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Original article

GLOBAL PREVALENCE OF *CHLAMYDIA* INFECTION AMONG WILD BIRDS AS PUBLIC HEALTH CONCERNS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Summary

Mamun, T. I., J. Rahman, M. J. Hossain, R. Hasan, M. T. Neoaj & M. M. Mia, 2023. Global prevalence of *Chlamydia* infection among wild birds as public health concerns: A systematic review and meta-analysis. *Bulg. J. Vet. Med.* (online first).

Chlamydia has a significant worldwide zoonotic impact and can infect a variety of animal species, including wild birds and humans. Birds are frequently susceptible to the *Chlamydia* pathogen, which can lead to avian chlamydiosis and the human disease ornithosis. The present study investigated wild birds *Chlamydia* prevalence using a systematic review and meta-analysis. Scientific papers from Scopus, PubMed, Google Scholar, and local journals were evaluated between November 1 and December 10, 2022. The investigators conducted a meta-analysis of 29 studies, which included 48 trials to assess the prevalence of *Chlamydia*. The pooled prevalence of the infection was 22% (95% CI: 15–28%) based on the findings of the meta-analysis. Following that, a survey of the continents was conducted, with Europe having the highest prevalence rate (26%), followed by Australia (22%), South America (22%), Asia (15%), and North America (13%). Italy had the highest prevalence rate, and Sweden had the lowest prevalence rate among European countries. Egypt had the highest prevalence rate of 75%, as shown in a single study. The prevalence rate for *Chlamydia* species was 21% (CI: 13–28%), with *Chlamydia psittaci* being the most common. This finding provides more evidence that wild birds are a potential reservoir for the spread of the *Chlamydia* disease, since they are known to be carriers of the infection. In order to acquire a more precise estimate of worldwide prevalence, further molecular studies are recommended.

Key words: *Chlamydia,* meta-analysis, prevalence, wild birds, zoonotic pathogens

INTRODUCTION

Chlamydia, a Gram-negative, obligate intracellular bacterium, is extensively distributed globally, causing a variety of illnesses in both humans and animals (Schachter, 1999). Fourteen species have been identified, with four more uncultured candidates proposed; *Chlamydia psittaci* being the most widely known zoonotic agent (Harkinezhad *et al.*, 2009; Cheong *et al.*, 2019; Laroucau *et al.*, 2019; 2020). *C. psittaci* is a pathogen with a wide range of distribution, which can affect more than 465 species of birds from 30 different orders including domestic, pet, and wild birds (Kaleta & Taday, 2003). Worldwide, 19.5% of birds were infected with *Chlamydia* (Sukon *et al.*, 2021).

Wild birds play an important role in the transmission of chlamydiosis across borders. Cleaning wild bird feeders and being exposed to bird faeces were found in two studies to be risk factors for human psittacosis in Sweden (Rehn *et al.*, 2013; Chereau *et al.*, 2018). It has long been believed that wild birds serve as a natural reservoir for *C. psittaci* infection (Andersen & Vanrompay, 2008). The majority of *C. psittaci* human cases have been associated with pet birds, but some have also been associated with free-living birds (Smith *et al.*, 2011; Kalmar *et al.*, 2014). Wild birds may be a potential source of chlamydial infections in poultry, which could be a health risk to farm workers and consumers. There have been links between wild birds and poultry infections (Hulin *et al.*, 2015; 2016). Wild birds may spread *Chlamydia* to horses, which could then be passed to humans, impacting *Chlamydia* epidemiology (Chan *et al.*, 2017; Jelocnik *et al.*, 2017). An earlier study's findings that *C. psittaci* can be transmitted from mammal to mammal led to additional testing of horses in Australia (Akter *et al.*, 2020; Polkinghorne & Branley, 2020; Anstey *et al.*, 2021). It can be inferred from the high number of different wild bird species that have contracted *C. psittaci* that all wild birds are susceptible to avian chlamydiosis (Kaleta & Taday, 2003). According to an earlier study, *Psittaciformes* had the highest prevalence of chlamydial infections, followed by *Passeriformes*, *Galliformes*, *Columbiformes*, and *Anseriformes* (Nemeth *et al.*, 2016). Infections with *Chlamydia* are most frequently observed among parakeets, love birds, cockatiels, Amazon parrots, and macaws that fall in the psittacine bird's category. Doves and pigeons, which are not psittacine birds, were found to have the highest rate of infection (Dahlhausen, 2007; Smith *et al.*, 2011). The zoonotic pathogen has the potential to infect humans by direct or indirect contact with contaminated bird excretions and droppings, resulting in pneumonia with up to 83% of cases and severe mortality without treatment (Telfer *et al.*, 2005; van Droogenbroeck *et al.*, 2009). *Chlamydia* can persist for months or even years in the respiratory and intestinal tracts of clinically infected birds, asymptomatic carriers, and even birds that have recovered from a symptomatic infection (Sachse *et al.*, 2015). *Chlamydia* infection diagnosis is difficult due to the lack of or diversity of clinical indications; for confirmation of a diagnosis, multimodal diagnostic testing should be performed (Balsamo *et al.*, 2017). The gold standard for *Chlamydia* diagnostics is PCR-based detection (Sachse *et al.*, 2009). Since *Chlamydia* infections are found in a wide range of bird species or orders all over the world and have zoonotic significance, it has become an urgent topic to know about the worldwide magnitude of *Chlamydia* infections. Meta-analysis is a powerful statistical tool that is used to synthesise and compare the results of numerous primary studies on the same topic (Borenstein *et al.*, 2021). Therefore, the present study focuses on evaluating the global prevalence of *Chlamydia* infection among wild birds, taking into account geographical diversity.

MATERIALS AND METHODS

Study design and literature search strategy

A systematic literature search was carried out from published articles in the period between 2000 and 2022, considering keywords such as follows: Prevalence, *Chlamydia*, Wild birds, Pet birds, *Chlamydia* infection, Incidence, and Avian chlamydiosis. Research papers were scrutinised from databases like Scopus, PubMed, and Google Scholar, as well as regional journals between November 1, 2022, and December 10, 2022. However, the search criterion was limited to English-language studies; finally, the eligible studies were extracted by reviewers for eliminating the bias. According to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) standards, the review was reported (Moher *et al.,* 2009).

Quality assessment of the study

The authors used standardised critical appraisal methods from the Joanna Briggs Institute (JBI) to conduct a comprehensive independent evaluation of each study before included it in the review (Munn *et al.*, 2015). Two researchers assessed the studies, and a third researcher was consulted in case of disagreement. Evaluation of the studies was done using checklists looking at context, sampling, screening, diagnosis, data processing, validity, and reliability. Studies with a score of 6–9 on the checklists were included in the meta-analysis.

Data selection and extraction

A review and meta-analysis of studies on wild bird species, published in English between 2000–2022 was conducted. Studies used tissue culture, PCR, ELISA, and included free living, zoo, pet and captive wild birds. We used two reviewers to evaluate titles and abstracts, then excluded review articles, duplicates, qualitative studies, case studies and non-peer-reviewed publications. Data were analysed in Microsoft Excel, including author, published year, location, diagnostic method, sample name and number of samples, case positives, and prevalence percentage.

Statistical analysis

Jamvoi software's meta-analysis (Major) packages (version 1.2.27) were used for statistical analysis. The constrained maximum-likelihood estimate for residual heterogeneity in the random effect model was utilised to calculate the prevalence followed by tau square, I2 (Higgin's I2), and P value to quantify the amount of variation among the different reports included in the study (Higgins *et al.,* 2003). Graphs were used to show the ratio of total studies to individual studies, which were displayed on forest plot. A funnel plot was also created to check for any publication bias. R Studio version 4.1.0 was utilised to create the forest and funnel plots. Standardised effect estimates were graphed against the inverse standard error as a scatter plot. Any points outside of the confidence intervals may indicate differing results between studies, suggesting that there is variability in the impact of the exposure-pathogen association due to factors such as study design and demographic variables (Bueno-Notivol *et al.*, 2021). A subgroup analysis was conducted to investigate the likely sources of variability in meta-analyses of observational data (Thompson & Higgins, 2002). Then, a chi-square test was performed to examine the relationship between variables in each subgroup. Afterwards, a paired *t*-test in a one-way ANOVA was employed in order to compare the continuous variables that had a significant P-value.

RESULTS

A total of 18,200 studies were found using the given keywords, of which 7,510 were relevant. The findings were customised based on the published year range from 2000 to 2022. After further filtering, 75 studies were identified after reading the title, abstract, and full text. Then 48 studies met the criteria for inclusion in the systematic review and meta-analysis, of which 29 were chosen (Fig. 1; Table 1).

Estimated pooled prevalence

A total of 29 studies were analysed, which included data from Asia, Europe, North America, South America, and Australia. The latest meta-analysis revealed that the overall worldwide prevalence of *Chlamydia* species in wild birds is 22% (95% CI: 15–28%), H2 value of 150.53 (Table 2; Fig. 2), and a funnel plot was generated (Fig. 3). When the results were broken down by continent (Table 3; Fig. 4), it was found that Europe had the highest rate at 26% (95% CI: 15–37%), followed by Australia 22% (95% CI: 02–50%), South America 22% (95% CI: 16–28%), Asia 15% (95% CI: 05–26%), North America 13% (95% CI: 04–22%), and Antarctica 13% (95% CI: 10–16%).

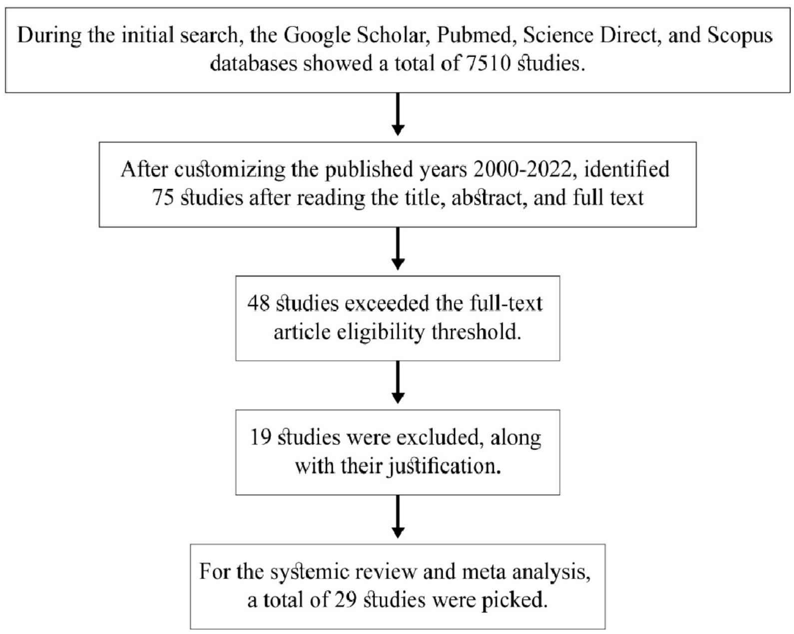

Fig. 1. Flow chart of the method of studies selection.

Table 1. Features of the studies included in the systemic review and meta-analysis

Authors' name, year	Country	Sample source	Bird species included	Test method	PC/TSS	Prevalence (%)	QAS
Isaksson *et al.*, 2015	Antarctica	Cloacal swabs	Chinstrap penguins, brown skuas, southern giant petrels, snowy sheathbills, franklin's gulls	PCR	67/527	12.713	8
Amery-Gale *et al.*, 2020	Australia	Swabs, liver	superb lyrebird, crimson rosella	PCR	2/299	0.669	8
Stokes *et al.*, 2020	Australia	Cloacal swabs	Wild parrot	PCR	49/123	39.837	8
Kalmar *et al.*, 2014	Belgium	Pharyngeal swabs	Long-eared owl, collared dove, northern lapwing, carrion crow, magpie, blackcap, herring gulls, common moorhens	Culture	11/42	26.190	7
de Freitas Raso *et al.*, 2006	Brazil	Tracheal and cloacal swabs	Amazon parrots, hyacinth macaws	PCR	14/77	18.182	7
Zhang *et al.*, 2015	China	Faecal and blood samples	Budgerigars, lovebirds, cockatiels, alexandrine parakeets	IHA	110/311	35.370	8
Li *et al.*, 2020	China	Faecal samples	Crested ibis	PCR	20/99	20.202	7
Sheleby-Elhas *et al.*, 2013	Costa Rica	Cloacal swabs	Green macaw, scarlet macaw, yellow naped parrot, Red-lored parrot, Finsch's parakeet, blue-fronted amazon, yellow-crowned amazon, southern mealy parrot, orange-chinned parakeet, grey parrot, yellow-headed parrot, blue-and-yellow macaw, military macaw, white cockatoo, olive-throated parakeet, scarlet macaw, lovebirds	PCR	4/117	3.419	7

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Szymacska-Czerwicska *et al.*, 2017	Poland	Cloacal swabs	Mallard, swan, Eurasian teal, common swift, white stork, pigeon, magpie, hooded crow, jack daw, gull	PCR	132/894	14.765	8
Jeong *et al.*, 2017	South Korea	Tracheal swabs	Woodcock, rook, Korean magpie, domestic pigeon	PCR	6/225	2.667	8
Ortega *et al.*, 2012	Spain	Conjunctivae/ choanae/cloacae	Common kestrel, Bonelli's eagle, Eurasian griffon vulture, Eurasian common buzzard, Eurasian eagle-owl	PCR	26/64	40.625	7
Blomqvist et al., 2017	Sweden	Cloacal swabs	Peregrine falcon, white-tailed sea eagle	PCR	4/319	1.254	8
Stalder *et al.*, 2020	Switzer-land	Cloacal swabs	Raptors, carrion crows, corvids	PCR	139/801	17.353	8
Liu *et al.*, 2019	Taiwan	Cloacal swabs	Malayan night heron	PCR	14/650	2.154	7
Beckmann *et al.*, 2014	England	Visceral organ (Liver/lung)	Dunnocks, great tits, blue tits, collared doves, robin	PCR	21/40	52.500	7
Docherty *et al.*, 2012	USA	Cloacal swabs	Mallard, American coot, Northern pintail, Northern shoveler, lesser scaup, gadwall	DCFT	13/61	21.311	7
Jouffroy *et al.*, 2016	USA	Conjunctivae/ choanae/cloacae	Osprey nestling, great horned owl, red-tailed hawk,	PCR	3/82	3.659	7
Dusek *et al.*, 2018	USA	Conjunctivae/ choanae/cloacae	Lovebirds, rocky doves	PCR	57/188	30.319	8
Seibert *et al.*, 2021	USA	Conjunctivae/ choanae/cloacae	Cooper's hawk, red-tailed hawk, Swainson's hawk, great horned owl	PCR	11/263	4.182	8

PC/TS: Positive cases/total sample size; QAS: Quality assessment score

Table 2. Pooled data regarding the studies

Parameters	Value
Total sample	18200
Total outcome	7510
Number of studies	29
Pooled prevalence (%)	22
95% CI:	15–28
H^2 value	150.53
Tau^2 value	0.0316
I^2 value	99.34

Country wise prevalence

After reviewing the data from each continent, we found that Italy had the highest prevalence rate and Sweden had the lowest prevalence rate among European countries. The prevalence was measured at 53%, 41%, 33%, 26%, 18%, 17%, and 12% for Italy, Spain, the Netherlands, Belgium, France, Switzerland, and Poland, respectively. Meanwhile, China, Iran, South Korea, and Taiwan reported prevalence rates of 31%, 13%, 3%, and 2%, respectively. According to a single study, Peru had the highest prevalence of 22% among South American nations, while Brazil had a prevalence of 18% and the USA had a prevalence of 14% across multiple studies. Lastly, Egypt had the highest prevalence rate of 75% based on a single study, while Australia and New Zealand had 1% and 32% prevalence rates, respectively.

Prevalence according to study time and Chlamydia species

Over the course of a first phase of research (2000–2015), 14 studies were conducted and reported a 23% prevalence of

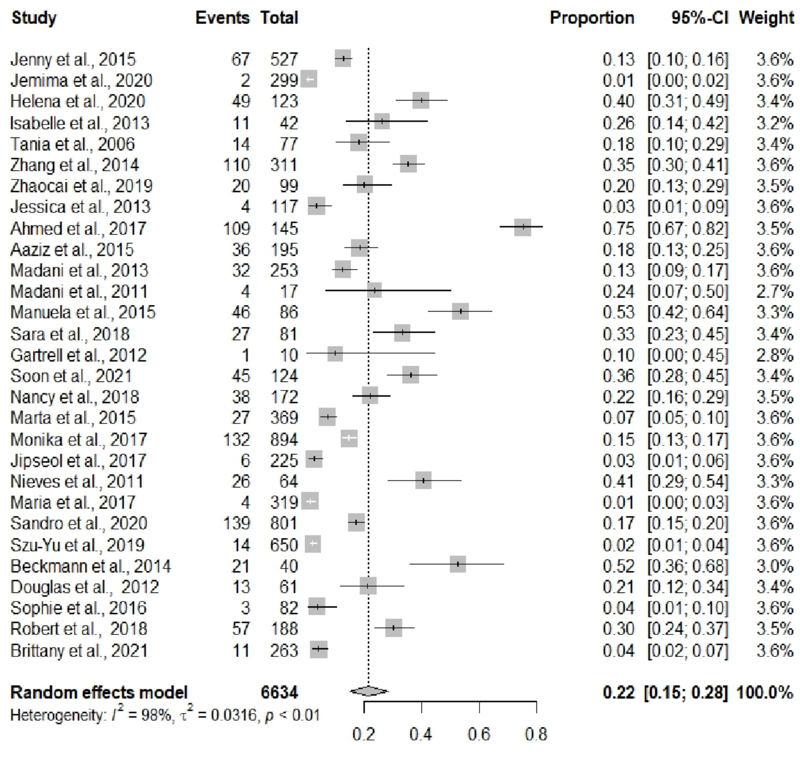

Fig. 2. Forest plot describing the pooled prevalence of *Chlamydia* infections in wild birds.

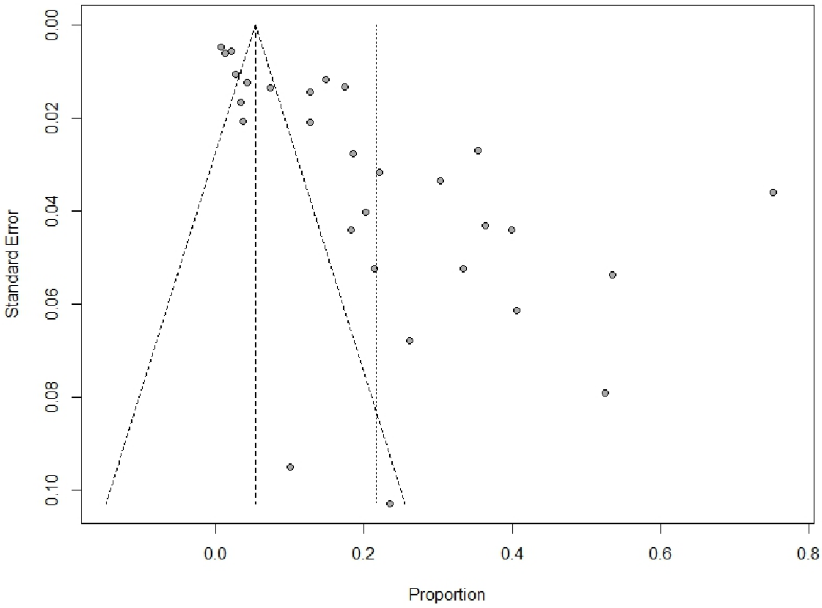

Fig. 3. Funnel plot describing studies' heterogeneity and assess publication bias.

Table 3. Continent wise prevalence of *Chlamydia* infection

Sample	No. of studies	Prevalence % (95% CI)	I^2 (%)	H^2	Tau^2	Chi-square test
Asia	06	15 (05–26)	98.54	68.53	0.016	P=0.026
Europe	10	26 (15–37)	99.00	100.35	0.030	
North America	06	13 (04–22)	96.00	25.02	0.012	
South America	02	22 (16–28)	–	–	–	
Australia	04	22 (02–50)	96.55	28.94	0.036	

risk factors (95% CI: 15–32) (Table 4). In the following phase (2016–2022), 15 studies were conducted and reported a 20% prevalence (95% CI: 10–30). In total, 29 studies examined the worldwide prevalence of 7 *Chlamydia* species (*C. psittaci; C. avium; C. gallinacea; C. ibidis; C. trachomatis; C. abortus; C. buteonis*), with *C. psittaci* being the most frequently reported; it had a prevalence rate of 21% (95% Cl: 13–28).

Prevalence according to difference source of sample

Four groups were sampled: a rehabilitation centre, a centre with a focus on wildlife, a nest, and a center for wildlife refuges. The wildlife refuge center had the highest prevalence of *Chlamydia* species, at 23%, and the nest had the lowest, at 2%. The Wildlife Specialised Centre had a prevalence of 7%, and the rehabilitation centre had 19% (Table 5).

Rate of prevalence from different samples

The sample size was splitted into two groups: 451–900 samples, and 450 or fewer. The latter group had a much higher incidence rate at 23%. The rate for studies with more than 451 samples was lower: at 12% (Table 5).

The prevalence of *Chlamydia* species was the highest in the visceral organ (liver/lung) samples at 45%, followed by conjunctivae/choanae/cloacae (18%), and cloacal swabs (19%) (Table 6). In the end, the study splitted the diagnostic tests into three groups: Polymerase chain reaction (PCR), Indirect haemagglutination (IHA)/ Direct complement fixation test (DCFT)/ Enzyme-linked immunosorbent assay (ELISA)/Giemsa, and culture. PCR had a prevalence rate of 18%, while the IHA/DCFT/ELISA/Giemsa test had a rate of 39%, and culture had a rate of 25% (Table 7).

DISCUSSION

A systematic review and meta-analysis were conducted to determine the world-wide prevalence of *Chlamydia* infection in wild bird populations. The study found that the global rate of *Chlamydia* infection was high, suggesting that people all over the world are vulnerable to contracting the disease from captive birds, pet birds, and wild birds. This is especially true for those who have close contact with wild birds. Researchers have found that various types of birds could potentially transmit a zoonotic disease known as psittacosis to humans. Psittacosis outbreaks have been reported in high-altitude areas of Austra-

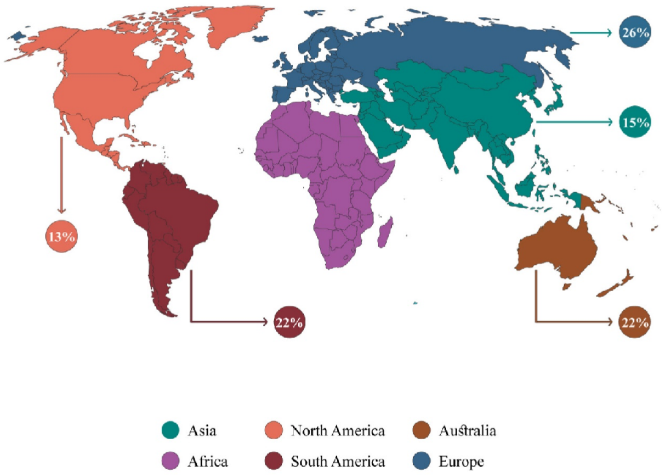

Fig. 4. Prevalence of *Chlamydia* infections in wild birds in different countries.

Table 4. Prevalence rate according to two research phases

Sample	No. of studies	Prevalence % (95% CI)	I^2 (%)	H^2	Tau^2	Chi-square test
2000–2015	14	23 (15–32)	96.74	30.70	0.022	P=0.853
2016–2021	15	20 (10–30)	99.68	317.35	0.040	

Table 5. Prevalence rate according to sample collection place, including more than two studies

Sample	No. of studies	Prevalence % (95% CI)	I^2 (%)	H^2	Tau^2	Chi-square test
Rehabilitation centre	5	19 (04–34)	98.73	78.50	0.028	P=0.297
Wildlife specialised centre	3	07 (04–19)	98.72	78.17	0.009	
Nest	2	02 (0–03)	–	–	–	
Wildlife refuge centre	2	23 (15–31)	–	–	–	

Table 6. Prevalence of *Chlamydia* species from different samples, reporting more than three studies

Sample	No. of studies	Prevalence % (95% CI)	I^2 (%)	H^2	Tau^2	Chi-square test
Cloacal swab	12	19 (10–28)	99.24	132.09	0.024	P=0.050
Conjuncti-vae/choanae/cloacae	6	18 (06–30)	97.09	34.32	0.020	
Viscera organ (Liver/lung)	3	45 (05–84)	99.00	99.58	0.034	

Table 7. Prevalence of zoonotic diseases based on different diagnostic tests

Diagnostic test	No. of studies	Prevalence % (95% CI)	I^2 (%)	H^2	Tau^2	Chi-square test
PCR	23	18 (12–25)	99.30	142.2	0.024	P<0.001
IHA/DCFT/ELISA/Giemsa	4	39 (14–63)	98.10	52.57	0.062	
Culture	2	25 (14–36)	–	–	–	

lia. These outbreaks are thought to be the result of indirect *C. psittaci* transmission from infected wild birds to humans through contact with a contaminated environment, with *Chlamydia* organisms persisting in colder, more shady mountainous habitats (Williams *et al.*, 1998; Telfer *et al.*, 2005; Branley *et al.*, 2014). The present study assesses the global prevalence of chlamydiosis reported in 22% (95% CI: 15–28) of the wild birds. Almost all investigations reported *C. psittaci* infections in birds. The main sources of *C. psittaci* were domestic and wild birds, which shed the organism in their excretions and pose a significant zoonotic risk to humans and native birds (Hegazy *et al.*, 2017). In the last few years, *C. gallinacea* has been discovered in the wild, in two parrot species in Australia and in a woodcock in South Korea (Jeong *et al.*, 2017; Stokes *et al.*, 2020). *C. avium* was first discovered in captive birds such as parrots and pigeons (Sachse *et al.*, 2014), and has since been found in wild doves (Mattmann *et al.*, 2019) and a wild ring-necked parakeet (Pisanu *et al.*, 2018).

After that, a study of the continents was done, with Europe having the highest prevalence rate, followed by Australia, South America, Asia, North America. Most Chlamydial observation has been carried out in Europe, where *Chlamydia* pathogens have been identified on every continent and isolated from at least 70 different types of wild birds (Stokes *et al.*, 2021). Italy had the highest prevalence rate among European countries, and Sweden had the lowest prevalence percentage, according to an assessment of country-specific statistics. A study conducted in Europe revealed that feral pigeons in 11 countries were infected with *C. psittaci* (Magnino *et al.*, 2009). There seemed to be a potential risk that humans or birds contract this zoonotic pathogen from collared doves, according to an Italian study that indicated that *Streptopelia decaocto* had a high prevalence of *C. psittaci* (61%) (Donati *et al.*, 2015). Egypt, the most populated country in Africa, showed the highest prevalence 75%. In our study, Egypt had the highest prevalence rate in the analysis of the single study. According to this, high *Chlamydia* species rates (67.3%) from migrating birds have been seen in Egypt in the earlier (Mousa *et al.*, 1987).

Similarly, among Asian nations, China, the continent's most populated nation, had a 31% prevalence rate. Taiwan is most likely the country with the lowest prevalence. One of the most popular pet birds in China is the parrot, which can carry *Chlamydia*, which is significant for both human and animal health (Zhang *et al.*, 2015). According to the results of the current meta-analysis, we have identified several *Chlamydia* species that are widely distributed, with *C. psittaci* being the most commonly reported (21%) causative pathogen. Pet and wild birds acted as the main reservoir for *C. psittaci*, which sheds in their excreta and poses a high zoonotic risk to humans and native birds (Hegazy *et al.*, 2017).

In addition, the various sources of the sample were classified to discover that the wildlife refuge centre had the highest prevalence rate of 23%. The nest, however, had the lowest prevalence rate, coming in at 2%. *C. psittaci* can spread by the ingestion or inhalation of ocular and nasal discharges as well as droppings from infected birds, increasing the risk of outbreaks at treatment centres (Heddema *et al.*, 2006; Beeckman & Vanrompay, 2009; Harkinezhad *et al.*, 2009).

Moreover, analysing the prevalence from different wild bird samples, we found a significant (45%) prevalence rate of *Chlamydia* species from visceral organs (liver/lung). A previous study found that *Chlamydia* was highly isolated from livers (78%) (Moore *et al.*, 1985). The prevalence of *C. psittaci* in wild hyacinth macaw nestlings can be different depending on the sample type or diagnostic method used; cloacal swabs showed 27% prevalence, while tracheal swabs only showed 9% in the same individuals (Raso *et al.*, 2006). Our research shows that the results of PCR and non-PCR methods can differ. This heterogeneity may be explained by the possibility of false positive errors in serological tests due to cross reactivity with other *Chlamydia* species (Nieuwenhuizen *et al.*, 2018).

Our research has some limitations. The studies do not cover all continents, with no studies from Africa. Additionally, the studies are not spread out evenly across continents, with only two from South America being excluded from the analysis. Furthermore, the tool used to assess the included studies' risk of bias and quality is limited. This study utilised a comprehensive approach to search the literature, analysed a large sample size, and incorporated climate variables into its methodology. However, the findings may be slightly off due to potential missed research and insufficient data in some studies. Therefore, further molecular research is recommended to obtain a more accurate estimate of global prevalence.

CONCLUSIONS

According to a meta-analysis of zoonotic pathogens in wild birds, the prevalence of *Chlamydia* species worldwide was 22% (95% CI: 15–28%). When data were broken down by continent, Europe had the greatest prevalence rate (26%). Seven *Chlamydia* species were evaluated in total by 29 studies, with *Chlamydia psittaci* being the most commonly reported. This study suggests that captive, pet, and wild birds may be a source of human infection with *Chlamydia* if there is direct contact, and recommends that policymakers assess the molecular profiles of these birds to aid in the implementation of protective measures to reduce the spread of this pathogen.

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