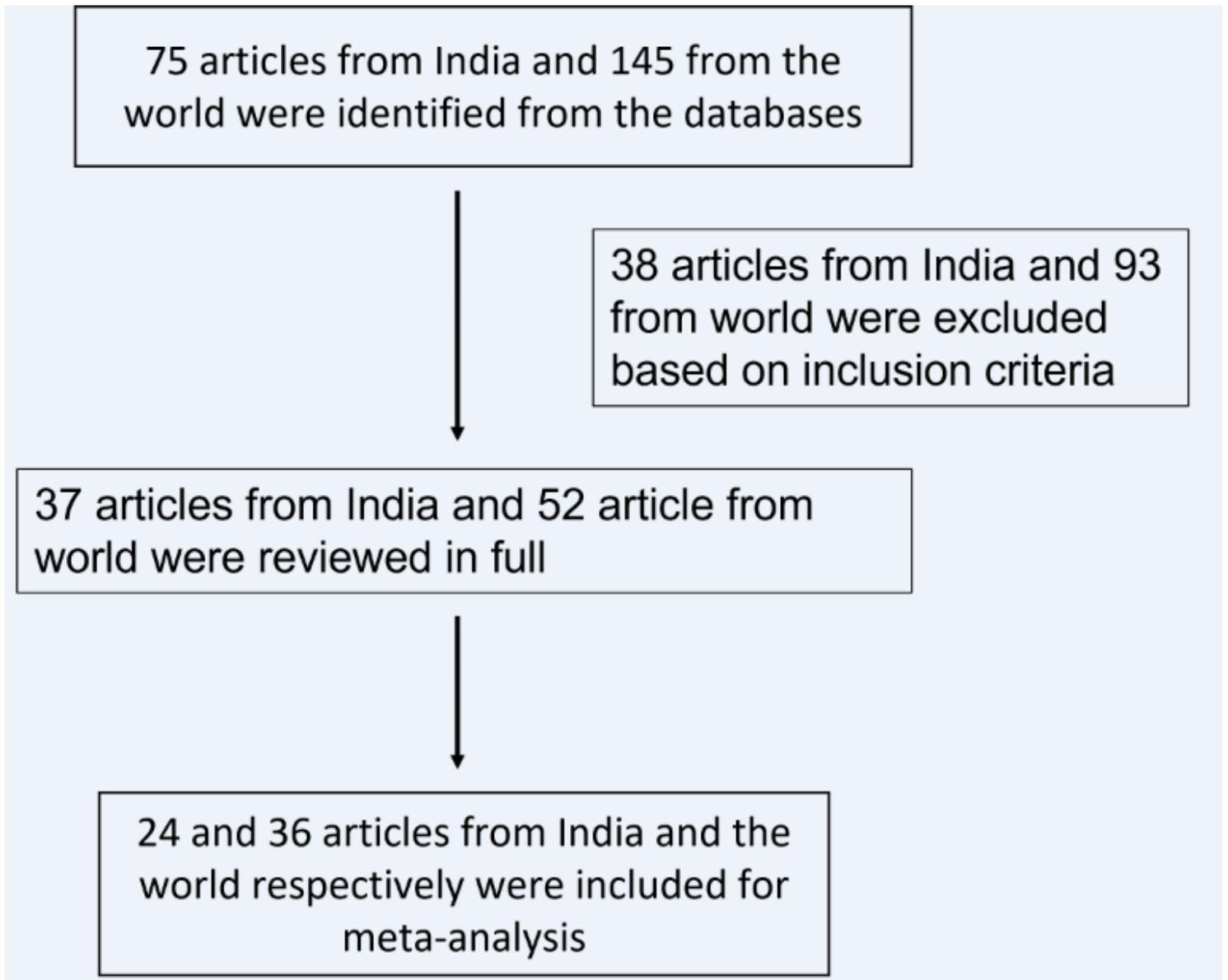
S. No	<i>Mycoplasma gallisepticum</i> (MG)	No. of studies	Pooled prevalence (CI at 95%)	I <sup>2</sup> %	Tau <sup>2</sup>	DF	Q statistics	P value
	<b>Overall prevalence</b>	17	25.97 (19.41-33.11)	97.2	0.0246	16	580.49	<0.0001
<b>1</b>	<b>Diagnostic method wise</b>							
a.	ELISA	7	35.44 (27.7-43.4)	96.2	0.0112	6	155.92	<0.0001
b.	PCR	9	19.97 (14.19-26.44)	89.6	0.0110	8	76.57	<0.0001
c.	Isolation	2	12.07 (8.6-15.9)	32.4	0.0007	1	1.48	0.2237
d.	SPA	2	35.23 (13-61.49)	96.3	0.0351	1	26.77	<0.0001
<b>2</b>	<b>Zone wise</b>							
a.	North	5	20.77 (12.81-30.01)	79.6	0.0113	4	19.58	0.0006
b.	South	6	37.37 (22.13-54)	98.7	0.0420	5	383.68	0.0001
c.	East	0	-	-	-	-	-	-
d.	West	1	10	-	-	-	-	-
e.	Central	2	17.83 (10.75-26.16)	36.3	0.0019	1	1.57	0.2101
f.	Multi-state	3	24.12 (15.76-33.63)	96.7	0.0083	2	59.84	<0.0001
S. No	<i>Mycoplasma synoviae</i> (MS)	No. of studies	Pooled prevalence (CI at 95%)	I <sup>2</sup>	Tau <sup>2</sup>	DF	Q statistics	P value
	<b>Overall prevalence</b>	13	29.34 (14.40-46.93)	99.2	0.1074	12	1555.95	0
<b>1</b>	<b>Diagnostic method wise</b>							
a.	ELISA	5	66.12 (48.29-81.90)	98.7	0.0390	4	313.5	<0.0001
b.	PCR	8	20.19 (6.31-39.03)	98.0	0.0844	7	352.86	<0.0001
c.	Isolation	3	49.22 (1.58-97.9)	98.7	0.2894	2	150.99	<0.0001
d.	SPA	2	35.49 (6.54-72.19)	97.3	0.0709	1	37.43	<0.0001
<b>2</b>	<b>Zone wise</b>							
a.	North	4	20.77 (12.81-30.01)	79.6	0.0113	4	19.58	0.0006
b.	South	5	37.37 (22.13-54.0)	98.7	0.042	5	383.68	<0.0001
c.	East	0	-	-	-	-	-	-
d.	West	1	20	-	-	-	-	-
e.	Central	0	-	-	-	-	-	-
f.	Multi-state	3	27.73 (12.29-46.54)	98.7	0.0292	2	159.52	<0.0001

**Table 2. Worldwide pooled prevalence of *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) in chicken**

S. No	<i>Mycoplasma gallisepticum</i> (MG)	No. of studies	Pooled prevalence (CI at 95%)	I <sup>2</sup> (%)	Tau <sup>2</sup>	DF	Q statistics	P value
	Overall	30	39.31 (25.76-53.74)	99.8	0.1597	29	11863	0
<b>1</b>	<b>Diagnostic method wise</b>							
a	ELISA	12	46.78 (22.88-71.48)	99.9	0.1993	11	9101	0
b	PCR	10	26.49 (13.94-41.29)	98.3	0.0594	9	540	<0.0001
c	Isolation	5	35.57 (19.69-53.19)	95.8	0.0354	4	94.13	<0.0001
d	SPA	10	42.16 (21.18-64.71)	99.5	0.128	9	1858	0
<b>2</b>	<b>Continent wise</b>							
a	Asia	13	37.94 (23.24-53.86)	99.6	0.0856	12	3344	0
b	Africa	11	50.09 (31.75-68.42)	98.7	0.0941	10	761	<0.0001
c	Europe	2	13.80 (0-62.34)	99.8	0.1452	1	500	<0.0001
d	North America	2	36.02 (0-97.28)	98.8	0.2830	1	83.63	<0.0001
e	South America	2	25.51 (0-83.58)	99.5	0.1996	1	186	<0.0001
f	Oceania	-	-	-	-	-	-	-
S.No	<i>Mycoplasma synoviae</i> (MS)	No. of studies	Pooled prevalence (CI at 95%)	I <sup>2</sup> (%)	Tau <sup>2</sup>	DF	Q statistics	P value
	Overall	23	42.52 (33.16-52.16)	99.4	0.0517	22	3643	0
<b>1</b>	<b>Diagnostic method wise</b>							
a.	ELISA	8	62.6 (46.53-77.38)	99.5	0.0506	7	1409	<0.0001
b	PCR	9	26.47 (14.09-41.02)	98.3	0.0515	8	479	<0.0001
c	Isolation	3	26.69 (18.89-	73	0.004	2	7.41	0.0246

d	SPA	7	35.26) 34.99 (16.24- 56.47)	99.3	0.0819	6	920	<0.0001
<b>2</b>	<b>Continent wise</b>							
a	Asia	8	35.29 (22.54- 49.18)	99.5	0.0385	7	1486	<0.0001
b	Africa	8	44.79 (23.26- 67.37)	98.6	0.1035	7	484	<0.0001
c	Europe	3	38.22 (9.19- 73.03)	99.7	0.0996	2	644	<0.0001
d	North America	1	69.04	-	-	-	-	-
e	South America	2	45.79 (0- 100)	99.7	0.4389	1	335	<0.0001
f	Oceania	1	68.75	-	-	-	-	-

## Figures



**Figure 1**

Flowchart showing the studies from India and world on prevalence of mycoplasmosis included for meta-analysis

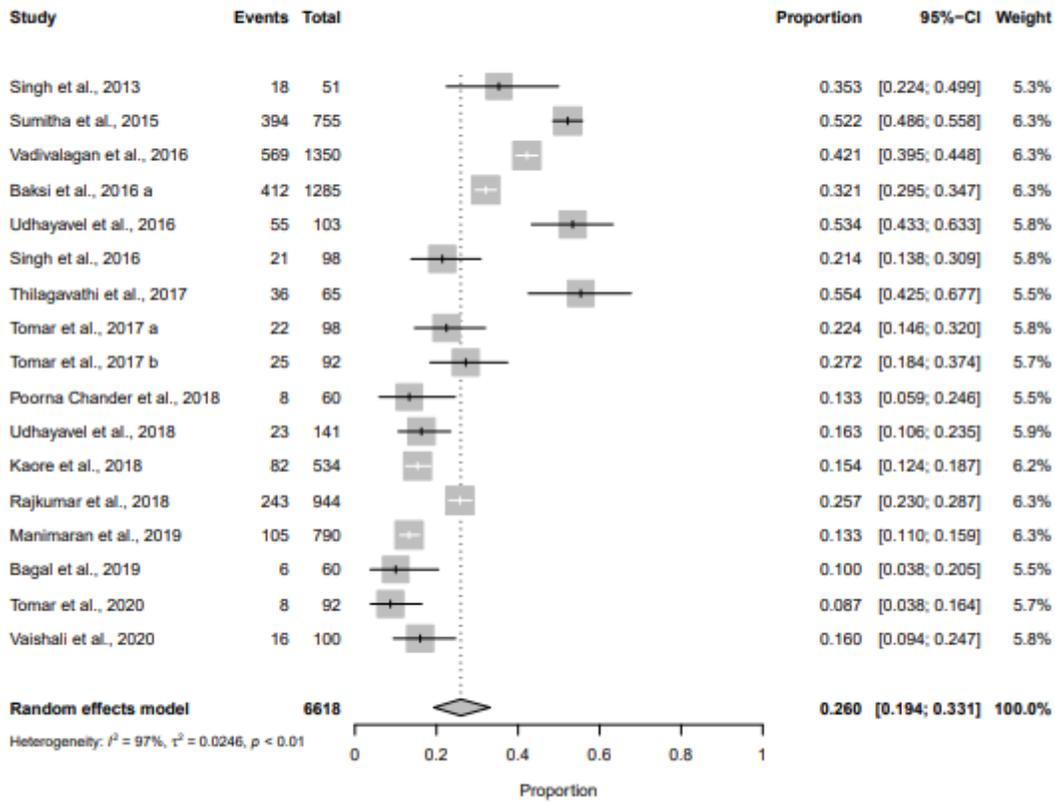


Figure 2

Forestplot of Mycoplasma Gallisepticum (MG) prevalence in chicken of India

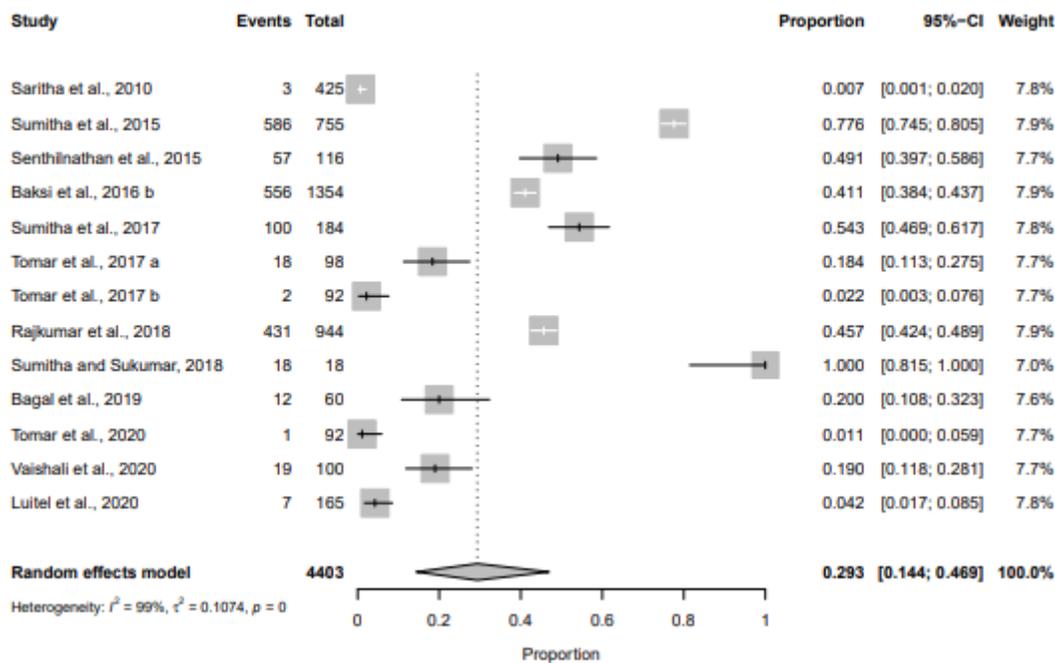


Figure 3

Forestplot of Mycoplasma Synoviae (MS) prevalence in chicken of India

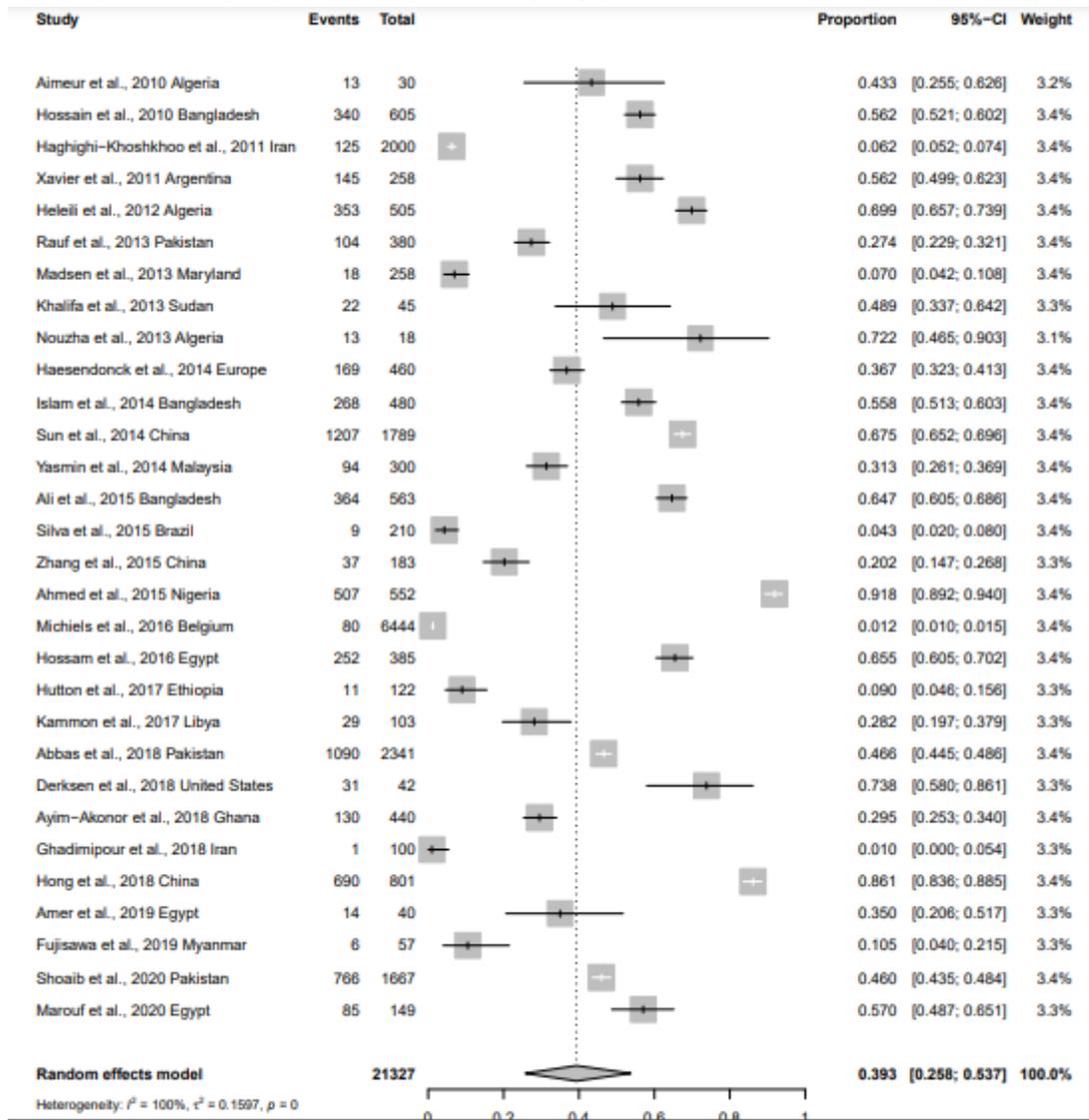


Figure 4

Forestplot of Mycoplasma Gallisepticum (MG) prevalence in world

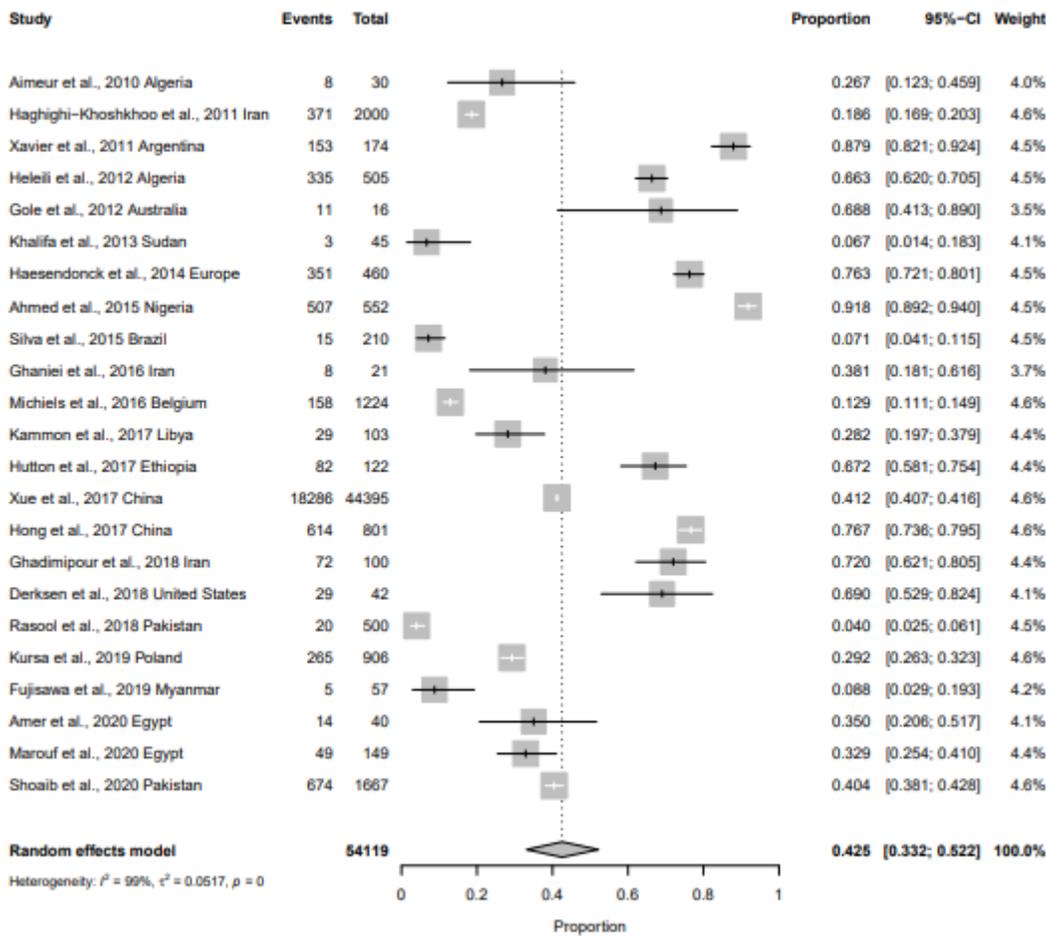


Figure 5

Forestplot of Mycoplasma Synoviae (MS) prevalence in world

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTable1Indiastudies.docx](#)
- [SupplementaryTable2worldstudies.docx](#)
- [Supplementaryfile1.docx](#)