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Prevalence of Avian Influenza H5N6 in Birds: A Systematic Review and Meta-analysis of Other Viral Zoonosis

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ABSTRACT

Avian influenza viruses (AIV) are zoonotic pathogens that can potentially affect humans and potentially be epidemic in a region. Birds (such as poultry and wild birds) serve as potential reservoirs for these viruses, highlighting the importance of determining AIV prevalence in the avian population. No systematic reviews have been published on this issue in the world so far. The present systematic literature review following the PRISMA standard, with meta-analysis, used three databases to globally assess the Influenza H5N6 infection in birds (including poultry and wild birds). A model of random-effects meta-analysis was performed to calculate the pooled prevalence and 95% Confidence Interval (95% CI) for the prevalence of Influenza H5N6 infection in birds. A total number of 14,605 articles published from 2015 to 2020 were retrieved. After screening the abstract/title, 37 articles were selected for full-text assessment, and 15 were included for qualitative and quantitative analyses. Of the total number of birds (n = 13,416 birds), the pool prevalence by RT-PCR was 3.5% (95% CI: 2.8-4.3%). From the total, 39.67% of the birds assessed were ducks (family Anatidae), in which pool prevalence was 7.7% (95% CI: 4.4-11.0). In chickens (*Gallus gallus domesticus*), the pool prevalence was 3.3% (95% CI 1.9-4.8). Vietnam was the country with the highest pool prevalence; 7.9% (95% CI 4.0-11.7%). Bangladesh was the country with the lowest pool prevalence of 0.4% (95% CI 0.2-0.7%). A considerable proportion of infected birds tested positive highlighted the relevance of individual animals as reservoirs of H5N6. Ducks and chickens were found to be positive by RT-PCR in over 3% of the cases. These data suggest their relevance in maintaining zoonotic transmission and their potential implications for epidemics and even pandemics in the near future.

Keywords: H5N6, Influenza, Meta-Analysis, Molecular diagnosis, RT-PCR, Systematic Review

INTRODUCTION

Avian influenza viruses (AIV) belong to the Alphainfluenzavirus genus in the Orthomyxoviridae family (Lefkowitz et al., 2018). They can be classified into Low-Pathogenic Avian Influenza Viruses (LPAIVs) with water birds as primary host reservoirs and Highly Pathogenic Avian Influenza Viruses (HPAIVs) with poultry as the main host reservoirs (Dhingra et al., 2018). These viruses can be brought long distances by aquatic birds, transmitted at chicken farms, and infect naive poultry (Bi et al., 2016). Highly Pathogenic Avian Influenza Viruses (HPAIVs) remain an underlying threat to global health and the economy. Some of these viruses carry potential pandemic risks (Swayne et al., 2017; Shin et al., 2020; Shittu et al., 2020).

H5N6, an AIV subtype, was first isolated from mallards in 1975 (Garcia et al., 1997). This virus has continuously evolved and reasserted to generate novel HPAIVs that have led to several epidemics incidents; in 2014, Laos and Vietnam reported an Influenza H5N6 outbreak that killed hundreds of birds (Shen et al., 2015), possibly imported from live poultry from China (Wong et al., 2015). In the same year, China reported the first fatal case of Influenza H5N6

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among its people (Pan et al., 2016). To date, 24 confirmed cases of human infection with influenza A (H5N6) virus have been reported to the World Health Organization (WHO) from China since 2014 resulting in seven deaths (World Health Organization, 2020).

Since birds play a pivotal role as natural hosts and reservoirs for this virus, a clearer understanding of bird-to-human transmission dynamics across wild, urban, and suburban settings is essential. Thus, a systematic review and meta-analysis of AIV were set to synthesize previously published data that assessed H1N6 infection in birds using the reverse-transcriptase polymerase chain reaction (RT-PCR). Then, this systematic review's main objective was to summarize the frequency of Influenza H5N6 infection in birds reported in currently available observational studies. Also, it was to examine the differences among the pool prevalence of H5N6 infections by animal, sample, and year.

METHODS

Protocol

The present systematic review followed the PRISMA statement's recommendations (Preferred Reporting Items for Systematic Review and Meta-Analysis, Moher et al., 2009).

Eligibility criteria

Published peer-reviewed articles that reported H5N6 infection in birds with serological or molecular confirmation by RT-PCR were included. The articles' language was not limited to English, and the publications were considered from January 1, 2002, to April 1, 2020, when the search ended. The exclusion criteria included review articles, opinion articles, correspondence articles or letters not presenting original data, and reports with incomplete information.

Information sources and search strategy

Medline/PubMed, Scopus, and Web of Sciences were used in the present systematic review. The search procedure was accomplished using the following terms "influenza", "avian influenza", "H5N6", "birds", and "influenza A". These words were used in combination while searching. Searching for this review ended on the date April 1, 2020, and a group of four different researchers evaluated the results yielded independently.

Study selection

The initial search strategy was first screened by title and abstract, as used in other systematic reviews (Rodriguez-Morales et al., 2020). The full text of relevant articles was examined for inclusion and exclusion criteria (Figure 1). When an article reported the same information from the same patient, both reports' information was combined to obtain complementary data, counted as a single case. Observational studies that reported the frequency of H5N6 infection in birds were included for quantitative synthesis (metanalysis).

Data collection and data items

Data extraction questionnaires, including information on the type of articles, publishing institution, country, year, and date of publication, as well as the number of infected animals assessed by RT-PCR, were filled independently by four researchers. An additional researcher checked the article list and data extractions to ensure no duplicate articles or duplicate information of the same study and resolved discrepancies about study inclusion. Regarding countries, the review found studies from China, Bangladesh, Myanmar, and Vietnam.

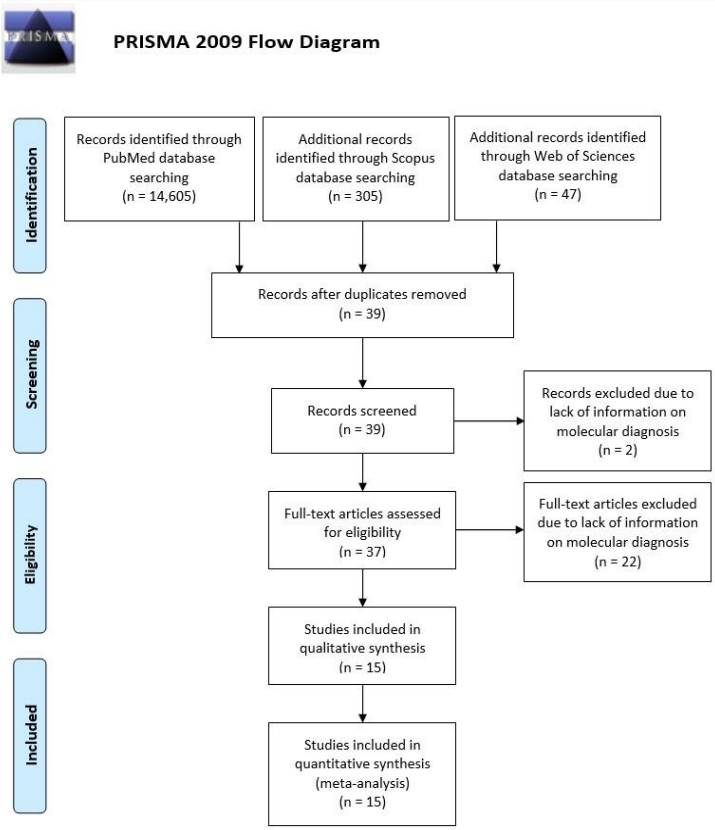

Figure 1. Study selection and characteristics of the articles included and considered for the qualitative and quantitative synthesis of data

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Assessment of methodological quality and risk of bias

To evaluate the quality of cross-sectional studies (AXIS), the critical appraisal tool from the Quality Appraisal of Case Series Studies Checklist of the Institute of Health Economics (IHE) was used (IHE, 2014; Downes et al., 2016). Publication bias was measured using a funnel plot. A model of random-effects was used to calculate the pooled prevalence and 95% CI, given variable degrees of data heterogeneity. The intrinsic heterogeneity in any systematic review of studies from published literature should be considered, then, also Egger's test was applied.

Statistical approach

Unit discordance for variables was resolved by converting all units into a standard measurement. The baseline data were analyzed using Stata version 14.0, licensed by Universidad Tecnológica de Pereira.

The meta-analysis was performed using Stata, Open Meta (Analyst) Software, and Comprehensive Meta-Analysis ve.3.3® licensed by Universidad Tecnológica de Pereira, Colombia. Pooled prevalences and their 95% confidence intervals (95% CIs) were used to summarize the weighted effect size for each study grouping variable using the binary random-effects model, considering the sample size of individual studies. For median ages, a continuous random-effect model was applied (DerSimonian-Laird procedure). A model of random-effects meta-analysis presumes that the effects being estimated in the different studies are not identical but follow some distribution. For random-effects analyses, the pooled estimate and 95% CIs refer to the center of the pooled prevalence distribution but do not describe the width of the distribution. Often the pooled estimate and its 95% CI are quoted in isolation as an alternative estimate of the quantity evaluated in a fixed-effect meta-analysis, which is inappropriate. The 95% CI from a random-effects meta-analysis describes uncertainty in the mean of systematically different prevalence in different studies.

Measures of heterogeneity, including Cochran's Q statistic, the I^2 index, and the tau-squared test, were estimated and reported, as elsewhere (Rodriguez-Morales et al., 2020). The subgroup analyses (sub-meta-analyses) were performed by diagnostic technique, animals, and countries.

RESULTS

Study selection and characteristics

A total of 14,605 articles were retrieved using the mentioned search strategy; 37 articles were selected for full-text assessment after screening by abstract and title. The rest were excluded as not containing relevant information and data for the systematic review. Twenty-two articles were excluded due to lack of information on molecular diagnosis, and 15 articles were finally included for the final qualitative and quantitative meta-analysis (Figure 1). Table 1 presents the main characteristics of the included studies.

The present review included 15 cross-sectional prevalence studies published from January 1, 2015, to April 1, 2020, which most of them were from China (81%), Vietnam (9%), Myanmar (9%), and Bangladesh (3%, tables 1 and 2), with a total of 13,416 birds assessed by RT-PCR. Three main variables (bird grouping, countries, and years) for the meta-analyses were analyzed (Table 2). Publication bias was reviewed with a funnel plot for the standard error by logit event, with no evidence of bias (Figure 2). Additionally, the Egger test suggested no substantial evidence of publication bias ($p = 0.568$).

Individual study characteristics

The mean number of included animals for RT-PCR per study was 407, with positive rates ranging from 0 to 53.8% (Tables 1-2).

Main findings

The RT-PCR pool prevalence for H5N6 was 3.5% (95% CI: 2.8-4.3%, Figure 3), 39.67% corresponded to ducks, with a pool prevalence of 7.7% (95% CI: 4.4-11.0, Figure 4), 35.63% corresponded to chickens, with a pool prevalence of 3.3% (95% CI: 1.9-4.8), and 19.15% of them were non-specified poultry birds, with the pool prevalence of 5.1% (95% CI: 0.0-14.8%, Table 2 and Figure 4).

Among the countries with the highest prevalence, Vietnam and China showed no significant differences. Vietnam with 538 animals and China with 4212 animals yielded a pool prevalence of 7.9% (95%CI: 4.0-11.7%) and 6.0% (95%CI: 4.3-7.8%). Myanmar and Bangladesh yielded a prevalence of 1.3% and 0.4%, respectively (Figure 5 and Table 2). The year 2018 had the highest prevalence (21.2%), followed by 2019 (8.3%) and 2020 (5.0%, Figure 6 and Table 2).

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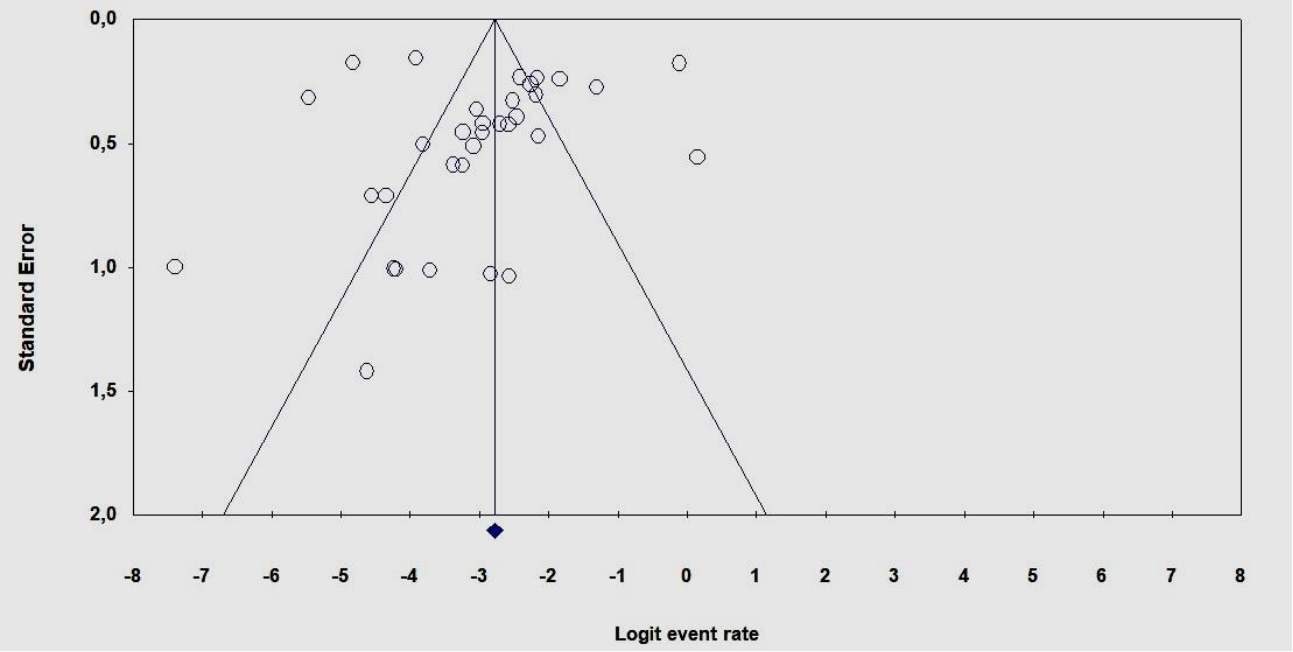

Figure 2. Funnel-plot for the standard error by logit event rate to assess the publication bias

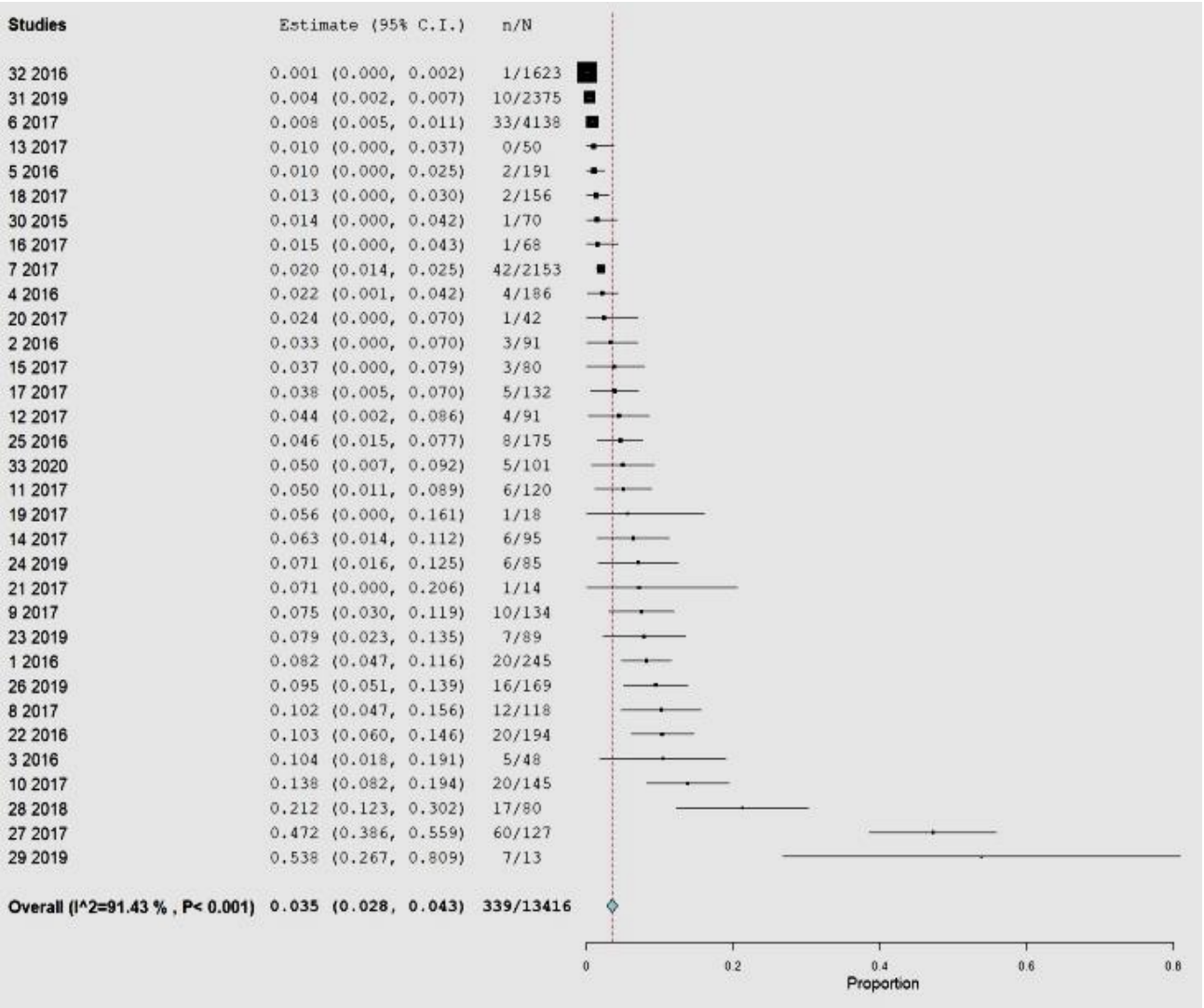

Figure 3. Forrest plot of the pooled prevalence meta-analysis of H5N6 infection in birds

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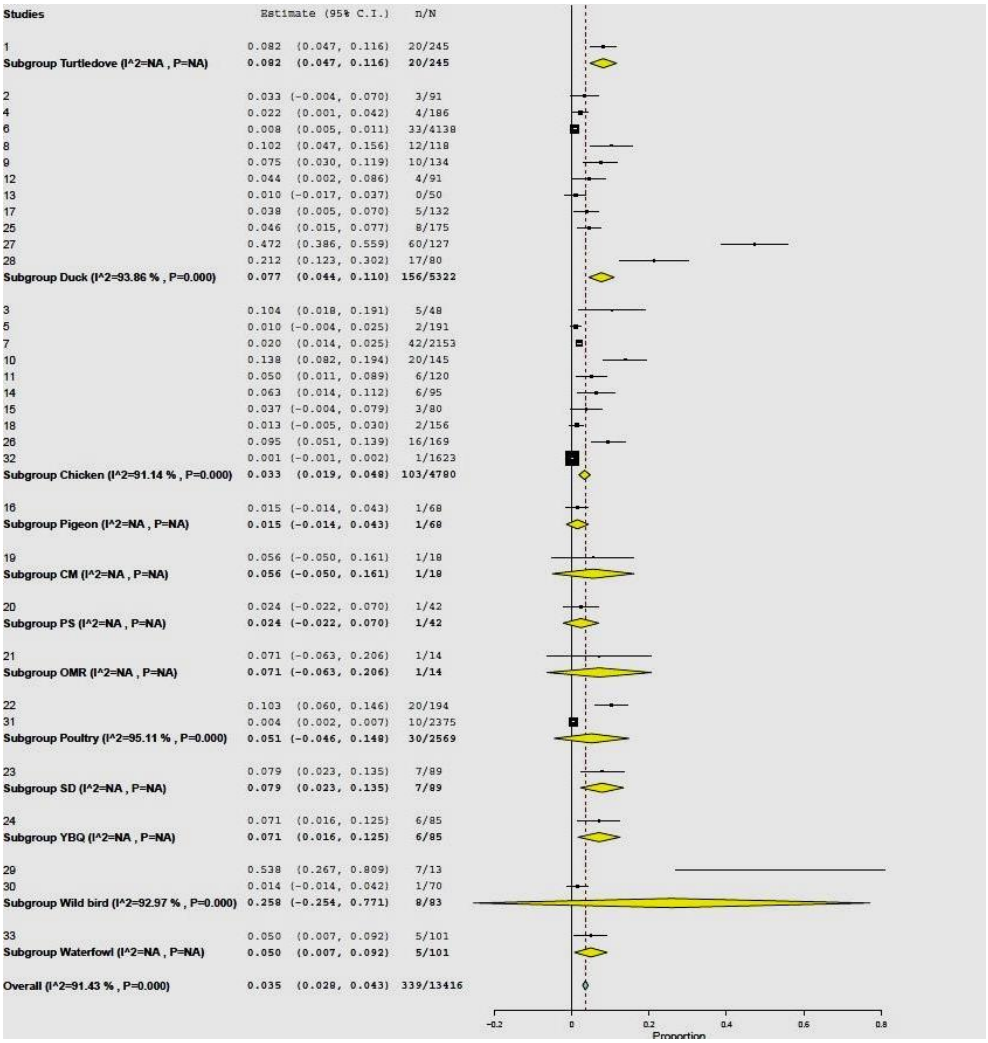

Figure 4. Forrest plot of the pooled prevalence meta-analysis of H5N6 infection in birds, by families or bird groups

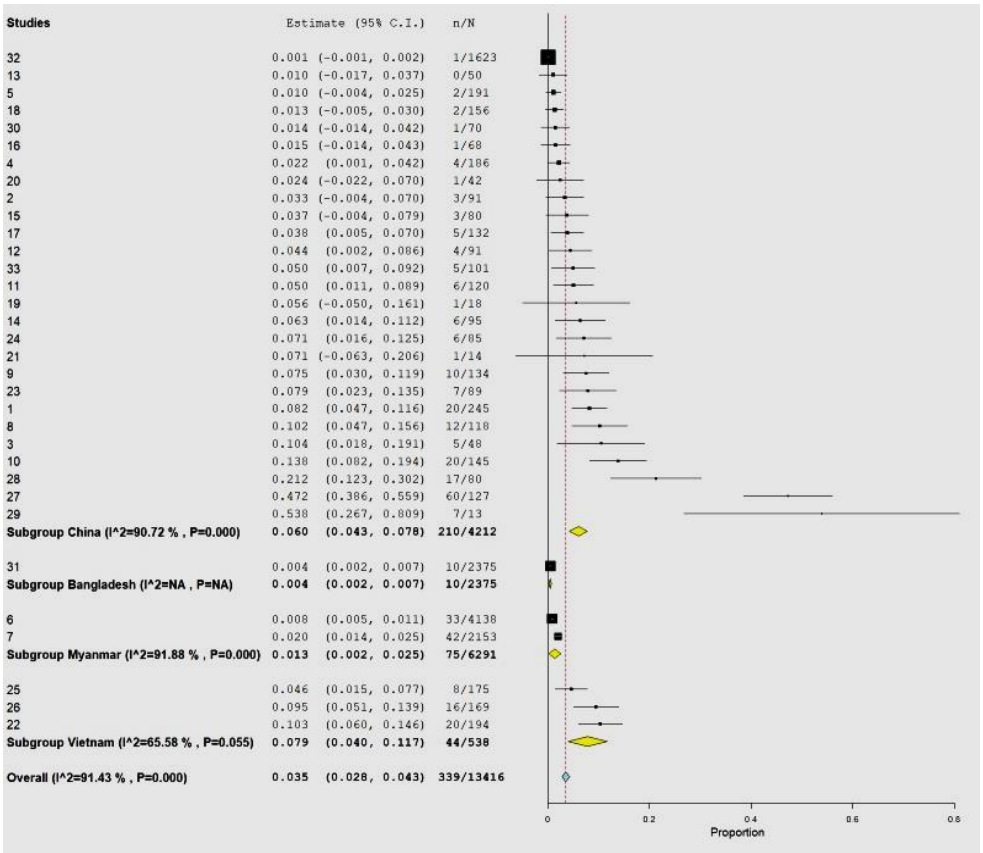

Figure 5. Forrest plot of the pooled prevalence meta-analysis of H5N6 infection in birds in terms of countries

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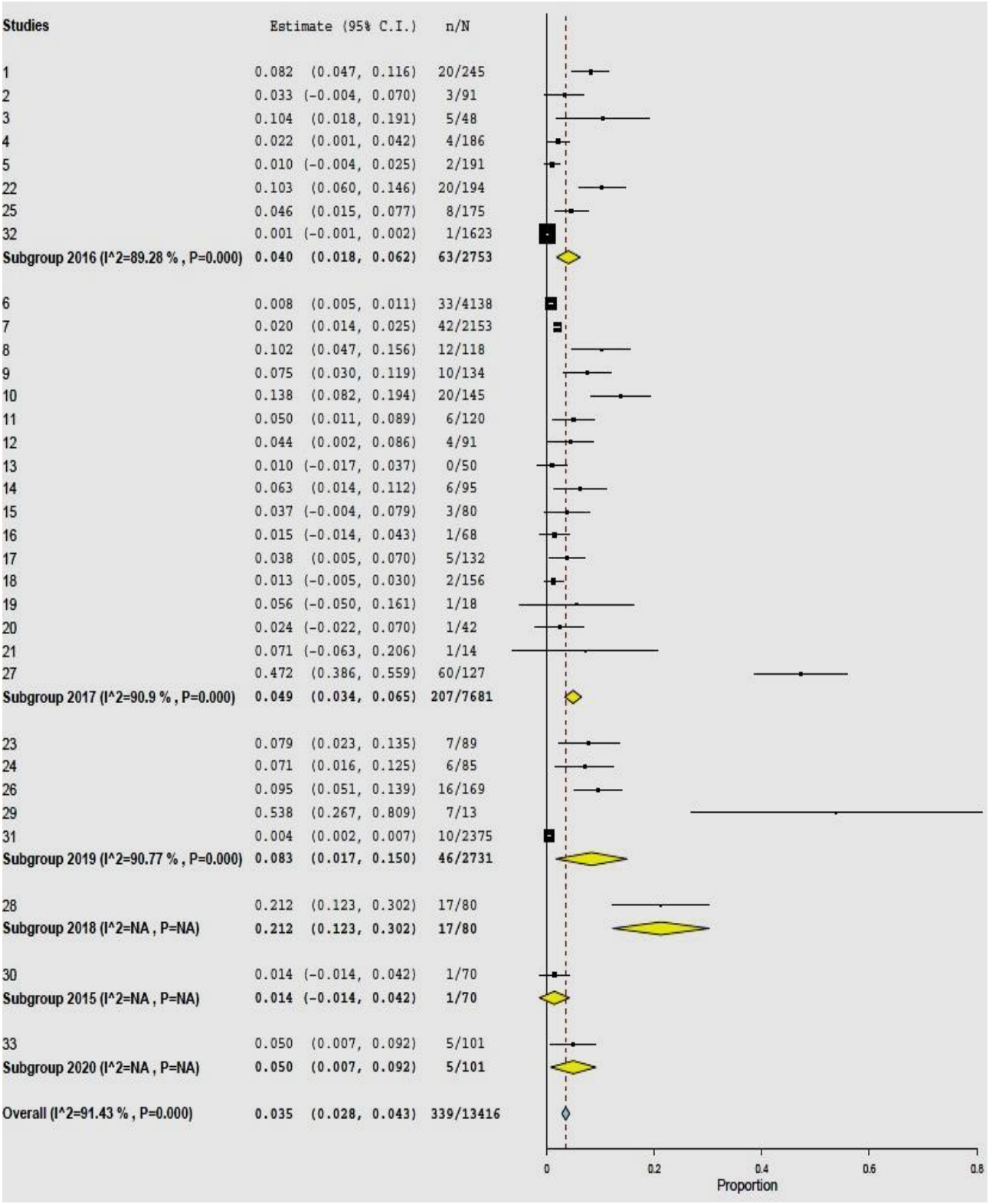

Figure 6. Forrest plot of the pooled prevalence meta-analysis of H5N6 infection in birds regarding years

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Table 1. Characteristics of the included studies on avian Influenza H5N1 in birds

Title of the study	Publication Year	Study Years	Country	Place	Birds Assessed	Sample	N	n (+)	Positive (%)	Reference
Diversity and evolution of avian influenza viruses in live poultry markets, free-range poultry, and wild wetland birds in China	2016	2014-2015	China	Hubei	Turtledove	Fecal	245	20	8.2	(Chen et al., 2016)
	2016	2014-2015	China	Hubei	Duck	Fecal	91	3	3.3	
	2016	2014-2015	China	Hubei	Chicken	Fecal	48	5	10.4	
	2016	2014-2015	China	Zhejiang	Duck	Fecal	186	4	2.2	
	2016	2014-2015	China	Zhejiang	Chicken	Fecal	191	2	1.0	
Emerging Zoonotic Influenza A Virus Detection in Myanmar: Surveillance Practices and Findings	2017	2014-2016	Myanmar	N/A	Duck	Oropharyngeal	4138	33	0.8	(Tun Win et al., 2017)
	2017	2014-2016	Myanmar	N/A	Chicken	Oropharyngeal	2153	42	2.0	
Diversity, evolution and population dynamics of avian influenza viruses circulating in the live poultry markets in China	2017	2014-2015	China	Hubei	Duck	Fecal	118	12	10.2	(Chen et al., 2017)
	2017	2014-2015	China	Hubei	Duck	Cloacal	134	10	7.5	
	2017	2014-2015	China	Hubei	Chicken	Fecal	145	20	13.8	
	2017	2014-2015	China	Hubei	Chicken	Cloacal	120	6	5.0	
	2017	2014-2015	China	Zhejiang	Duck	Fecal	91	4	4.4	
	2017	2014-2015	China	Zhejiang	Duck	Cloacal	50	0	0.0	
	2017	2014-2015	China	Zhejiang	Chicken	Fecal	95	6	6.3	
	2017	2013-2015	China	Zhejiang	Chicken	Cloacal	80	3	3.8	
	2017	2014-2015	China	Zhejiang	Pigeon	Cloacal	68	1	1.5	
	2017	2014-2015	China	Jiangxi	Duck	Fecal	132	5	3.8	
	2017	2014-2015	China	Jiangxi	Chicken	Fecal	156	2	1.3	
Highly pathogenic H5N6 influenza A viruses recovered from wild birds in Guangdong, southern China, 2014–2015	2017	2014-2015	China	Guandong	CM	Fecal	18	1	5.6	(Kang et al., 2017)
	2017	2014-2015	China	Guandong	PS	Fecal	42	1	2.4	
	2017	2014-2015	China	Guandong	OMR	Fecal	14	1	7.1	
Shifting Clade Distribution, Reassortment, and Emergence of New Subtypes of Highly Pathogenic Avian Influenza A(H5) Viruses Collected from Vietnamese poultry from 2012 to 2015	2016	2012-2015	Vietnam	Vietnam	Poultry	Oropharyngeal	194	20	10.3	(Nguyen et al., 2017)
First Detection of a Novel Reassortant Avian Influenza A(H5N6) Clade 2.3.2.1c Virus, Isolated from a Wild Bird in China	2019	2016	China	N/A	SD	Fecal	89	7	7.9	(Zhang et al., 2019)
	2019	2016	China	N/A	YBQ	Fecal	85	6	7.1	
Genetic and antigenic characterization of H5, H6, and H9 avian influenza viruses circulating in live bird markets with intervention in the center part of Vietnam	2016	2014	Vietnam	Thua Thien Hue	Duck	Fecal	175	8	4.6	(Chu et al., 2016)

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Title of the study	Publication Year	Study Years	Country	Place	Birds Assessed	Sample	N	n (+)	Positive (%)	Reference
Poultry trading behaviors in Vietnamese live bird markets as risk factors for avian influenza infection in chickens	2019	2017	Vietnam	northern Vietnam	Chicken	Oropharyngeal	169	16	9.5	(Sealy et al., 2019)
Identification of two novel avian influenzas a (H5N6) viruses in wild birds, Shanghai, in 2016	2017	2016	China	Shanghai: Chongming Dongtan, Nanhui Dongtan, Jiuduansha	Duck	CTS	127	60	47.2	(He et al., 2017)
Genetics, pathogenicity, and transmissibility of novel reassortant H5N6 highly pathogenic avian influenza viruses first isolated from migratory birds in western China	2018	2015	China	Changshantou	Duck	OCS	80	17	21.3	(Lu et al., 2018)
First Detection of a Novel Reassortant Avian Influenza A(H5N6) Clade 2.3.2.1c Virus, Isolated from a Wild Bird in China	2019	2016	China	Suichuan County	Wild bird	TCS	13	7	53.8	(Zhang et al., 2019)
Fatal H5N6 Avian Influenza Virus Infection in a Domestic Cat and Wild Birds in China	2015	2014	China	Sichuan province	Wild bird	Feces	70	1	1.4	(Yu et al., 2015)
Detection of highly pathogenic avian influenza A(H5N6) viruses in waterfowl in Bangladesh	2019	2016-2017	Bangladesh		Poultry	TCS	2375	10	0.4	(Yang et al., 2019)
Novel H7N2 and H5N6 Avian influenza A viruses in sentinel chickens: A sentinel chicken surveillance study	2016	2014	China	Jiangsu Province	Chicken	CTS	1623	1	0.1	(Zhao et al., 2016)
Novel H5N6 Avian Influenza Virus Reassortants with European H5N8 Isolated in Migratory Birds, China	2020	2017	China	Ningxia Hui Autonomous Region	Waterfowl	OCS	101	5	5.0	(Sun et al., 2020)

N: Total number assessed, n: number of positive, CM: Common moorhen, PS: Pallas's sandgrouse, OMR: oriental magpie-robin, SD: Streptopelia decaocto, YBQ: Yellow-legged button quail, CTS: Cloacal and tracheal swab, OCS: Oropharyngeal and cloacal swabs, TCS: Tracheal and cloacal swab, N/A: Not available or reported.

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Table 2. Meta-analysis outcomes (random-effects model) (prevalences of influenza H5N6, overall and subanalyses)*

Subgroups	Number of Studies	Pool Prevalence (%)	95% CI	n	Q†	I^{2} ‡	t^{2} §	p value
All the studies	15	3.5	2.8-4.3	13,416	373.460	91.431	0.001	< 0.001
Prevalence by Bird grouping								
Ducks	11	7.7	4.4-11.0	5,322	126.786	93.86	0.001	< 0.001
Chickens	10	3.3	1.9-4.8	4,780	101.601	91.14	0.001	< 0.001
Non-specified poultry	2	5.1	0.0-14.8	2,569	20.439	95.11	0.001	< 0.001
Prevalence by Countries								
Vietnam	3	7.9	4.0-11.7	538	5.811	65.58	0.100	0.05
China	28	6.0	4.3-7.8	4,212	298.457	90.72	0.001	< 0.001
Myanmar	2	1.3	0.2-2.5	6,291	12.320	91.88	0.001	< 0.001
Prevalence by Years								
2019	5	8.3	1.7-15.0	2,731	43.323	90.77	0.001	< 0.001
2017	17	4.9	3.4-6.5	7,681	175.786	90.9	0.001	< 0.001
2016	8	4.0	1.8-6.2	2,753	65.279	89.28	0.001	< 0.001

* 95% CI: 95% confidence interval. † Cochran's Q statistic for heterogeneity. ‡ I^{2} index for the degree of heterogeneity. § Tau-squared measure of heterogeneity.

DISCUSSION

Recent studies suggest that HPAI outbreaks from 2016 to 2018 caused by novel reassortant clade 2.3.4.4 H5N6 viruses resulted in the death of one billion birds in South Korea (Shin et al., 2020). In 2020, the clade 2.3.4.4B was reported in Iran after complete-genome sequencing of 28 H5Nx viruses circulating in the country from 2016 to 2018 (Abdollahi et al., 2020). In the same year, a study reported the first African case of HPAI (H5N6) virus (clade 2.3.4.4b) in a duck identified at a live-poultry market (LPM) in Nigeria whose genome was nearly linked to the European H5N6 viruses (2017-2018) (Shittu et al., 2020). However, as observed in the current meta-analysis, a tremendous prevalence burden exists within the Asian continent.

The prevalence rates of H5N6 infection using RT-PCR were reported up to 7.7%, with an upper limit of the confidence level of 11.0% in ducks and 3.3% in chickens. Both birds (ducks and chickens) share close contact with human beings, especially in the Asian live-poultry market (Fang et al., 2016). Ducks play a critical role in viral preservation and dissemination throughout different settings and environments. Thus, the control of H5N6 within LMPs is pivotal to eradicate influenza from poultry (Chen et al., 2019; de Vries et al., 2018). The continued interaction between humans and poultry in these settings poses a significant risk for human spillover infection and potential emerging health threats of epidemic or pandemic proportions. The increased H5N6 prevalence in LPMs has shifted public health efforts towards sustained LPM surveillance to retrieve relevant epidemiological information and provide early warnings of human infection with AIV. Interventions, such as live-poultry market surveillance and closings, should be implemented to mitigate the potential risk of infection in humans when these viruses are detected widely (Fang et al., 2016).

H5N6 Influenza is just one example of a current systematic phenomenon. The complete focus of public health towards Coronavirus disease 2019 (COVID-19) detracts nearly all attention away from other latent but relevant infectious diseases. As observed in the present meta-analysis, from 2015 to 2020, there has been consistent evidence of approximately 3% prevalence rates by RT-PCR for H5N6 Influenza in birds.

In 2020, a study reported a patient infected with the avian influenza A (H5N6) virus by aerosol exposure in China (Li et al., 2020). That case had no history of exposure to LMPs but had a record of exposure to live poultry placed in a car with closed doors and windows. The samples collected from the patient's lower respiratory tract and remaining frozen chicken meat were positive for the influenza A (H5N6) virus (Li et al., 2020). Earlier that year, a fatal case of H5N6 in an obese 9-year-old Chinese girl was reported. She was initially presented with fever and coughing, and then pneumonia, Acute Respiratory Distress Syndrome, and respiratory failure were developed. Aspirates from the lower respiratory tract and anal swabs were taken serially in that patient up to the death. A novel reassortant H5N6 virus was isolated, and genome sequencing and phylogenetic analysis were performed. Except for the polymerase acid protein (PA) gene, all the other seven genes of the virus belonged to H5N6 genotype A (S4-like virus) (Chen et al., 2020). Given these alarming cases in China, extraordinary measures should be implemented to mitigate or avoid future outbreaks of avian influenza.

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Although the current meta-analysis found a pool prevalence of 3.5%, some selected studies reached more than 21% (Figure 3). Considering the number of assessed birds, these findings should be considered relevant. A significant concern is raised, even more considering that there are no effective vaccines to prevent human H5N6 Influenza infection, although some candidates have been recently tested (Chen et al., 2019; de Vries et al., 2018). One of them, the rDEVus78HA vaccine, efficiently protected ducks against challenges with isolated heterologous H5N6 and H5N8 viruses (Chen et al., 2019). Another vaccine candidate, rMVA-H5 (Clinical trials registration: NTR3401), seemed to be effective against antigenically distinct H5 viruses (de Vries et al., 2018).

Primary prevention is critical in diseases with a high case fatality rate, typical for many HPAI infection cases (Bi et al., 2016). H5N6 virus induces large economic losses to poultry breeding industries in developing regions worldwide, especially in Asia. HPAI H5 clade 2.3.4.4 viruses were introduced in Europe in late 2014 and re-introduced in late 2016, following detections in Asia and Russia (Poen et al., 2019). Recent outbreaks reported in captive *Pavo cristatus* in Jiangxi Province, China, suggest avian influenza as a critical latent threat to public health (Li et al., 2019).

The present results highlighted the relevance of individual birds as reservoirs for H5N6. Ducks and chickens were found positive by RT-PCR in over 3% of the cases, showcasing their relevance in maintaining zoonotic transmission with the consequent risk of disease outbreaks. Additional research and enhanced LPM in China and other countries worldwide should be promptly considered to prevent more subsequent outbreaks.

DECLARATIONS

Present study was a part of the thesis of Veterinary Medicine and Zootechnics of D.C. Erazo-Arana, at Universidad Tecnológica de Pereira, Pereira, Risaralda, Colombia, under the supervision of D.K. Bonilla-Aldana and A.J. Rodriguez-Morales.

Authors' contributions

D. Katterine Bonilla-Aldana conceived the idea of the study. Yeimer Holguin-Rivera, Isabella Cortes-Bonilla, María C. Cardona-Trujillo, Alejandra García-Barco, Hugo A. Bedoya-Arias, Leidy Jhoana Patiño-Cadavid, Mateo Aguirre-Florez, Graciela J. Balbin-Ramon, Delcy C. Erazo-Arana collected data. Alfonso J. Rodriguez-Morales and D. Katterine Bonilla-Aldana analyzed the data. Alfonso J. Rodriguez-Morales wrote the first draft. All authors wrote and revised the subsequent drafts. All authors approved the final submitted version and the data analysis.

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Competing interests

All authors declare no competing interests to be reported.

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