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Infectious bursal disease virus in free-living wild birds: a systematic review and meta-analysis of its sero-viroprevalence on a global scale

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SUMMARY

Infectious bursal disease virus (IBDV) is an economically important pathogen for poultry, whereas knowledge of its occurrence in non-poultry hosts is limited. The objective of this systematic review and meta-analysis is to summarize the up-to-date knowledge about the sero-viroprevalence of IBDV in wild birds on a global scale. A computerized literature research was performed on PubMed, Scopus, CAB Direct and Web of Science to find relevant publications, along with the screening of reference lists. Journal articles, book chapters, scientific correspondences, conference proceedings and short communications on IBDV virological and/or serological surveys in free-living wild birds published between 1970 and 2021 were considered as eligible. Among 184 studies found, 36 original contributions met the pre-established criteria. A random-effect model was applied to calculate pooled seroprevalence estimates with 95% confidence intervals, whereas the paucity of virological studies (n = 6) only allowed a qualitative description of the data. The pooled seroprevalence was estimated to be 6% (95% C.I.: 3%-9%) and a high heterogeneity was detected (I^2=96%). Sub-group analyses were not performed due to the scarcity of available information about hypothetical moderators. With respect to virological studies, IBDV was detected in *Anseriformes*, *Columbiformes*, *Galliformes*, *Passeriformes* and *Pelecaniformes* and different strains related to poultry infection were isolated.
Our estimates of serological data showed a moderate exposure of wild birds to IBDV. The susceptibility of different species to IBDV infection underlines their potential role in its epidemiology at least as carriers or spreaders. Indeed, the isolation of IBDV in healthy wild birds with a migratory attitude might contribute to a long-distance spread of the virus and to strain diversity. Whilst a wild reservoir host could not be clearly identified, we believe our work provides useful insights for conducting future surveys which are needed to broaden our knowledge of IBDV occurrence in wild birds.

1. INTRODUCTION

Infectious bursal disease (IBD) or Gumboro disease (GD) is an economically impacting disease of the global poultry industry caused by Infectious bursal disease virus (IBDV), a highly contagious bi-segmented double stranded RNA virus. IBDV belongs to the genus *Avibirnavirus* within the family *Birnaviridae* (Lefkowitz et al., 2018) and two serotypes can be identified by cross-neutralization assays, namely serotype 1 and serotype 2. Serotype 1 includes pathogenic strains for chickens and serotype 2 includes non-pathogenic strains which naturally occurs in turkeys (McNulty et al., 1979; McFerran et al., 1980) and are also detected in other avian species (Candelora et al., 2010; Gough et al., 2002). IBDV transmission commonly happens through the fecal-oral route (Benton et al., 1967).

Being a non-enveloped RNA virus, IBDV is also extremely resistant in the environment and has the potential to be spread by different fomites (Crespo et al., 2016) and mechanical vectors (Howie & Thorsen, 1981; McAllister et al., 1995; Pagès-Manté et al., 2004; Park et al., 2010).
Regardless of the pathogenicity of the strain, IBDV infection damages the bursa of Fabricius and causes an immunosuppression which is more severe the younger the animals affected (Lupini et al., 2020; Rautenschlein et al., 2003; Silveira et al., 2019; Sharma et al., 2000). IBDV-related immunodeficiency indeed leads a flock to higher susceptibility to secondary bacterial infections and decreases the efficiency of vaccination programs routinely applied (Aricibasi et al., 2010). Because of viral evolution through mutation (Aliyu et al., 2021), reassortment (Jackwood et al., 2016) and recombination (Jackwood, 2012; He et al., 2009), diverse IBDV genotypes are detected and classified in numerous genogroups (Michel & Jackwood, 2017; Islam et al., 2021). Infections with antigenically different strains can significantly impact the poultry production system due to the potential limited efficacy of implemented vaccination plans. Furthermore, the origin of newly emergent IBDV strains can be unobvious and epidemiological surveys can lead to inconclusive epidemiological links (Felice et al., 2017; Lupini et al., 2016; Thai et al., 2021).
Despite an increasing awareness of the role of wildlife in poultry pathogens' ecology, little is known about the role played by wild birds in the IBDV epidemiology. Since 1980, scientific papers have demonstrated that other avian species apart from chicken (*Gallus gallus*) and turkey (*Meleagris gallopavo*) are susceptible to IBDV (Gough et al., 1998; McFerran et al., 1980; Wang et al., 1997; Zhou et al., 1998). It has therefore been hypothesized that wild birds could be epidemiologically relevant to the genetic evolution of circulating IBDV strains (Hon et al., 2006; Tammiranta et al., 2018; Yamaguchi et al., 1997) or could act as spreaders between infected farms (Gilchrist, 2005).
Wild birds can exhibit extremely heterogeneous patterns of movement according to species and populations. Given their ability to fly over long distances, wild migratory birds interconnect different parts of the globe and can deliver pathogens from one country to another (Jourdain et al., 2007). In addition to natural habitats, some species are well adapted to human-driven environments and can act as bridges between pristine ecosystems and anthropogenic ones (Patankar et al., 2021; Wille et al., 2020).
Considering the continuous detection of diverse strains of IBDV, disentangling the potential role of wild birds in the epidemiology of this pathogen is pivotal. In this study we summarize the up-to-date evidence of IBDV sero-virological prevalence in wild birds on a global scale conducting a systematic review and meta-analysis. To the best of our knowledge, this is the first review that focuses on IBDV in wild species rather than in poultry (Alkie & Rautenschlein, 2016; Dey et al., 2019; Berg, 2000; Mahgoub et al., 2012).

2. MATERIALS AND METHODS

A systematic review and meta-analysis were performed to estimate the seroprevalence of the IBDV in free-living wild birds. Given the small number of studies using molecular or virological methods to define the viral prevalence, it was determined that these could not be considered together. Thus, the analysis was limited to a systematic review and the studies' characteristics were displayed in a descriptive table.
Our work followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P) (Moher et al., 2015) and the PRISMA 2020 Statement (Page et al., 2020) (Supporting information 1).

2.1 Information sources and search strategy

Four databases were accessed from 01/10/2020 to 24/04/2021. These included: PubMed (https://pubmed.ncbi.nlm.nih.gov), Scopus (https://www.scopus.com/), CAB Direct (https://www.cabdirect.org) and Web of Science (https://apps.webofknowledge.com/). The reference

lists of eligible studies were also screened to find other relevant contributions by hand (Higgins et al., 2021).
The search strategy used for each database is reported in Table 1. Boolean operators 'OR' and 'AND' were used in all the computerized databases and MeSH Terms related to IBDV were used in PubMed. Eligible studies were merged and managed in a Microsoft Excel 2021 sheet (Version 16.49).

2.2 Selection process

Literature searches and the screening process of the papers were independently performed by two reviewers (G.G. and A.F.). Eligible studies were selected by applying pre-established inclusion criteria to title and abstract of each work (Table 2). Portuguese and Chinese manuscripts were screened after translation into English through Google Translate (https://translate.google.com). After removing duplicate papers, the reasons for exclusion of any other study were recorded. If disagreements occurred, a third experienced author in the avian pathology field was consulted (E.C.).

2.3 Data collection process

Data extraction from each eligible study was performed by two independent reviewers (G.G. and A.F.) and the quality of the data was double checked by a third author (C.L.). Whenever two different diagnostic methods were applied, only the outcomes from the test used as confirmatory were considered. If prevalence data retrieved from original articles was expressed as percentage, raw numbers were obtained converting the percentages to the closest integers.
Two data extraction sheets were created, one for the seroprevalence surveys and another for viral prevalence ones. The first was filled with the following information: title, first author last name, year of publication, country, language, sampling period, host identification (order, family and species), age classes and sex, sample type, total number of animals tested, total number of positive cases, serological method, serological cut-off used to define a sample as positive, specificity and sensitivity of the method applied and serotype characterization (serotype 1 or serotype 2). The second with: title, first author last name, year of publication, country, language, sampling period, host identification (order, family and species), age classes and sex, sample type, total number of animals tested, total number of positive cases, molecular method, viral isolation method, specificity and sensitivity of the methods applied and GenBank accession number (http://www.ncbi.nlm.nih.gov/).

2.4 Study risk of bias assessment

Despite the availability of different tools to evaluate the publication bias, the authors could not find a suitable one to the given dataset (Hunter et al., 2014). Considering the heterogenous study designs, diversity of species tested, different sample sizes and zero positive cases reported in some original papers it was decided to include all the eligible full texts retrieved. A comment section was added to the data extraction sheets to report anything that could affect the interpretation of the results (Fanelli et al. 2020). Moreover, outliers were identified through leave-one-out analysis and excluded from the meta-analysis (Viechtbauer & Cheung, 2010).

2.5 Summary outcomes

The principal summary outcome was the overall seroprevalence of IBDV in free-living wild birds without restriction to any geographical area. As mentioned before, data from virological studies were not pooled due to the paucity of original articles available and virological prevalence was therefore calculated for each individual study.

2.6 Synthesis methods

2.6.1 Statistical synthesis methods

All statistical calculations were performed on R software version 3.5.2 (R Core Team, 2018).
Visual representation of search results was realized with the *wordcloud* package. The *meta* version 4.18-2 and *metaphor* version 2.4.0 packages were used to perform the meta-analysis of serologic data as described in the comprehensive tutorial of Wang (2018). Briefly, the observed proportions were transformed using the double arcsine method to normalize the data (Freeman & Tukey, 1950; Miller, 1978). For reporting, the pooled transformed proportions and its 95% confidence interval (C.I.) was converted back to the observed proportions. The between-study variance was estimated through the random effects method using the restricted maximum-likelihood estimator (REML). The heterogeneity between studies was quantified using the inconsistency index (I^2) and interpreted as small, medium or high according to <25%, 25-50% and >75% values respectively (Higgins & Thompson, 2002). After visual inspection of the pooled seroprevalence forest plot, outliers were identified performing leave-one-out analysis and therefore removed.
Considering the virological prevalence, the *prevalence* package version 0.4.0 was applied to calculate this estimate and its 95% C.I. using the "exact" method.

2.6.2 Other analyses

Countries were grouped into sub-regions according to the standards of the Statistics Division of the United Nations Secretariat (https://unstats.un.org/unsd/methodology/m49/). Eventual inconsistencies in the taxonomic nomenclature used by the Authors were harmonized according to current nomenclature following the latest Avibase version (Lepage & Warnier, 2014). The taxonomic order of the hosts, the age classes and the geographical area of the studies were hypothesized as possible moderators. However, we decided not to perform sub-group analyses because of the scarcity of data available and heterologous age attribution techniques. Moreover, the studies were not pooled according to serological methods given the lack of any information about the sensitivity and specificity of the different tests applied.
With respect to virological studies, sequence analysis of IBDV Viral Protein 2 from wild birds was performed. Reference strains reported in Aliyu et al. (2021) were obtained from NCBI GenBank and used to represent IBDV genogroups (Michel & Jackwood, 2017). Sequences were aligned by MUSCLE algorithm. Best partition scheme, substitution models and maximum likelihood phylogenetic reconstruction were performed on the IQ-TREE web server using 1000 ultrafast bootstrap replicates (Trifinopoulos et al. 2016).

3. RESULTS

3.1 Search summary

A total of 165 articles were identified through the computerized literature research on PubMed, CAB Direct, Scopus and Web of Science. After duplicates removal (n = 67), 98 articles were assessed for eligibility through the screening of titles and abstracts. Eventually, 23 studies met the pre-established criteria and were full-text accessed. Of these, one article out of 23 was afterwards excluded due to lack of necessary information on the species tested (Pawar et al., 2009). 22 eligible studies were therefore obtained from literature research on selected databases.
Another 19 articles were added after reference lists reading. Two of these studies were excluded due to unsuccessful retrieval of the full texts (Oña et al., 2000; Edgar & Cho, 1965). Three more studies were rejected after full text reading: 1) Wang et al. (1997) and 2) Zhou et al. (1998) surveyed the same population that was raised and held in captivity, 3) Candelora et al. (2008) presented the

preliminary results of Candelora et al. (2010), only the latter was therefore considered. As result, 14 studies retrieved through reference lists reading were considered eligible.
To conclude, 36 original articles about virological and serological surveys of IBDV in wild birds were included in the systematic review here presented (Figure 1). After full-text retrieval, articles applying direct (n = 3) or indirect (n = 30) diagnostic methods were sorted into two different Microsoft Excel sheets as previously described. Watts et al. (2009), Vargas-Castillo et al. (2019) and Kasanga et al. (2008) which applied both serological and virological diagnostic methods were included in both databases.

3.2 Study characteristics

We identified 36 eligible articles on IBDV surveys in free-living wild birds published between 1978 and 2020 and covering 22 countries (Figure 2). An overview of the scientific publications is provided in Table 3 and Table 4, respectively for the use of indirect or direct methods for IBDV diagnosis.
Regarding serological surveys, the studies retrieved (n = 33) were published between 1978 and 2020 and covered 20 countries of the world. A total amount of 7556 sera of free-living wild birds were examined and 1112 resulted positive. The serological methods applied included the enzyme-linked immunosorbent assay (ELISA) performed with both in-house and commercial kits, the viral neutralization test (VNT) and the agar gel immunodiffusion assay (AGID). None of the studies reported the sensitivity and specificity of the methods with regards to the species tested. The most common technique was the VNT (n = 13) whose details on strains and cell cultures applied are listed in Table 5, followed by the ELISA (n = 10). Four studies (Campbell, 2001; Grimaldi et al., 2018; Nunes et al., 2012; Wilcox et al., 1983) used two different serological tests in parallel or subsequently. Only eight out of 33 studies distinguished the IBDV serotype as serotype 1 or 2 for the positive cases (Candelora et al., 2010; Gauthier-Clerc et al., 2002; Hollmén et al., 2000; Kasanga et al., 2008; Miller et al., 2008; Ogawa et al., 1998; Watts et al., 2009; Wilcox et al., 1983). Regarding the host, a total of 17 orders of birds were sampled (Table 1A, Supporting information 2). Most studies concerned *Sphenisciformes* (12/33 papers), *Passeriformes* (10/33), *Columbiformes* (9/33), and *Charadriiformes* (7/33) (Figure 3). *Sphenisciformes* (n = 4050 birds), *Galliformes* (n = 753 birds) and *Anseriformes* (n = 459 birds) were the groups most frequently sampled. The highest rate of positive cases per order was retrieved for *Falconiformes* (33.3%), *Anseriformes* (32.9%); *Gruiformes* (32.7%), *Sphenisciformes* (16.8%), *Accipitriformes* (12.9%) and *Charadriiformes* (11.1%).
After statistical analyses, the following studies were identified as outliers and therefore removed from the summary of results: Dwight et al. (2018), Hollmén et al. (2000) and Miller & Shellam (2007) (Supporting information 3).
The six molecular/virological studies retrieved were published between 2009 and 2020 and covered 6 countries (Figure 2). Bursa of Fabricius (n = 3), cloacal swabs (n = 2) and cecal tonsils (n = 1) were sampled for attempting IBDV diagnosis. The total number of animals sampled were 244 of which 10 resulted positives in four different studies (Curland et al., 2018; Jeon et al., 2008; Kasanga et al., 2008; Naggar et al., 2020). The results from Watts et al. (2009) were not included in the count of the positive cases due to a hypothetical positive control contamination of the reverse-transcriptase PCR (RT-PCR) as assessed by the Authors of the paper. Regarding the technique applied, the RT-PCR or RT nested PCR assays were used as a screening method in all the studies. Primers and genes amplified are listed in Table 6. Naggar et al. (2020) and Jeon et al. (2008) both attempted viral isolation *via* chorioallantonic membrane (CAM) inoculation of embryonated specific pathogen free (SPF) chicken eggs. Whereas the CAM harvested material in Naggar et al. (2020) was confirmed of IBDV isolation through a Real-Time quantitative RT-PCR (qRT-PCR), Jeon et al. (2008) used both RT-PCR and AGP (agar gel immunoprecipitation test) for confirmation. Strains isolated by Jeon et al. (2008) and Naggar et al. (2020) and detected by Kasanga et al. (2008) were sequenced in Viral Protein 2 (VP2) gene, whereas Curland et al. (2018) did not further characterized the IBDV positive RT-PCR products. With respect to the host, eight different orders of birds were investigated and the

Columbiformes and *Galliformes* were surveyed in 3 out of 6 papers (Table 2A, Supporting information 2). The most frequently sampled groups were *Anseriformes* (n = 92 birds), *Passeriformes* (n = 43 birds) and *Galliformes* (n = 37 birds). The highest rate of positive cases per order was retrieved for *Pelecaniformes* (33.3%) and *Anseriformes* (5.4%).

3.3 Results of individual serological and virological studies

Effect estimates and confidence intervals for each seroprevalence study are reported in the forest plot in Figure 4. The results from individual studies on serological and molecular/virological prevalence are shown in Table 3 and Table 4, respectively.

3.4 Risk of bias in serological and virological studies

No relevant risk of bias were identified by reviewers.

3.5 Pooled seroprevalence of serological studies

The pooled seroprevalence of IBDV in free-living wild birds was 6% (C.I. 95%: 3-9%). The I^2 statistic showed high heterogeneity ($I^2 = 96\%$, $p < 0.01$).

3.6 Phylogenetic analysis

Phylogenetic reconstruction was performed using VP2 sequences from wild bird strains by applying a TIM2+F+G4 substitution model to the first codon position and a TIM2e+G4 substitution model to the second and third codon positions, as implemented on the IQ-TREE web server. As represented in Figure 6, IBDV strains detected in wild birds clustered with IBDV genogroup 1 and genogroup 3 strains.

4. DISCUSSION

4.1 Summary of evidence

To the best of our knowledge this is the first systematic review and meta-analysis of the sero-viroprevalence of IBDV in wild birds on a global scale. Considering the persistence of serum antibodies over time and the ease of revealing both past and current infection, the majority of relevant papers retrieved from literature concerned serological surveys rather than virological ones.
With respect to seroprevalence, the results showed that wild birds from different taxonomic groups were exposed to IBDV with an overall pooled prevalence estimate of 6% (95% C.I.: 3-9%). The meta-analysis also highlighted a high degree of heterogeneity ($I^2 = 96\%$, $p < 0.01$) thus our estimate is important to be considered together with its 95% C.I.. Hollmén et al. (2000), Dwight et al. (2018), and Miller & Shellam (2007) were identified as outliers. Although these studies reported the highest percentages of wild birds with antibody titers against IBDV (69.2%, 69.7% and 63.6% respectively), none of the positive cases were epidemiologically linked to outbreaks in poultry. Hollmén et al. (2000) found a high seroprevalence of IBDV in spectacled eiders (*Somateria fischeri*) living in remote areas of western Alaska, speculating that they might act as carrier hosts of IBDV. Dwight et al. (2018) carried out a sero-survey on wild and pen-reared pheasants (*Phasianus colchicus*). High seroprevalence found in both populations was interpreted by the Authors as a potential false positivity due to unvalidated diagnostic test applied or as a potential unexploited epidemiological role of pheasants in the ecology of IBDV. Miller & Shellam (2007) detected IBDV antibodies in adults and 1 year-old king penguins (*Aptenodytes patagonicus*) and adult royal penguins (*Eudyptes schlegeli*) on a sub-Antarctic island, thus implying an endemic circulation of IBDV in these populations.

Despite the rigorous statistical methods applied, our findings need to be cautiously interpreted. Several variables could have been hypothetically considered as moderators (e.g. geographic areas, host characteristics, diagnostic methods), however we could not perform any sub-group analyses due to the scarcity of available data. This can be considered a limit of the review hereby presented. With regards to the spatial distribution of data, we included 33 eligible studies from 11 different world sub-regions. More than one sero-survey per area were retrieved only for South America, Western Africa, Eastern Asia and Antarctica therefore we were not able to pool the data according to this factor. Concerning the avian host characteristics, free-living wild birds can be frequently found around rural and commercial poultry farms (Burns et al., 2012). Moreover, some species could be considered as more exposed to possible IBDV infection due to their ecology and feeding habits. Higher taxonomic categories could have been considered as a possible grouping factor to investigate the heterogenous results generated from the diversity of species tested. Unfortunately, several orders of birds were underrepresented or included exclusively in one survey. Still, we hereby discuss relevant findings for guiding future surveys. *Sphenisciformes* was the most studied order (12/33 papers) due to the increasing attention of men-driven threats to the Antarctic ecosystem. IBDV seropositivity was frequently observed in wild penguins (16.8%), possibly implying an endemic circulation (Miller & Shellam, 2010; Watts et al., 2009) or a diagnostic cross-reactivity with closely related viruses (Gauthier-Clerc et al., 2002; Gilbert et al., 2013; Grimaldi et al., 2018). Currently available data though is not sufficient for considering these birds as a possible natural IBDV reservoir. *Falconiformes* and *Anseriformes* appear to be the orders with the highest rates of seropositive animals (33.3% and 32.9%, respectively), however: 1) in *Falconiformes* only 3 animals were sampled; 2) the major contribution to positive cases in *Anseriformes* (127 cases over 151 total positives) arose from spectacled eiders tested solely by Hollmén et al. (2000). *Gruiformes* also showed an high rate of seropositive animals (32.7%), although this order was investigated in just two out of the 33 sero-surveys retrieved (Candelora et al., 2010; Assam et al., 2014). Candelora et al. (2010) found IBDV antibodies against serotype 2 in sandhill cranes (*Grus canadensis*) in Florida, USA. The Authors hypothesized that these infected cranes could interact with the endangered whooping cranes (*Grus americana*) living in the same habitat and be a potential carrier of infection for the latter, with unknown consequences for the species' conservation. A 12.9% overall seropositivity was found in *Accipitriformes,* with positive cases originated from only one (Höfle et al., 2001) of the two studies where representatives of this order were included (Assam et al., 2014). This *taxon* comprises diurnal predators with feeding behaviors that might expose them to IBDV spillover from poultry farms with low biosecurity levels, however current data is insufficient to drive any conclusion. Lastly, *Charadriiformes* showed an 11.1% overall seropositive IBDV rate. This order includes aquatic birds as gulls, terns, plovers and other shorebirds which are often gregarious and migrants. Hollmén et al. (2000) hypothesized that herring gulls (*Larus argentatus*) might be IBDV carriers due to their exposure to poultry waste and their opportunistic feeding behaviors. The trophic plasticity of *Lariidae* species might indeed be a factor to be taken into account when arranging epidemiological surveys in wild birds. Furthermore, skuas (*Stercorariidae*) might migrate and introduce pathogens in Antarctica by stealing food resources from other birds and scavenging around fishing boats (Miller et al., 2008; Nunes et al., 2012).

Another possible source of the heterogeneity observed in our meta-analysis could be found in the age or sex of the tested birds. Demographic data is indeed believed to be an important tool for comparing the outcomes from different studies. Unfortunately, we could not investigate age or sex related patterns of seroprevalence since many studies lacked information or applied incomparable criteria of age classification.

Also, the use of different serological methods with different performances could have affected the obtained results. None of the studies reported about the sensitivity and the specificity of the test used with regards to the species. ELISA is frequently chosen for conducting sero-epidemiological surveys and is considered as a sensitive and rapid diagnostic tool, however the commercial kits validated for poultry could yield false-positives when applied to non-poultry hosts (Karesh et al., 1999; Nunes et

al., 2012; Parsons et al., 2016; Smith et al., 2008; Travis et al., 2006b; Uhart et al., 2020). The virus neutralization test is recognized as a very sensitive and very specific serological test and has the advantage of being possibly used with the sera of any species (Phalen, 2002). VNT can also differentiate antibodies from exposure to IBDV serotype 1 or 2. The latter is considered apathogenic for fowl, however high titers against IBDV serotype 2 had been associated with high mortality and morbidity events in captive-reared whooping cranes (*G. americana*) during a reintroduction program in Florida, USA (Spalding et al., 2008). Only few studies distinguished between IBDV serotypes (8/33 articles), still this differentiation is advised to give a more exhaustive epidemiological picture. Grimaldi et al. (2018) and Nunes et al. (2012) combined two different serologic methods (an in-house competitive ELISA and the VNT and a commercial ELISA kit and the AGID, respectively) to confirm the positivity observed with the ELISA test and overcome methodological limitations.

With respect to viro-prevalence studies, we could not perform a meta-analysis due to the limited number of relevant articles retrieved (n = 6). Virus detection proves active viral shedding and allows further characterization of the isolated strains, we therefore consider it relevant to review the available data even if scarce. As reported by Naggar et al. (2020), the isolation of IBDVs in alive wild birds with a migratory attitude highlights the chance of a long-distance spread of the virus. It is also relevant to notice that these positive individuals did not show any sign of disease. IBDV strains were also detected in a black-billed magpie (*Pica hudsonia*) (Jeon et al., 2008), a wild pigeon (*Columba livia*) (Kasanga et al., 2008) and a wild pheasant (*P. colchicus)* (Curland et al., 2018) which are common species around poultry farms. According to sequence analyses, wild bird isolates of IBDVs are closely related to IBDV genogroup 3 strains (Jeon et al., 2008; Kasanga et al., 2008; Naggar et al., 2020) or IBDV genogroup 1 strains (Naggar et al., 2020), suggesting epidemiological links between domestic chicken and wild birds. Unique aminoacidic residues found in the VP2 hypervariable region (HVR) of some isolates also indicates that IBDV can undergo mutational changes in the wild hosts (Kasanga et al., 2008, Naggar et al., 2020). Furthermore, wild birds might harbor strains that could eventually reassort with poultry ones leading to the emergence of novel IBDV outbreaks in the future.

4.2 Limitations

Among the constrains identified regarding the systematic review presented, we acknowledge the scarcity of publications on IBDV in free-living wild birds in comparison with the abundance of studies available for poultry. We also imply the existence of possible research which may not be accessible through the search strategy here adopted. With respect to the statistical analysis, the scarcity of relevant articles about IBDV virological prevalence prevented us from pooling the data and performing a meta-analysis. Furthermore, we did not apply any test neither to assess nor to quantify the publication bias because of the absence of specific tools appliable to our research question (Fanelli et al., 2021).

5. CONCLUSION

Considering the continuous evolution of IBDV strains and their spatial distribution over time, we feel that the role of free-living wild birds has implications that remain unexplored. Our systematic review and meta-analysis provide an up-to-date synthesis of the available literature related to IBDV sero-viroprevalence in wild hosts. Whereas it is not possible to currently identify any wild bird species as a reservoir of IBDV, it is still important to assess the role that free-living birds could have in the epidemiology of this virus considering their movements and aggregation patterns. An effective screening strategy should combine, whenever possible, serological and virological methods of diagnosis to increase the significance of the outcomes. Increasing attention has been placed on synanthropic wildlife as bridge hosts, potentially vehiculating viruses from natural maintenance hosts to poultry, between different poultry farms (Shriner et al., 2016) or from poultry to other wild birds.

To better interpret the epidemiological links existing among different IBDV host species, it is crucial to perform a genetic characterization of the isolates. We also consider of main importance collecting exhaustive information on the individuals tested, such as species, sex and age classes according to international standards. Whenever positive results are presented, a thorough examination of the host ecology may help for further epidemiological considerations. Due to men-driven environmental changes, pathogens' ecology at the wildlife-domestic interface is evolving fast. Further research is necessary to better understand the role that wild birds might play in the eco-epidemiology of this virus and in driving changes in the way IBDV impacts poultry production.

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TABLES

Table 1. Search lines used for the computerized literature research and number of studies retrieved from each database (PubMed, Scopus, CAB Direct, Web of Science) before duplicates removal.

Database	Search line	No. of studies retrieved
PubMed	(((((infectious bursal disease*[Title/Abstract]) OR "infectious bursal disease virus"[MeSH Terms]) OR "gumboro disease"[Title/Abstract]) OR "ibdv"[Title/Abstract]) OR "birnaviridae infections/veterinary"[MeSH Terms]) AND ((animal, wild[MeSH Terms]) OR “free living”[Title/Abstract]) AND (((“birds”[MeSH Terms]) OR bird*[Title/Abstract]) OR "wild birds"[Title/Abstract])	14
Scopus	TITLE-ABS("infectious bursal disease virus") OR TITLE-ABS("infectious bursal disease") OR ("Gumboro") OR TITLE-ABS("IBDV") AND (TITLE-ABS("wild") OR TITLE-ABS(*free-living*)) AND TITLE-ABS(*bird*)	49
CAB Direct	(title:("infectious bursal disease virus") OR ab:("infectious bursal disease virus") OR title:("Gumboro disease") OR ab:("gumboro disease") OR title:("IBDV") OR ab:("IBDV")) AND ((title:("wild") OR ab:("wild")) OR (title:(free-living) OR ab(free-living)) AND (title:("bird") OR ab:("bird"))	42
Web of Science	ALL=(("infectious bursal disease" OR "infectious bursal disease virus" OR "avian infectious bursitis" OR "gumboro disease" OR "IBDV") AND (((wild) OR (free-living)) AND (bird*)))	60

Table 2. Inclusion criteria applied for screening titles and abstracts of the publications retrieved.

All studies (journal articles, book chapter, scientific correspondence, conference proceedings and contributions, short communication) on natural infections of IBDV published after 1970
All languages
Full text access
Virological/molecular study (direct diagnostic methods)
Serological study (indirect diagnostic methods)
Samples: blood, tissue samples, swabs
Free-living wild birds: species of free-range wild birds not living in captivity when the study occurred, exception for wild birds admitted to rehabilitation center
Sample size > 10
Population not included in more than one study

Table 3. Overview of the serological studies on IBDV in wild birds included in the qualitative synthesis of results and in the meta-analysis.

Authors and publication year	**Geographic area**[†]	**Study period**	**Method**	**Host identification**	**Total no. birds (total cases)**	**Apparent prevalence (%)**	**Sero-type**
Leite et al., 2020	South America	n.r.	ELISA[a]	*Columbiformes, Passeriformes*	30(0)	0	n.a.
Uhart et al., 2020	South America	1994-2008	AGID	*Sphenisciformes*	337(0)	0	n.a.
Orakpoghenor et al., 2020	Western Africa	2017	ELISA[b]	*Columbiformes, Pelecaniformes, Passeriformes*	150(3)	2	n.a.
Vargas-Castillo et al., 2019	South America	2012-2013	ELISA[a]	*Apodiformes, Columbiformes, Passeriformes*	29(0)	0	n.a.
Dwight et al., 2018	Northern America	2014-2015	ELISA[a]	*Galliformes*	33(23)	69.7	n.a.
Grimaldi et al., 2018	Antarctica	2010-2013	ELISA and VNT	*Sphenisciformes*	424(10)	2.4	n.r.
Adamu et al., 2017	Western Africa	2014	ELISA[a]	*Columbiformes, Passeriformes, Pelecaniformes*	195(12)	6.2	n.a.
Parsons et al., 2016	Southern Africa	2007; 2009; 2010-2013	ELISA[a]	*Sphenisciformes*	443(12)	2.7	n.a.
Assam et al., 2014	Western Africa	2012	AGID	*Accipitriformes, Anseriformes, Charadriiformes, Ciconiiformes, Columbiformes, Falconiformes, Galliformes, Gruiformes, Passeriformes, Pelecaniformes, Piciformes*	95(0)	0	n.a.

Table 3. *Cont.*

Iman et al., 2013	Northern Africa	2011-2012	serum	AGID	*Anseriformes, Charadriiformes, Columbiformes, Coraciiformes, Gruiformes, Passeriformes, Pelecaniformes*	41(0)	0	n.a.
Nunes et al., 2012	South America	2012	serum	ELISA[a] and AGID	*Sphenisciformes*	89(42)	47.2	n.a.
Candelora et al., 2010	Northern America	2004-2007; 1992-1998	serum	VNT	*Galliformes, Gruiformes*	757(87)	11.5	2
Miller & Shellam, 2010	Antarctica	2008	serum	VNT	*Sphenisciformes*	560(187)	33.4	n.r.
Watts et al., 2009	Antarctica	1996-2002	serum	VNT	*Charadriiformes, Sphenisciformes*	1395(190)	13.6	1
Kasanga et al., 2008	Eastern Africa	2005	serum	VNT	*Columbiformes, Galliformes*	11(0)	0	1
Miller et al., 2008	Antarctica	1999	serum	VNT	*Charadriiformes*	118(20)	16.9	1
Smith et al., 2008	South America	1992-1994	serum	AGID	*Sphenisciformes*	20(0)	0	n.a.
Miller & Shellam, 2007	Antarctica	2006	serum	VNT	*Sphenisciformes*	313(214)	68.4	n.r.
Travis et al., 2006a	South America	2003	serum	AGID	*Suliformes*	68(0)	0	n.a.
Travis et al., 2006b	South America	2003-2004	serum	AGID	*Sphenisciformes*	75(0)	0	n.a.
Deem et al., 2005	South America	2000	serum	AGID	*Psittaciformes*	22(0)	0	n.a.
Gauthier-Clerc et al., 2002	Antarctica	1996-1997	serum	VNT	*Sphenisciformes*	302(14)	4.6	1; 2
Höfle et al., 2001	Southern Europe	n.r.	serum	VNT	*Accipitriformes, Falconiformes, Strigiformes*	37(4)	10.8	n.r.

Table 3. *Cont.*

Campbell, 2001	Northern Europe	n.r.	serum	AGID and/or ELISA[a]	*Anseriformes, Columbiformes, Galliformes, Passeriformes*	41(10)	24.4	n.a.
Hollmén et al., 2000	Northern Europe; Northern America	1998	serum	VNT	*Anseriformes, Charadriiformes*	211(146)	69.2	1
Fagbohun et al., 2000	Western Africa	n.r.	serum	ELISA	*Columbiformes,Pelecaniformes*	75(14)	18.7	n.a.
Karesh et al., 1999	South America	1994	serum	AGID	*Sphenisciformes*	30(0)	0	n.a.
Ogawa et al., 1998	Eastern Asia	1989-1997	serum	VNT	*Anseriformes,Charadriiformes, Columbiformes, Falconiformes, Passeriformes, Pelecaniformes, Strigiformes*	739(51)	6.9	1; 2
Gu et al., 1998	Eastern Asia	n.r.	serum	AGID	*Galliformes*	70(14)	20	n.a.
Gardner et al., 1997	Antarctica	1991; 1995-1996	serum	VNT	*Sphenisciformes*	364(39)	10.7	n.r.
Ezeifeka et al., 1992	Western Africa	n.r.	serum	AGID	*Passeriformes, Piciformes*	35(0)	0	n.a.
Wilcox et al., 1983	Australia and New Zealand	1977-1979	serum	AGID and VNT	*Anseriformes, Charadriiformes, Procellariiformes*	397(14)	3.5	1; 2
Nawathe et al., 1978	Western Africa	1977	serum	AGID	*Passeriformes,Piciformes*	50(6)	12	n.r.

[†]Countries of origin of the studies were grouped into sub-regions as stated by the Statistics Division of the United Nations Secretariat (https://unstats.un.org/unsd/methodology/m49/overview/)

[a]ELISA kit: IDEXX IBD®, IDEXX Laboratories – specificity and sensitivity on chicken serum (%): 100 and 88, respectively. (De Wit et al., 2001)

[b]ELISA kit: IDEXX IBD-XR®, IDEXX Laboratories – specificity and sensitivity on chicken serum (%): 95.4 and 100, respectively (De Wit et al., 2001)

n.a.: not applicable; n.r.: not reported

Table 4. Overview of the molecular/virological studies on IBDV in wild birds included in the qualitative synthesis of results.

Authors and publication year	Geographic area	Study period	Sample type	Molecular method	Viral isolation	GenBank accession number	Host identification	Total no. birds (total cases)	Prevalence % (95% C.I.)
Naggar et al., 2020	Northern Africa	2019	cloacal swab	RT-PCR	CAMs (qRT-PCR ‡)	MT304668 MT304669 MT304670	*Anseriformes, Pelecaniformes, Galliformes*	28(3)	10.7 (0%-20%)
Vargas-Castillo et al., 2019	South America	2012-2013	cloacal swab	RT-PCR	-	-	*Apodiformes, Columbiformes, Passeriformes*	48(0)	0
Curland et al., 2018	Western Europe	2011-2014	bursa of Fabricius	RT-PCR	-	-	*Galliformes*	27(1)	3.8 (0%-11%
Watts et al., 2009	Antarctica	2000	bursa of Fabricius	RT nested PCR	CAMs	-	*Sphenisciformes*	23(3) †	0†
Kasanga et al., 2008	Eastern Africa	2005	bursa of Fabricius	RT-PCR	-	AB306716	*Galliformes*	11(1)	9.1 (8%-27%)
Jeon et al., 2008	Eastern Asia	2006-2007	cecal tonsil	RT-PCR	CAMs (RT-PCR‡; AGP‡)	EU493342 EU493343 EU493345 EU493344 EU493341	*Anseriformes, Charadriiformes, Columbiformes, Passeriformes*	107(5)	4.7 (0.7%-9%

† Watts et al. (2009) reported 3 IBDV positive samples interpreted as a possible positive-control contamination after genetic characterization therefore the prevalence (%) was considered as 0.
‡ Diagnostic tests used to confirm IBDV isolation with CAMs.

Table 5. IBDV strains and cell cultures used for virus neutralization test (VNT) as serological screening of IBDV in wild bird samples in the eligible studies which reported the information.

Reference	**IBDV strain (serotype)**	**Cell culture**
Grimaldi et al., 2018	GT101 strain (1)	Chicken embryo fibroblast (CEF)
Candelora et al., 2010	Ohio strain (2)	Chicken embryo fibroblast (CEF)
Watts et al., 2009	GT101 strain (1)	Chicken embryo fibroblast (CEF)
Kasanga et al., 2008	GBF-1E strain (1)	Chicken embryo fibroblast (CEF)
Miller et al., 2008	GT101 strain (1)	Chicken embryo fibroblast (CEF)
Gauthier-Clerc et al., 2002	CT strain (1); TY89 strain (2)	Chicken embryo fibroblast (CEF)
Höfle et al., 2001	D78 strain (1)	Chicken embryo liver (CEL)
Ogawa et al., 2008	GBF-1E strain (1); OH strain (2)	Chicken embryo fibroblast (CEF)
Wilcox et al. 1983	PBG98 strain (1); TY89 strain (2)	Chicken embryo fibroblast (CEF)

Table 6. Primers and genes amplified for molecular screening of IBDV in wild bird samples in the eligible studies considered.

Reference	**Gene(s) amplified**	**Primers 5'-3'**	**Location of 5' nucleotide**
Naggar et al., 2020	Viral Protein 2	Forward: GCCCAGAGTCTACACCAT Reverse: CCCGGATTATGTCTTTGA	717 1459
Vargas-Castillo et al., 2019	Viral Protein 3; Viral Protein 4	Forward: GTRACRATCACACTGTTCTCAGC Reverse: GATGTRAYTGGCTGGGTTATCTC	804 1050
Curland et al., 2018	Viral Protein 2	Forward: GCCCAGAGTCTACACCAT Reverse: CCCGGATTATGTCTTTGA	717 1459
Watts et al., 2009	Viral Protein 2	Outer set of primers: Forward: TCACCGTCCTCAGCTTA Reverse: TCAGGATTTGGGATCAGC Inner set of primers: Forward: GCCCAGAGTCTACACCATAACTGC Reverse: GCGACCGTAACGACAGATC	 587 1212 717 1174
Kasanga et al., 2008	Viral Protein 2	Forward: CCAGAGTCTACACCATAA Reverse: CCTGTTGCCACTCTTTCGTA	719 1189
Jeon et al., 2008	Viral Protein 2	Forward: GCCCAGAGTCTACACCAT Reverse: CCCGGATTATGTCTTTGA	717 1459

FIGURE LEGENDS

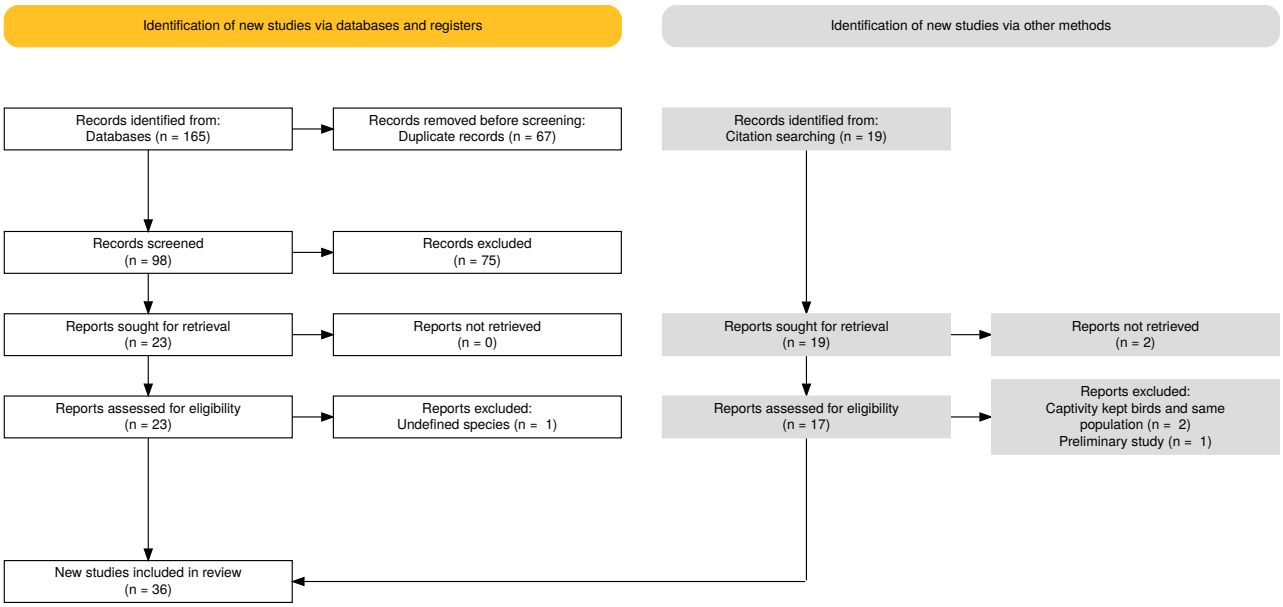
Figure 1. PRISMA flow chart of the systematic review method applied to the occurrence of IBDV in free-living wild birds on a global scale (https://estech.shinyapps.io/prisma_flowdiagram/).

Figure 2. Global distribution of IBDV publications in free-living wild birds included in our study. Green = both direct and indirect diagnostic methods applied in the study; orange = direct diagnostic methods; blue = indirect diagnostic methods. Created with QGIS 3.6.0 (Development Team, 2017).

Figure 3. Word cloud showing the number of serological studies on IBDV according to different taxonomic orders of wild birds. Data are ordered from low to high, with light colors for lower number of studies on a specific order and darker color for higher values.

Figure 4. Forest plot of the random-effects meta-analysis of IBDV serological prevalence. I^2 (inverse variance index), τ^2 = the between study variance, χ^2 and p-value of the Cochran's Q test for heterogeneity.

Figure 5. Phylogenetic tree of IBDV VP2 sequences of reference strains and wild bird strains retrieved from eligible virological studies (red square). IBDV strains are clustered into genogroups from G1 to G7 and into serotype 1 and serotype 2. Values for nodes with bootstrap >70 are showed.



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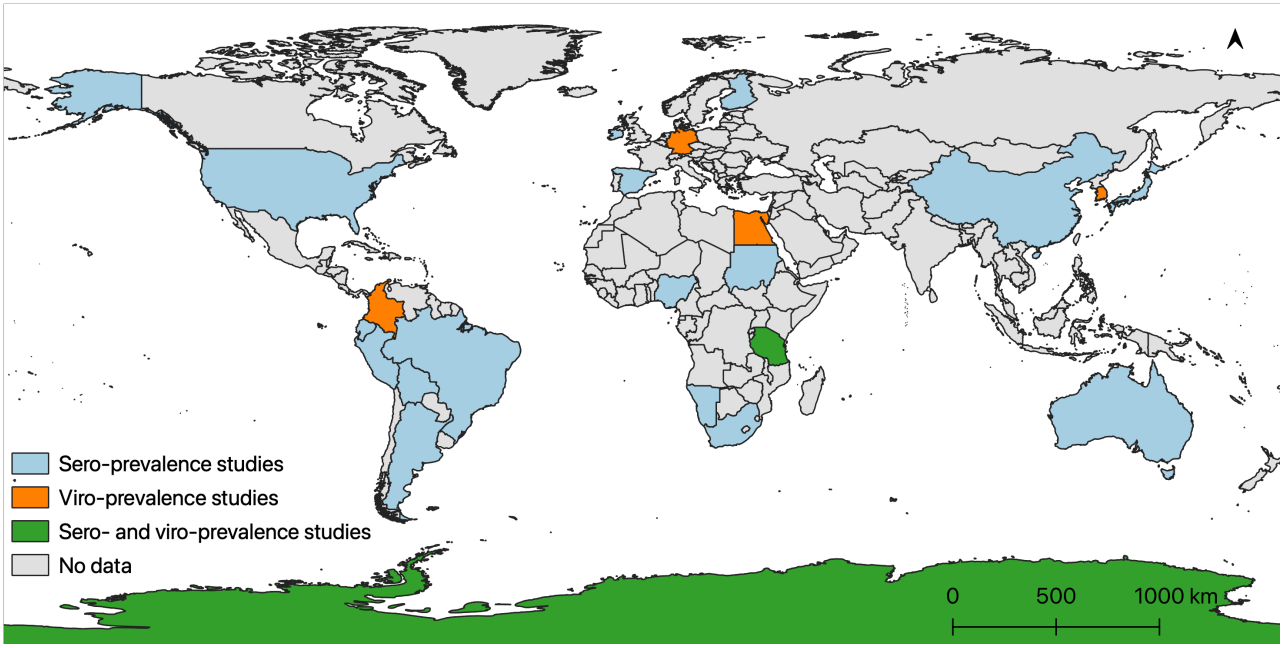


Figure 2. Global distribution of IBDV publications in free-living wild birds considered as "eligible" according to pre-established criteria applied as screening method of literature. Green = both direct and indirect diagnostic methods applied in the study; orange = direct diagnostic methods; blue = indirect diagnostic methods. Created with QGIS 3.6.0.

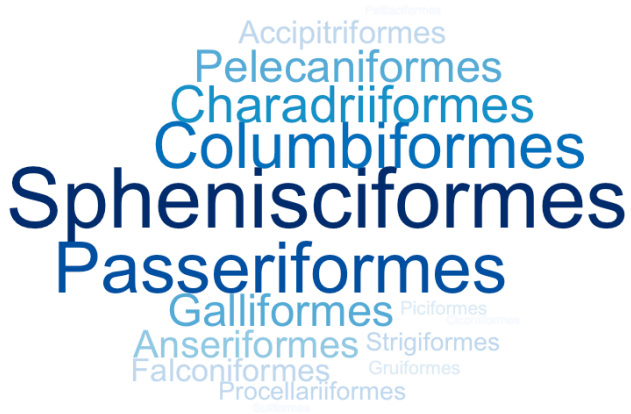
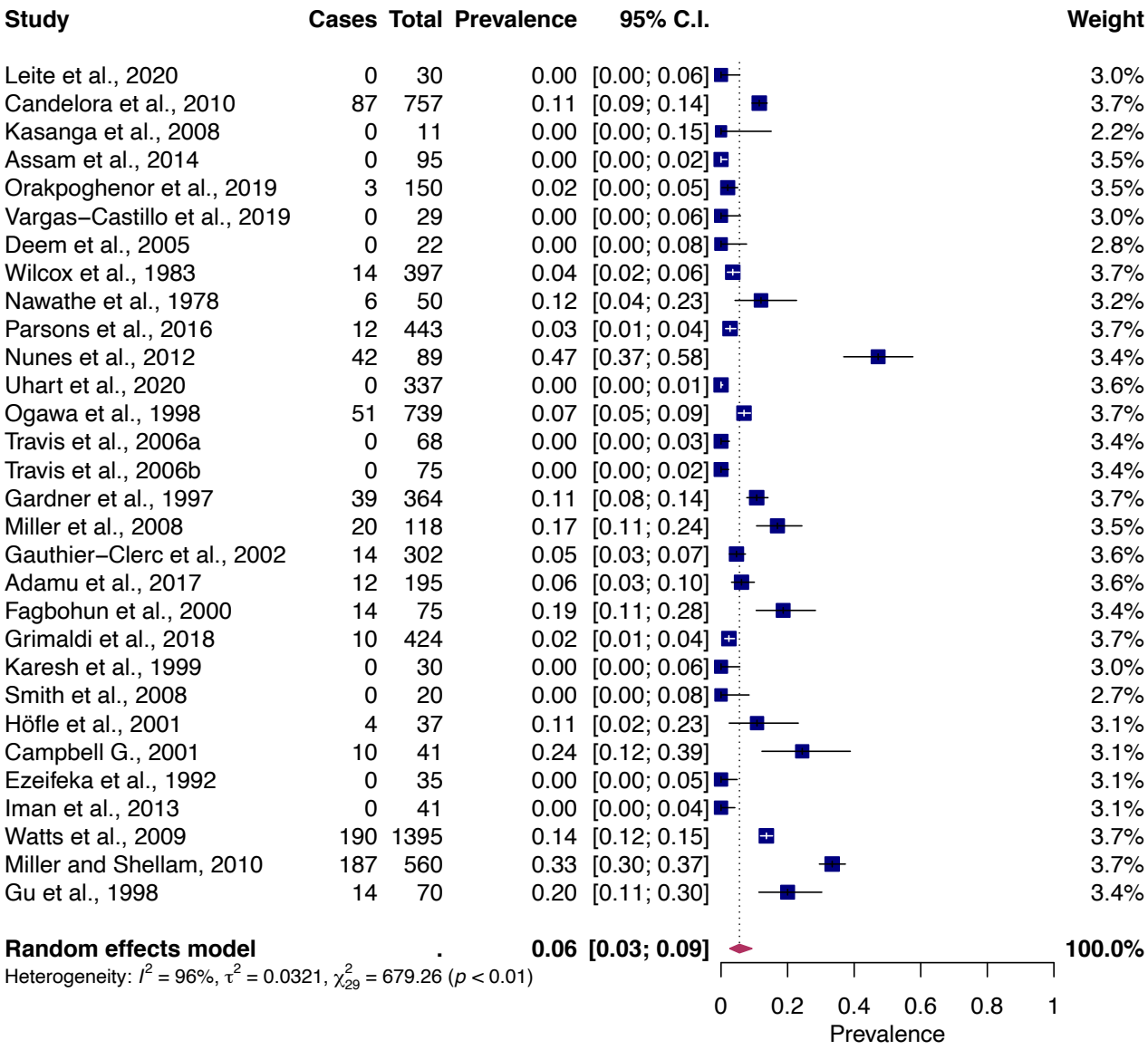


Figure 3. Word cloud showing the number of serological studies on IBDV according to different taxonomic orders of wild birds. Data are ordered from low to high, with light colors for lower number of studies on a specific order and darker color for higher values (*Sphenisciformes* n = 12 studies; *Columbiformes* n = 9 studies; *Passeriformes* n = 8 studies; *Charadriiformes* n = 7 studies; *Pelecaniformes*, *Galliformes* n = 6 studies each; *Anseriformes* n = 5 studies; *Accipitriformes*, *Falconiformes* n = 4 studies each; *Procellariiformes*, *Strigiformes* n = 3 studies each; *Gruiformes*, *Piciformes* n = 2 studies each. *Ciconiiformes*, *Psittaciformes* and *Suliformes* with n = 1 study each are not represented).



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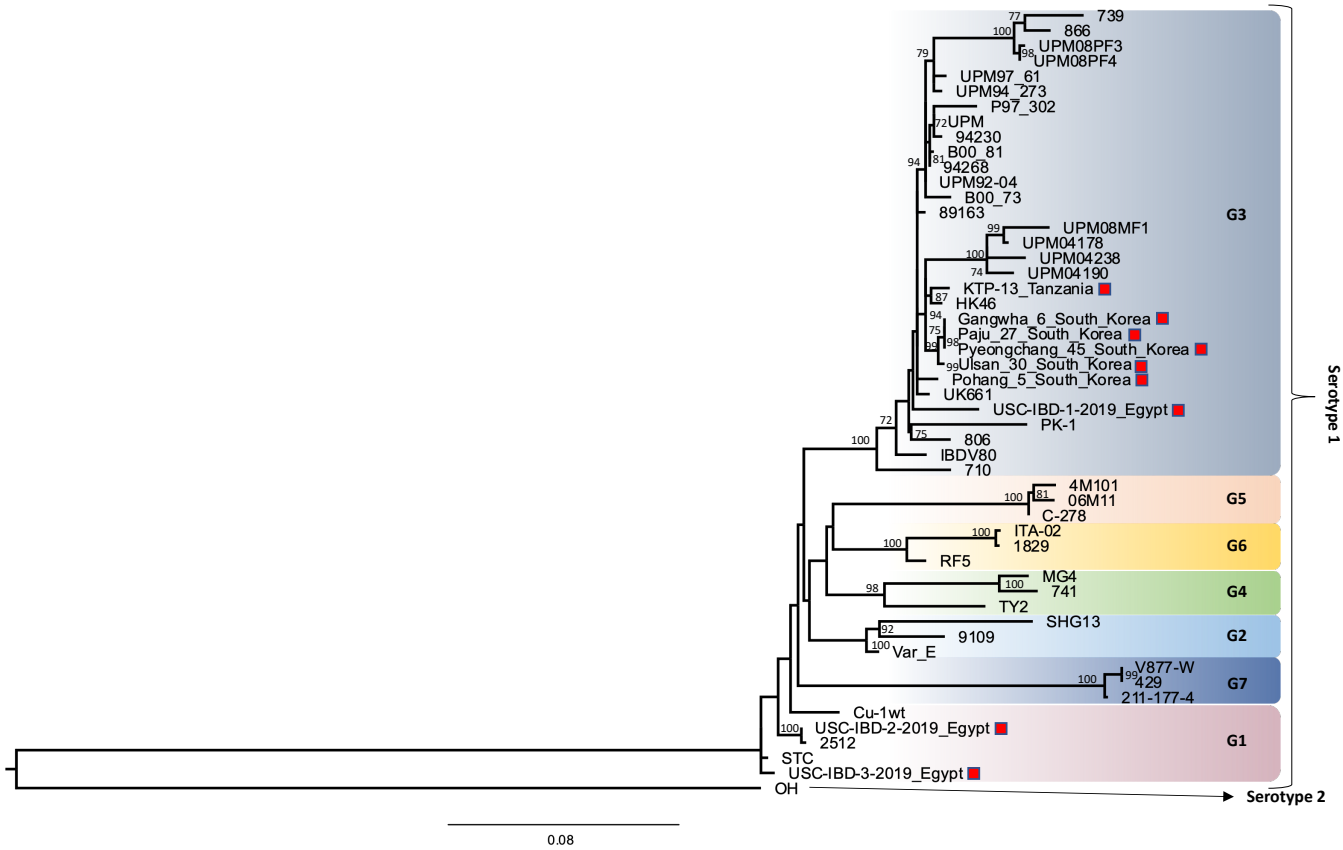

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