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REVIEW

Pooled molecular occurrence of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in poultry: A systematic review and meta-analysis

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Abstract

Worldwide, *Mycoplasma gallisepticum* (MG) and *M. synoviae* (MS) are the main agents responsible for chronic respiratory disease in poultry. Therefore, we conducted a systematic review and meta-analysis to estimate their occurrence. We searched electronic databases to find peer-reviewed publications reporting the molecular detection of MG and MS in poultry and used meta-analysis to estimate their pooled global occurrence (combined flock and individual), aggregating results at the regional and national levels. We performed a subgroup meta-analysis for subpopulations (broilers, layers, breeders and diverse poultry including turkeys, ducks and ostriches) and used meta-regression with categorical modifiers. We retrieved 2294 publications from six electronic databases and included 85 publications from 33 countries that reported 62 studies with 22,162 samples for MG and 48 studies with 26,413 samples for MS. The pooled global occurrence was 38.4% (95% CI: 23.5–54.5) for MS and 27.0% (20.4–34.2) for MG. Among regions, Europe and Central Asia had the lowest occurrence for both pathogens, while MG and MS were highly prevalent in South Asia and sub-Saharan Africa, respectively. At the national level, MG occurrence was higher in Algeria, Saudi Arabia and Sudan, whereas China, Egypt and Ethiopia reported higher values of MS. Among the poultry subpopulations, MS and MG were more prevalent in the breeders and layers (62.6% and 31.2%, respectively) than in diverse poultry. The year of publication, the sample size and the level of ambient air pollution (measured indirectly by PM2.5) were associated with the occurrence of both mycoplasmas. Our study revealed high and heterogeneous occurrence values of MG and MS and justifies the need for early detection and improved control measures to reduce the spread of these pathogens.

KEYWORDS
avian mycoplasmosis, avian respiratory disease, bacterial infection, molecular detection, systematic study

1 | INTRODUCTION

Avian mycoplasmosis is caused by several pathogenic mycoplasmas among which *Mycoplasma gallisepticum* (MG) and *M. synoviae* (MS) are the most important. Both pathogens are listed and notifiable to the World Organization for Animal Health (World Organization for Animal Health [OIE], 2019). MG causes chronic respiratory disease in poultry and stands as one of the major causes of economic losses to this industry (Hennigan et al., 2012). Economically, MG is the most important species of mycoplasma because it might cause estimated annual losses of 140 million dollars in the United States for egg production alone (Peebles et al., 2006), while in broilers a single company in North Carolina, USA, estimated losses due to MG as high as $750,000 over 6 months (Evans et al., 2005). MS has been traditionally considered as the second most important avian mycoplasma species of commercial chickens from the clinical and economical point of view (Feberwee et al., 2009). MS infection is generally associated with poor growth and significant downgrading of carcasses (Landman, 2014), a decline in egg production (Stipkovits & Kempf, 1996), and the induction of eggshell apex abnormalities (Kursa et al., 2019). MS infection frequently occurs as a subclinical upper respiratory disease, though it can cause severe airsacculitis or synovitis when coinfections occur with other viral or bacterial pathogens (Landman & Feberwee, 2001). Thus, a major inflammation caused by MS is synovitis that occurs in the synovial tendon sheath and joint synovium (Morrow et al., 1990). Chickens with infectious synovitis may exhibit pale combs, lameness, and retarded growth and swellings may occur around joints (World Organization for Animal Health [OIE], 2019), which usually contain a viscous creamy exudate in the joint and the tendon sheaths, along with hepatosplenomegaly and mottled swollen kidneys (Kleven & Ferguson-Noel, 2008). Some of the pathological changes include extensive scattered and focal inflammatory cell infiltration of the tendon sheath synovial layer (Xu et al., 2020).

The efficient containment of these two pathogens requires identifying infected birds to reduce the risk of transferring the infection to healthy birds, as well as to prioritize care and control measures on geographical regions in which MG and MS are highly prevalent. Currently, there are several reports of the seroprevalence of mycoplasmas in poultry farms around the globe (Ali et al., 2015; Feberwee et al., 2008; Rehman et al., 2018; Xue et al., 2017). However, they are based on a serological test that may lack specificity or sensitivity (World Organization for Animal Health [OIE], 2019) or also might be inaccurate for the detection of avian mycoplasmosis because the serological tests cannot be used to determine whether the presence of antibodies is due to infection with a field strain or the result of immunoprophylaxis (Kursa et al., 2016). Besides, serological screening might not detect subclinical MS infections, and monitoring programs that depend solely on detecting seroconversion might be inadequate (Kleven et al., 2001).

An alternative for the conventional diagnostic techniques is the use of molecular methods that allow a reliable and precise detection of specific strains of mycoplasma in poultry (World Organization for Animal Health [OIE], 2019). Nevertheless, molecular methods should not be a substitute but a complement for the traditional serologic surveys and the identification of mycoplasma pathogens by standard culture techniques. The use of molecular methods allows not only quick and precise laboratory diagnostics in birds by detecting mycoplasma infections, but also enables further characterization of the field isolates, as well as confirmation or exclusion of the results from serological tests (Kursa et al., 2016). Nevertheless, no study has focused on estimating the pooled molecular occurrence of MG and MS reported in the published scientific literature. Such information might be useful at the regional and national levels to (1) estimate the magnitude of the molecular occurrence of mycoplasmosis in poultry, (2) mapping the regions and countries in which MG and MS are highly prevalent, and (3) design tailored strategies for identifying flocks at higher risk for contamination or establishing breeding programs with pathogen-free flocks (Moronato et al., 2018) as well as provide prophylactic measures such as vaccination and medication (Zhao et al., 2018).

In the present study, we conducted a systematic review and meta-analysis to estimate the pooled global occurrence of MG and MS infection in poultry. We aimed to summarize the available literature that reports the molecular occurrence of these two pathogens in poultry to both assess the magnitude of the infection and further serve as a point of comparison to the available seroprevalence estimations of avian mycoplasmosis. The results from this study might provide a valuable guide for selecting regions and countries of high occurrence in which to implement and improve regular surveillance and intervention strategies aimed to diminish the burden and the economic impact of avian mycoplasmosis.

2 | METHODS

2.1 | Protocol and objectives

This study was conducted following a protocol developed a priori by MACI and DD according to the Preferred Reporting Items for Systematic reviews and Meta-analysis Protocol (PRISMA-P) statement (Moher et al., 2015). The protocol is available on the Open Science Framework website (https://osf.io/kya5c/?view_only). We conducted a systematic review and meta-analysis to address the question: What is the pooled, regional and national occurrence of MG and MS infection in poultry? Our study was conducted according to Cochrane guidelines (Higgins et al., 2019) and is reported following the PRISMA statement (Liberati et al., 2009).

2.2 | Eligibility criteria

The inclusion criteria for the publications were as follows: (1) the publication included a population of poultry differentiated according to the zootechnical function in layers, broilers, breeders (for both broilers and layers), or a category named 'diverse poultry' that included other bird species such as turkey, duck or ostrich but excluded wild, song or ornamental birds, (2) the samples from the bird populations (individual or

flock levels) were tested for the presence of MG or MS infection by molecular methods, (3) the publication reported the occurrence of MG- or MS-positive birds/samples or presented raw data from which the occurrence was estimated (number of positives/total assessed) and (4) the studies were primary peer-reviewed publications in English, Portuguese or Spanish available in full-text and the types of studies were cross-sectional, case study or retrospective studies without imposing temporal or regional limitations. To ensure optimal methodological comparability among studies, we included journals considered mostly in the Scimago Journal & Country Rank (SJR) and no grey literature (i.e. unpublished studies, reports, conference proceedings or thesis) was included in the study (van Driel et al., 2009).

2.3 | Information sources and search methodology

PubMed, Web of Science, CAB Abstract, Science Direct, Virtual Health Library (VHL) and SciELo were searched to find relevant publications. This three-stage process was completed from September 1 to October 2, 2019: first, the review team defined a thesaurus composed of a broad variety of terms; second, MACI and DD tested these terms in pilot searches to select the most relevant search terms that allowed more specific and sensitive searches; third, MACI conducted the final electronic database searches. The final search terms were defined according to the population (poultry OR chick OR breeder OR broiler OR bird OR turkey), the study factor (chronic respiratory disease OR avian mycoplasma OR avian respiratory pathogen OR mycoplasma gallisepticum OR mycoplasma synoviae OR mycoplasmosis) and the outcome (occurrence OR epidemiology OR molecular epidemiology OR molecular occurrence OR molecular surveillance OR PCR-occurrence OR molecular confirmation OR 16S gene). Boolean operators (AND, OR and NOT) were used for the search command, which was defined as follows: (population) AND (study factor) AND (outcome) and methodological filters within each database were used to refine the search process. Representative full searches per database are presented in Web Appendix 1 of the supplementary material.

2.4 | Selection process

After the searches were completed, the records were gathered into a single EndNote X9 file (Thomson Reuters, USA) and then the duplicates were removed both automatically and manually by MACI. Once duplicates were removed, this reviewer conducted the screening process, first based on the title and then on the abstract. Next, MAIC and DZV independently used a standardised questionnaire based on the eligibility criteria to select publications for final inclusion (Web Appendix 2). Before trial eligibility, the questionnaire was piloted in 10% of the publications randomly selected from the database. Before discrepancies between MAIC and DZV were corrected by DD, we found a moderate agreement rate according to a Cohen's Kappa value of 0.618 ($T = 7.34$, $p < .000$).

2.5 | Data extraction

MACI extracted data from the selected publications using a predefined format based a priori on the eligibility criteria (Web Appendix 3). The standardised questionnaire was piloted in 10% of the randomised publications from the database. The main characteristics of the publications were extracted to construct summary tables in Excel that included study (author and year of publication), the geographic region where the study was conducted, characteristics of the population and zootechnical function (breeders, laying hens, broilers and diverse poultry 'ducks, turkeys or ostriches'), type of sample assessed (tissue or swab), diagnostic technique, genomic region target and outcome (number of birds positive for MG or MS infection divided by the total size of the samples assessed). The occurrence of MG or MS infection was considered the only outcome measure. In the cases where publications included data at both the individual and flock level, we chose individual data because we found a more consistent and accurate estimation in comparison to flock level data. Publications that assessed independently both MG and MS were extracted separately and counted as one study per species. Also, some studies reported an overall occurrence for MG or MS and included occurrence values stratified by subpopulation, that is, breeders, laying hens, broilers and diverse poultry. In addition, we included a 'mixed subgroup' to indicate those studies that included at least two categories of poultry (laying, broilers, breeders or diverse poultry) and did not report differentiated occurrence values per subpopulation. Therefore, for some studies, we extracted up to eight occurrence values distributed in the two pathogens and the four subpopulations. There was no confirmation of the data with the authors. However, DZV confirmed the extracted information for all studies and corrected any discrepancies.

2.6 | Assessment of the risk of bias in individual studies

We used a modification of the Cochrane evaluation tool (Higgins et al., 2019) to assess the risk of bias in the individual studies. The studies were rated as having a low, high or unclear risk of bias according to the following criteria: (1) appropriate definition of the population included in the study, (2) alternative technique for diagnosis of avian mycoplasmosis, (3) use of a molecular method that specifies the target genomic region of MG or MS in poultry, (4) evaluation of all the positive and negative samples included in the study, (5) consistency of the report (no discrepancies in results) and (6) selective reporting (omission of missing data by samples without tracking).

2.7 | Summary measures and statistical analysis

The occurrence of MG and MS in poultry was quantitatively summarized using a meta-analysis of proportions to obtain a pooled estimation from individual studies using the Freeman–Tukey double

arcsine transformation to stabilize variance (Barendregt et al., 2013) with 95% exact confidence intervals (95% CI) (Nyaga et al., 2014). Because of the expected heterogeneity across the studies, we defined a priori a random-effects model (D-L) (Nikolakopoulou et al., 2014). We used subgroup meta-analyses to aggregate independent studies first at the national level and second at the regional level according to the seven regions defined by the World Health Organization (WHO) (WHO, 2003): sub-Saharan Africa, Middle East and North Africa, North America, Latin America and the Caribbean, Europe and Central Asia, South Asia and East Asia and Pacific. As described elsewhere (Diaz et al., 2019; Romo-Barron et al., 2019), we used a significance test for overall effect with z statistic (effect size = 0) and assessed significant heterogeneity across trials with Cochran's Q statistic (X^2 test). Finally, we used the I^2 statistic to determine the proportion of variation in the effects due to variations in true effects rather than sampling error (Borenstein et al., 2017). We did not perform publication bias assessment with funnel plots because they are inaccurate in a meta-analysis of proportions (Hunter et al., 2014).

2.8 | Meta-regression

To determine whether the study characteristics partially explained the heterogeneity in the estimated Mycoplasma occurrence, we used a random-effects meta-regression analysis (Harbord & Higgins, 2008). We constructed several univariable models including each covariate and then selected those that were significant. We included the avian subpopulation, the quartile of the avian population for each country (FAOSTAT, 2020), the year of publication, the sample size of the study, the alternative techniques used for the diagnosis (serological, bacterial isolation, clinical/histological or molecular), the continent, the latitude, the mean variation in temperature. Additionally, we included the official reports of the annual mean concentration of particulate matter lower than 2.5 microns (PM2.5, $\mu g/m^3$) per country (WHO, 2020), we chose these particles as a proxy of the exposure to ambient air pollution because this particle size penetrates deeply into the respiratory tract and therefore constitute a risk for both animal and human health (WHO, 2020). All analyses were performed using Stata 12 (StataCorp, TX, USA), and the graphs were constructed using Prism 9 (GraphPad, Inc., CA, USA). A value of $p < .05$ was considered significant.

3 | RESULTS

3.1 | Study selection

A total of 2294 records were retrieved from electronic database searches. Web of Science, Science Direct and CAB abstracts provided 78.2% of the records. After duplicate removal, 1669 records remained for screening based on title and abstract, of which 145 publications were retrieved in full text for eligibility assessment. A total of 85 publications met the inclusion criteria and were selected for inclusion in the narrative synthesis. These 85 publications included 62 and 48 studies

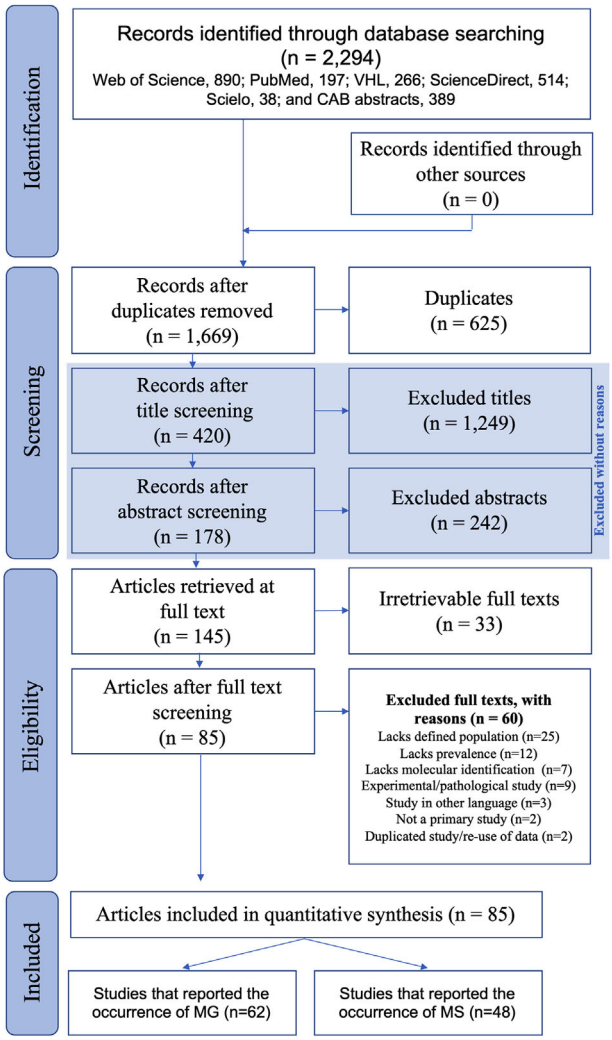

FIGURE 1 PRISMA flow chart for the selection of studies included in the systematic review and meta-analysis

that specifically assessed MG and MS, respectively (Figure 1). Among the 60 publications that were excluded, lacking the defined population and the absence of the outcome were the main reasons for exclusion. A list with the primary reasons for exclusion is summarised in Web Appendix 4, whereas the references for the 85 publications included in our study are shown in Web Appendix 5.

3.2 | Main characteristics of the studies

The 85 publications belong to 33 countries distributed across the seven WHO regions, mainly from Asia and Europe (42 and 16 articles, respectively). Pakistan, Iran, Brazil, Egypt and Turkey provided 40/85 articles. The 62 and 48 studies that specifically assessed MG and MS provided data on 22,162 and 26,413 bird samples respectively, with a median for both species of 64 samples per study (25.5 and 190, 25th and 75th percentile, respectively). As summarized in Table 1, for both pathogens, the publications from the Middle East and North Africa region contributed the highest number of studies (25), followed by publications from the

TABLE 1 Pooled global, regional and national occurrence of MG and MS in poultry, estimated from results extracted from 85 publications that included 110 studies (62 for MG and 48 for MS) from 33 countries

WHO Region/country	Studies	Positives/samples	MG occurrence (95% CI)	Studies	Positives/samples	MS occurrence (95% CI)
Pooled	62	3935/22,162	27.0 (20.4–34.2)	48	19,783/26,413	38.4 (23.5–54.5)
Sub-Saharan Africa	4	21/72	26.5 (1.6–62.8)	2	13/22	61.5 (39.4–81.6)
Ethiopia	1	2/11	18.2 (2.3–51.8)	1	10/11	90.9 (58.7–99.8)
Ghana	1	0/20	0.0 (0.0–16.8)	–	–	–
Sudan	1	8/11	72.7 (39.0–93.9)	1	3/11	27.3 (6.0–60.9)
Zimbabwe	1	11/30	36.7 (19.9–56.1)	–	–	–
North America	4	105/397	22.1 (10.3–36.5)	3	142/333	44.6 (33.8–55.6)
Canada	1	35/151	23.2 (16.7–30.7)	1	54/151	35.8 (28.1–43.9)
USA	3	70/246	21.4 (3.9–46.8)	2	88/182	48.3 (41.0–55.7)
Latin America and the Caribbean	8	107/1340	17.2 (3.1–38.1)	8	584/1528	40.8 (28.8–53.3)
Brazil	7	71/1249	14.3 (1.6–34.6)	7	541/1437	39.7 (26.1–54.1)
Colombia	1	36/91	39.6 (29.5–50.4)	1	43/91	47.2 (36.7–58.0)
South Asia	13	2241/6415	37.3 (26.2–48.9)	7	480/900	56.0 (34.0–76.9)
Bangladesh	–	–	–	1	219/365	60.0 (54.8–65.1)
India	4	151/929	28.5 (13.7–45.9)	2	68/134	50.7 (42.1–59.4)
Pakistan	9	2090/5486	40.7 (27.8–54.3)	4	193/401	55.7 (14.9–92.3)
East Asia and Pacific	6	352/1679	23.2 (15.0–32.6)	5	17,503/18,702	30.8 (0.0–86.3)
China	2	54/213	24.4 (18.8–30.6)	2	17,398/18,103	96.8 (96.5–97.0)
Malaysia	2	239/959	24.7 (21.9–27.5)	–	–	–
Myanmar	1	6/57	10.5 (3.9–21.5)	1	5/57	8.8 (2.9–19.3)
Taiwan	–	–	–	1	29/92	31.5 (22.2–42.0)
Thailand	1	53/450	11.8 (8.9–15.1)	1	71/450	15.8 (12.5–19.5)
Europe and Central Asia	12	403/9923	13.9 (6.1–24.0)	12	727/3548	22.9 (16.1–30.7)
Belgium	1	80/7464	1.1 (0.8–1.3)	1	158/1224	12.9 (11.1–14.9)
Czech Republic	1	0/45	0.0 (0.0–7.9)	1	2/45	4.4 (0.5–15.1)
France	3	48/237	18.8 (0.0–57.7)	2	75/231	31.5 (25.7–37.8)
Netherlands	–	–	–	1	3/8	37.5 (8.5–75.5)
Poland	–	–	–	1	265/906	29.2 (26.3–32.3)
Portugal	–	–	–	1	24/36	66.7 (49.0–81.4)
Russia	1	50/287	17.4 (13.2–22.3)	1	70/287	24.4 (19.5–29.8)
Turkey	5	182/1759	17.9 (4.9–36.1)	3	116/680	14.2 (5.6–25.9)
United Kingdom	1	43/131	32.8 (24.9–41.6)	1	14/131	10.7 (5.9–17.3)
Middle East and North Africa	15	736/2336	37.8 (22.3–54.6)	11	334/1380	37.7 (26.1–50.0)
Algeria	1	9/9	100.0 (66.4–100)	1	2/9	22.2 (2.8–60.0)
Egypt	4	257/611	34.9 (14.4–58.9)	2	15/18	91.6 (71.7–100)
Iran	4	161/772	22.8 (2.7–53.8)	6	261/903	37.4 (27.8–47.6)
Iraq	1	26/38	68.4 (51.4–82.5)	–	–	–
Israel	1	37/183	20.2 (14.6–26.8)	–	–	–
Jordan	2	48/465	9.5 (6.9–12.4)	2	56/450	11.8 (8.9–14.9)
Kuwait	1	29/50	58.0 (43.2–71.8)	–	–	–
Saudi Arabia	1	169/208	81.2 (75.3–86.3)	-	-	–

region of Europe and Central Asia (24 studies) and publications from the South Asia region (20 studies).

A detailed summary of the main characteristics per publication is presented in Supplementary Data 1 and 2 for MG and MS, respectively. Of the 110 studies (62 and 48 for MG and MS, respectively), 32 included an undefined subpopulation, 22 assessed broilers, 17 assessed a mixed population, 15 included laying hens, 14 studies included breeders, and 10 used diverse poultry. Among the 110 studies, PCR, real-time PCR and multiplex PCR were the main diagnostic techniques used for the detection of Mycoplasma (74, 19 and 7 studies, respectively). Whereas the main targeted regions were: 16S rRNA, 48 studies; *mgc*2 gene, 16 studies; *vlh*A gene, 15 studies; commercial kits, 13 studies; lipoprotein, 7 studies; *gap*A, 2 studies and others, 9 studies.

3.3 | Risk of bias assessment

Among the 85 publications, 18.8% of the studies had a high risk of bias for not presenting an alternative technique for diagnosing avian mycoplasmosis, as well as 17.6% of the studies were assessed with a high risk of bias for not including an appropriate definition of the population, and 16.5% of the studies with a high risk of bias for selective reporting of the results (Web Appendix 6).

3.4 | Pooled, regional and national molecular occurrence of MG and MS infection in poultry

Overall, the pooled global occurrence for MG was 27.0% (95% CI, 20.4–34.2) in 62 studies with a significant test of effect size ($z = 12.3$, $p = .00$), significant heterogeneity across studies ($X^2 = 6836.3$, df = 61; $p = .00$), and a significant proportion of variation attributable to heterogeneity as judged by the I^2 value of 99.1% ($p = .00$). For MS, the pooled global occurrence was estimated in 38.4% (23.5–54.5) in 48 studies with a significant test of effect size ($z = 7.4$, $p = .00$) and evidence of significant heterogeneity across the studies according to the X^2 statistic (16859.9, df = 47; $p = .00$) with 99.7% of variation attributable to heterogeneity (I^2, $p = .00$). As summarized in Table 1, in the seven WHO regions, the occurrence of MG was highest in South Asia (37.3%, 26.2–48.9) and lowest in Europe and Central Asia (13.9%, 6.1–24.0). For MS, the occurrence was highest in sub-Saharan Africa (61.5%, 39.4–81.6) and lowest in Europe and Central Asia (22.9%, 16.1–30.7).

At the national level, the occurrence of both pathogens showed a highly heterogeneous distribution pattern that varied according to both the country and the pathogen (Figure 2a). For instance, Algeria and Sudan had higher occurrence values for MG than for MS, whereas China, Egypt and Ethiopia showed higher values for MS than for MG. As depicted in Figure 2b, the top five countries with the highest occurrence for MG were Algeria (100%), Saudi Arabia (81.2%), Sudan (72.7%), Iraq (68.4%) and Kuwait (58%). Besides, for MS, the countries ranked in the top five were China (96.8%), Egypt (91.6%), Ethiopia (90.9%), Portugal (66.7%) and Bangladesh (60%). The occurrence was similar and below the global average in the Czech Republic (0.0% and 4.4%), Myanmar (10.5% and 8.8%), Jordan (9.5% and 11.8%), Turkey (17.9% and 14.2%) and Thailand (11.8% and 15.8%) for MG and MS, respectively.

3.5 | Occurrence according to the poultry subpopulation

In subgroup analysis according to subpopulations of poultry, the pooled occurrence for MG showed less variation among the five categories that varied between 11.9% and 31.9% (Figure 3a). In contrast, the pooled occurrence found for MS showed broad heterogeneity according to a range from 20.1% to 62.6% among the five subpopulations (Figure 3b). Furthermore, diverse poultry showed the lowest occurrence for both MG (11.9%, 1.9–26.5) and MS (20.1%, 8.8–33.7), while MS was highly prevalent in breeders (62.6%, 30.2–90.5), and MG had the highest occurrence in laying hens according to a pooled occurrence of 31.9.1% (14.2%–52.3%). Web-Appendices 7 and 8 show the forest plots for MG and MS, respectively.

3.6 | Impact of selected covariates on the occurrence

Before commenting on these results, we must clarify that the variables included in meta-regression should not be interpreted as causal risk factors for infection, but as covariates that could explain part of the heterogeneity seen in the estimations (Lean et al., 2009). For MG, the meta-regression analysis showed that both the year of publication of the study (coefficient = 0.013, $p = .05$; Figure 4a) and the level of exposure to ambient air pollution measured as the concentration of PM2.5 $\mu g/m^3$ ($c = 0.004$, $p = .000$; Figure 4b) had a significant positive association with the occurrence. In consequence, the occurrence of MG was greater in more recent studies or countries with a higher level of exposure to ambient air pollution. In contrast, we found a significant decreasing trend in the occurrence of MG as the sample size of the study increased ($c = -0.0002$, $p = .015$; Figure 4c). For MS, there was a positive association between the occurrence value and the level of contamination (PM2.5, $\mu g/m^3$) in the country where the study was conducted ($c = 0.0032$, $p = .05$; Figure 4d). In the remaining covariates, we did not find a significant association with the estimated occurrence of MG or MS.

4 | DISCUSSION

4.1 | Summary and implications of the evidence for the occurrence of MG and MS

In our study, we found a pooled global occurrence of 27.0% for MG and 38.4% for MS. With these results and taking into account the current poultry population (FAOSTAT, 2020), we estimate that at least 7830

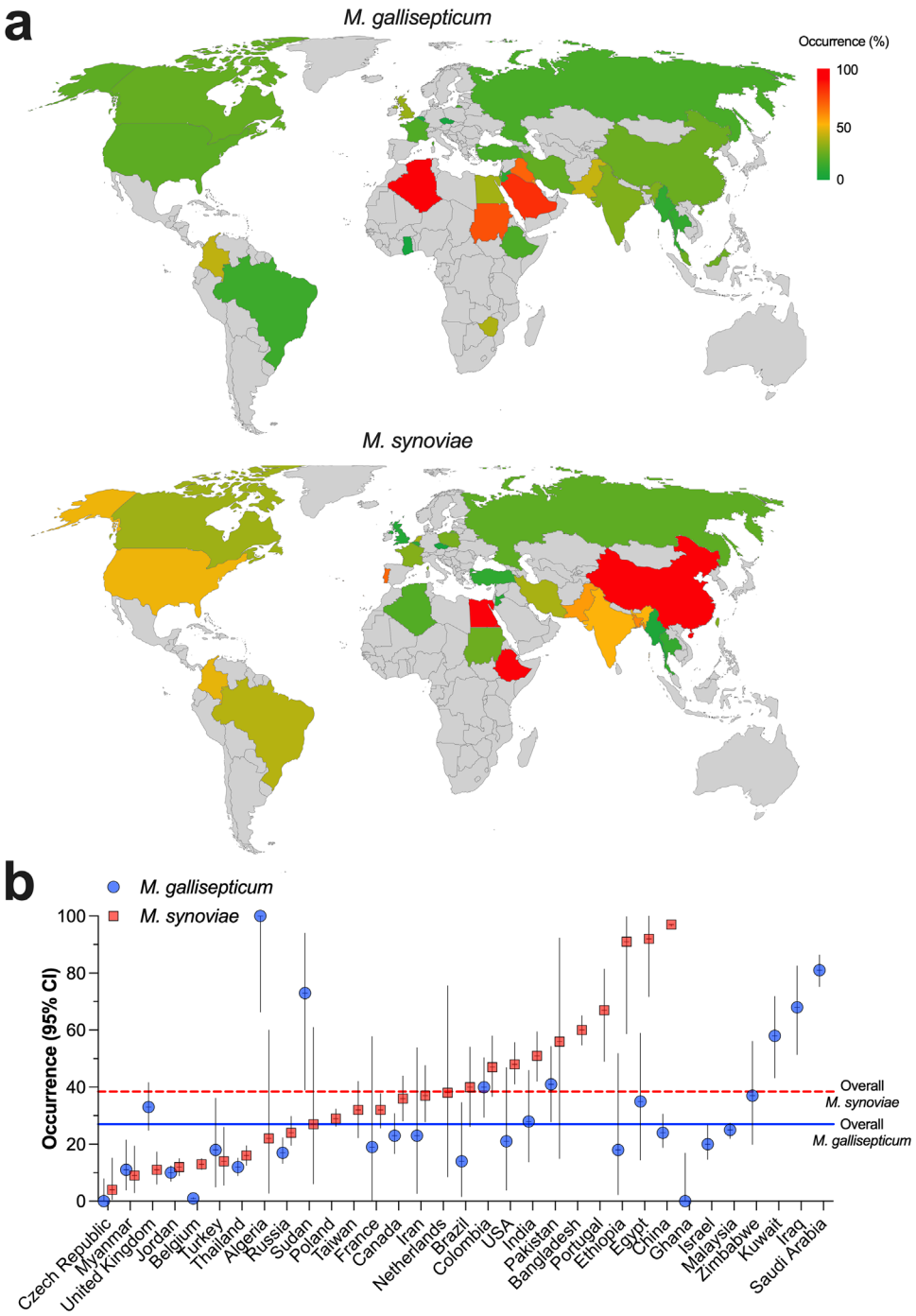

FIGURE 2 (a) Spatial distribution of the estimated occurrence per pathogen and (b) national estimated occurrence and 95% CI for *M. gallisepticum* and *M. synoviae*

and 11,136 million birds might be carriers of MG and MS worldwide, respectively. Our results revealed a heterogeneous pattern of occurrence that varied according to the mycoplasma species and the level of aggregation (regional or national). Indeed, we found that the overall occurrence of MS was higher than that of MG, thus coinciding with previous studies (Mettifogo et al., 2015; Rajkumar et al., 2018) but disagreeing with others that have shown the opposite trend (Ball et al., 2018; Rehman et al., 2018; Tomar et al., 2017). MG and MS had the lowest pooled occurrence (13.9% and 22.9%, respectively) in studies from the region of Europe and Central Asia. In contrast, studies from the region of South Asia and sub-Saharan Africa had the highest occurrence of MG (37.3%) and MS (61.5%), respectively. The higher occurrence of MS seen in sub-Saharan Africa could be related to an increased vertical transmission, which is the most important transmission route for MS in layer pullet flocks (ter Veen et al., 2020). On the opposite, the lower occurrence observed for MG could be partially explained due to

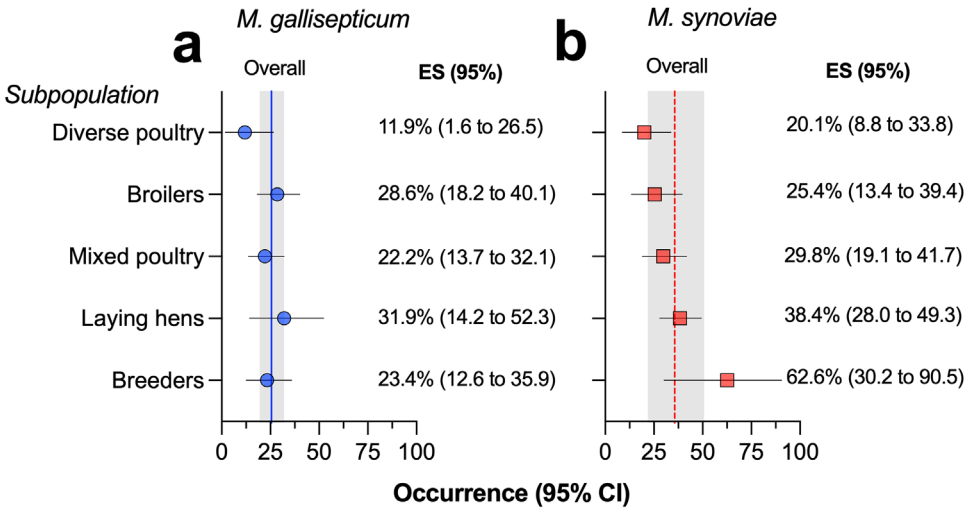

FIGURE 3 Occurrence estimates with 95% CI per subpopulation for (a) *M. gallisepticum* and (b) *M. synoviae*

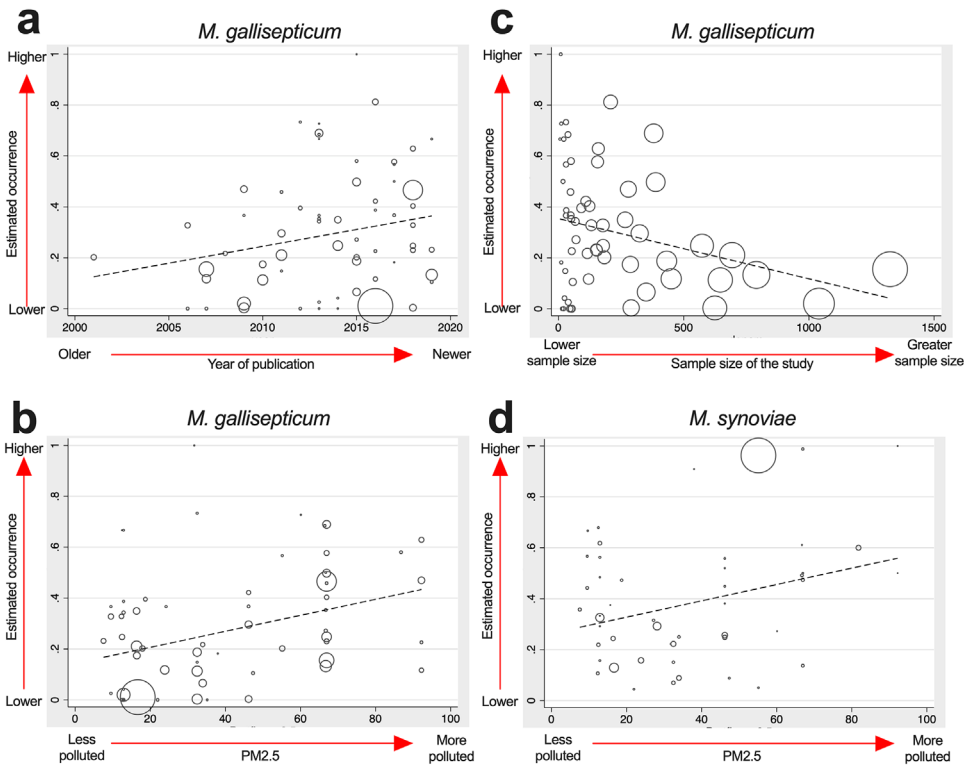

FIGURE 4 Results of the meta-regression analysis according to (a) year of publication for MG, (b) level of ambient air pollution (PM2.5, $\mu g/m^3$) for MG, (c) sample size assessed per study for MG, and (d) level of ambient air pollution (PM2.5, $\mu g/m^3$) for MS. We included only significant covariates for each pathogen

the use of live attenuated vaccines (El Gazzar et al., 2011; Ferguson-Noel & Williams, 2015).

The results from subgroup analysis revealed that diverse poultry had the lowest pooled occurrence of avian mycoplasmosis (11.9% for MG and 20.1% for MS) among the five categories of subpopulations. Such a result might be associated with the production practices for this category, such as a lower density of birds, less overcrowded habitat and less stressful conditions in comparison to commercial poultry. In contrast, the subpopulation of breeders was largely affected by MS according to an estimated occurrence of 62.6%, which contrasted with the 23.4% estimated for MG. The high occurrence of MS in breeders might be caused by the high concentration of breeder farms in some regions in conjunction with a lack of sanitary barriers (Moreira et al., 2017). Besides, the longer life span of layer and breeder flocks

could also increase the chance of getting mycoplasma infection in the field (Gharaibeh & Al Roussan, 2008), because the infection pressure slowly increases due to the accumulation of pathogenic bacteria in the environment (Feberwee et al., 2017). Possibly, management and sanity practices of the poultry subpopulations could be explored in a future systematic review of the extrinsic and intrinsic risk factors associated with MG and MS infection (de Sa et al., 2015); such information will allow a better understanding of the differences seen in the occurrence among these groups. Next, we discuss some of the aspects that might partially explain the differences seen in the epidemiological patterns for these two pathogens.

The preferred control method against MG and MS should be the maintenance of pathogen-free flocks and vaccination should be considered only on multiage farms (World Organization for Animal Health [OIE], 2019). However, in the areas where complete eradication is difficult, live vaccines are used as an alternative control strategy. Currently, there are three live vaccines commercially approved against MG (6/85 strain, ts-11 and F strain) and a K-strain that has been tested in some studies (Ishfaq et al., 2020). For MS, vaccination is performed especially in breeders and layers with two live attenuated vaccines available (Kaboudi & Jbenyeni, 2019). Concerning the use of antibiotics is not a suitable measure for control because this type of treatment does not eliminate mycoplasma infections (Buim et al., 2009). Nevertheless, further studies are needed to assess whether the efficacy of the three main forms of control measure for avian mycoplasmosis varies according to the poultry subpopulation or the country (Ferguson-Noel & Williams, 2015; Kleven, 2008) and how each strategy differentially impacts the occurrence of these pathogens.

Although both MG and MS are pathogens notifiable to the OIE, MS was added later in 2008 to the manual of diagnostic tests and vaccines for terrestrial animals (World Organization for Animal Health [OIE], 2019). Possibly, this could have contributed to the regional and national variations seen in the occurrence for both pathogens, mostly due to disparities in the schemes used for controlling these two mycoplasma species. Control measures for MG begun early in the 1950s–'60s, whereas the controlling measures for MS hardened during the last two decades. In the Netherlands, the control and eradication of MG started in the mid-'60s in broiler breeders and gradually expanded to layers and turkeys (Landman, 2014). In Brazil, the reduction of MG began in 1980 and was boosted by the establishment of the National Program for Avian Health, whereas in layers the use of the vaccine against MS is still very limited and for breeder farms participating in the program, vaccinations are not allowed and all infected flocks are eliminated (Buim et al., 2009). In the United Kingdom, infection with both species is now controlled through vaccination (Ball et al., 2018). Despite the policies adopted to control MG in the United States, the measures have proven inefficient and thus have maintained this country as a relevant route for the dissemination of MG (De la Cruz et al., 2020). Indeed, Tomar et al. (2021) found that the MG isolates found in poultry from India clustered with the US strain, a result that suggests that the MG strain from North America might be already circulating in the Haryana region of this country. In Iranian poultry farms, the attempts to eradicate the MG infection commenced earlier than for MS (Pourbakhsh et al., 2010). Voluntary MS control and eradication programs have been performed in some countries like the United States and the United Kingdom, whereas the Dutch poultry industry implemented in 2013 a mandatory control and eradication program for MS for all poultry categories except broilers (Feberwee et al., 2017). Mycoplasma monitoring is targeting breeding and commercial laying farms and national certification programs have contributed to the control of Mycoplasma infections in many countries, though the implemented programs vary widely across countries, regions and farms (Kaboudi & Jbenyeni, 2019).

Before the year 2000, MS was associated mainly with subclinical respiratory infections and was considered to have a low clinical and economic impact in broilers (Feberwee et al., 2008). However the high occurrence of MS shown in our estimations together with the capability of this pathogen to decline egg production (Stipkovits & Kempf, 1996) and the emergence of more virulent strains that might act synergic with other pathogens to induce a more severe disease (Landman, 2014) highlight the importance of MS infection and evidence the need to consider improving control measures.

According to the World Organization for Animal Health (OIE) (2019), due to the lack of specificity and sensitivity found in the common serological test, these are recommended to monitor flocks rather than for testing individual birds. In our study, when studies reported data for the individual and flock levels, we choose individual-level data because we found a more consistent and accurate estimation in comparison to flock level data. After comparing the estimates found at each level, we found a discrepancy higher than 15% between levels for breeders (both MG and MS) and layers (MS) (Web Appendices 9–11), whereas the remaining estimations were quite consistent. Even though we identified a potential source of variation for these estimates, both levels of sampling represent apparent prevalence and it is expected an overestimation in the occurrence at the flock level given that finding a single positive bird is required to define a flock as positive. Thus, more studies are needed at each level to perform further comparisons and establish whether individual or flock testing should be preferred for the molecular methods.

Additionally, we identified three more potential causes that could have contributed to the heterogeneity of the pooled estimates. First, the disparity in the number of data sets collected across the WHO regions. East Asia and the Pacific region contributed the highest share (44.8%) of the data sets, followed by Europe and Central Asia (29.7%). In contrast, a limited proportion of data sets was provided by sub-Saharan Africa (0.2%) and North America (1.6%). Second, for some countries, a limited number of publications were available for the estimations; consequently, this lack of data sets might cause part of the heterogeneity because, for some countries, the estimations were constrained to a single data set. Third, the diversity of health/disease conditions of the birds assessed in each study (i.e. diseased, mixed, healthy/diseased, unknown and healthy) avoided identifying a clear difference among healthy and diseased birds. Nevertheless, we performed an additional meta-analysis to compare the occurrence of MG

and MS between the studies that sampled diseased birds and the overall estimations. The results showed that in studies that sampled diseased birds, the occurrence of MS (36.53%, 21.25–53.11) and MG (27.95%, 21.47–34.84) was very similar regarding the global estimates for MS (38.4%) and MG (27.0%). However, the molecular frequency of avian mycoplasmosis would be expected to be higher in diseased birds having a history of respiratory disease or those who are seropositive for the pathogens, in contrast to healthy birds or whose health condition was unknown. In consequence, more studies that compare the occurrence of these pathogens in groups of birds with different health/disease statuses are needed.

We only included studies that assessed the presence of avian mycoplasmosis with a molecular approach. Real-time PCR, or quantitative PCR (qPCR), is used frequently for the detection of infectious agents because it provides a sensible, safe closed-tube assay with quantitative information not available from conventional PCR or other 'endpoint' amplification methods. The quantitative capability of qPCR allows the distinction of subclinical levels of infection (qualitatively positive by conventional PCR) from higher levels with pathological consequences (Buckingham, 2019). However, there exists a high discrepancy among laboratories regarding their ability to detect Mycoplasma by PCR (Hess et al., 2007); thus, the occurrence rates could be over- or underestimated. Although serological tests are not sufficient alone for the detection of avian mycoplasmosis, mostly because antibodies remain for a long time after the infection (World Organization for Animal Health [OIE], 2019), it is important to contrast the pattern of molecular occurrence that we report in this study to the seroprevalence profiles reported in other studies, especially from countries whose mycoplasma occurrence estimations were extreme, had a small sample size or reported a limited number of molecular studies.

Our systematic review and meta-analysis of the molecular occurrence of MG and MS in poultry add to previous secondary studies published recently for MG and MS in poultry (Yadav et al., 2021) and MG in wild birds (Sawicka et al., 2020). In the study by Yadav et al. (2021), the authors summarize epidemiological studies based on a molecular and serological tests for detection of the pathogen, highlighting the economic significance, diagnosis and prevention and control of avian mycoplasmosis with a special focus in India. In agreement with our results, the authors found a broad variability in the occurrence of avian mycoplasmosis globally and report variability across the geographical areas of India. Besides, the authors emphasize the use of several biosecurity and management practices that include acquisition of Mycoplasma-free fertile eggs and chicks, continued epidemiological surveillance of the flocks, and culling of positive birds as measures to prevent further infections and spreading of avian mycoplasmosis. Sawicka et al. (2020) meta-analysed the occurrence of MG in wild birds according to three different detection techniques (serology, culture and molecular) and found variability in the estimations from these techniques as well as a broad distribution of the pathogen in 56 species of birds that could serve as potential reservoirs.

Our study included 62 and 48 studies for MG and MS that, in conjunction, evaluated more than 48,500 samples collected across 33 countries from the seven WHO regions. To improve reliability and confidence about our findings, we used rigorous methodological and statistical procedures to estimate the occurrence and included publications that in the majority were judged with low risk of bias. Consequently, the appraisal of the quality of the overall body of evidence was rated as having a high quality according to the GRADE system (Balshem et al., 2011). Our mapping of WHO regions and countries of the high occurrence of MG and MS presents a view of the distribution of MG and MS infection and might provide further guidance regarding where to focus tailored measurements aimed at reducing avian mycoplasmosis occurrence. Also, identifying poultry subpopulations at higher risk in conjunction with control options for avian mycoplasmosis (Bennett et al., 2013) should increase success in the effort to eradicate this infection.

4.2 | Limitations of the study

The results of this review must be interpreted cautiously, considering the following limitations: (1) although this meta-analysis of the occurrence of MG and MS infection in poultry is the largest thus far, only eligible published studies from a reduced number of countries were included. Therefore, these estimates might not be representative of several nations and justify the need for conducting future studies in more countries; (2) subgroup analysis did not reduce the heterogeneity across studies, and the source of this heterogeneity could not be identified based on moderator analysis because, for MG, only 3/9 covariates assessed (year of publication, sample size and the exposure to ambient air pollution) were significantly associated with the occurrence, whereas for MS, only the exposure ambient air pollution was significant; also, we did not include vaccination status because of data scarcity as only eight studies reported vaccination history and eight more studies reported no vaccination in poultry and (3) the diversity of laboratory conditions and procedures to test the presence of avian mycoplasmosis is a common cause of heterogeneity across the studies.

5 | CONCLUSIONS

The results of this meta-analysis demonstrate that one-third of the birds worldwide might have a high probability of infection with MS and a quarter of the global poultry might be infected with MG, mostly in the regions of South Asia and the Middle East and North Africa for MG and the sub-Saharan African region for MS. Therefore, the need to prevent and control avian mycoplasma infections is still a priority for the poultry industry. The estimated occurrence of 38.4% of MS infections should serve as a reminder to animal health experts and policymakers about the need to rethink control measures in certain countries where intervention programs have not been implemented or have been ineffective for this pathogen. The series of results presented in our review justify the need for an improved early detection system and rethink control measures to reduce the spread of both MG and MS avian pathogens.

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ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this is a review article with no original research data.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data and material are available from the supplementary material that can be accessed online at the journal website.

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