

Generalizability and comparability of prevalence estimates in the wild bird literature: methodological and epidemiological considerations


Review

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Abstract

Wild birds have been the focus of a great deal of research investigating the epidemiology of zoonotic bacteria and antimicrobial resistance in the environment. While enteric pathogens (e.g. *Campylobacter*, *Salmonella*, and *E. coli* O157:H7) and antimicrobial resistant bacteria of public health importance have been isolated from a wide variety of wild bird species, there is a considerable variation in the measured prevalence of a given microorganism from different studies. This variation may often reflect differences in certain ecological and biological factors such as feeding habits and immune status. Variation in prevalence estimates may also reflect differences in sample collection and processing methods, along with a host of epidemiological inputs related to overall study design. Because the generalizability and comparability of prevalence estimates in the wild bird literature are constrained by their methodological and epidemiological underpinnings, understanding them is crucial to the accurate interpretation of prevalence estimates. The main purpose of this review is to examine methodological and epidemiological inputs to prevalence estimates in the wild bird literature that have a major bearing on their generalizability and comparability. The inputs examined here include sample type, microbiological methods, study design, bias, sample size, definitions of prevalence outcomes and parameters, and control of clustering. The issues raised in this review suggest, among other things, that future prevalence studies of wild birds should avoid opportunistic sampling when possible, as this places significant limitations on the generalizability of prevalence data.

Introduction

Wild birds have been implicated in the transmission of pathogenic organisms such as *Campylobacter*, *Salmonella*, and *Escherichia coli* O157:H7 between livestock, people, and the environment (Wallace *et al.*, 1997; Skov *et al.*, 2008; Hald *et al.*, 2016). Continuing encroachment on wildlife habitat by human beings through urbanization and agriculture increases the opportunities for contact between wild birds, domestic animals, and people (Lejeune and Pearl, 2014). Moreover, certain wild bird species have successfully adapted to anthropogenic environments, including farms and cities, and frequently come into close contact with livestock, domestic animals, and people (Cole *et al.*, 2005; Guenther *et al.*, 2010). Additionally, the highly mobile nature of many wild bird species may also facilitate dissemination of these microorganisms in the environment (Reed *et al.*, 2003; Wang *et al.*, 2017). Many bird species which breed in northern latitudes travel vast distances as part of an annual migration and are thus capable of introducing microorganisms from one region to another (Jourdain *et al.*, 2007). In addition to major enteric pathogens, such as *Campylobacter*, *Salmonella*, and *E. coli* O157:H7, a variety of parasites (e.g. *Giardia* and *Cryptosporidium*) and viruses (e.g. avian influenza virus and West Nile virus) of public health importance have been isolated from a number of wild bird species (Reed *et al.*, 2003; Ferens and Hovde, 2011; Reboredo-Fernández *et al.*, 2015). In some cases, bacteria isolated from wild birds have exhibited resistance to antimicrobials considered of highest importance to human medicine (World Health Organization, 2017), including resistance to carbapenems, quinolones, macrolides, glycopeptides, and polymyxins (Jiménez Gómez *et al.*, 2004; Silva *et al.*, 2011; Radhouani *et al.*, 2012; Liakopoulos *et al.*, 2016; Vittecoq *et al.*, 2017).

Knowledge and understanding of the prevalence of these potentially harmful microorganisms in wild birds is necessary to guide public health policies concerning the prevention and

control of foodborne and zoonotic illness in human beings. Similarly, prevalence estimates play an important role in the surveillance of antimicrobial resistance (AMR), with wild birds, among other wildlife, serving as an important source of data concerning the environmental burden of AMR (Wang *et al.*, 2017). In order to draw sound conclusions from prevalence estimates, it is important to understand their methodological and epidemiological underpinnings. These aspects of a prevalence estimate determine the appropriate inferences that can be made, including generalizations to other target populations. Similarly, appropriate or meaningful comparisons between prevalence estimates from different studies require careful attention to their methodological and epidemiological inputs. An understanding of these inputs is particularly important for the interpretation of prevalence estimates in the wild bird literature, and perhaps the wildlife literature more generally, for a number of reasons. For example, the sampling strategy used in wild bird studies is often opportunistic (i.e. non-probability sampling), which can introduce bias and thereby limit the generalizability of such estimates. In addition, small sample sizes are common and sometimes unavoidable in this research field. Small sample sizes restrict the generalizability and comparability of prevalence estimates and may exacerbate other limitations related to study design and microbiological methods. Finally, the methods used to collect and process samples from wild birds are heterogeneous, thereby complicating the comparison of prevalence estimates from different studies. This review examines these and other methodological and epidemiological considerations in detail in the context of the wild bird literature, using examples from three major foodborne bacteria (*Campylobacter*, *Salmonella*, and *E. coli*) and associated AMR to illustrate their importance. Our overall aim is to inform and guide the interpretation of prevalence estimates in this literature. The considerations advanced in this review may also be used in the design of future primary research studies and research synthesis methods in this field. Finally, some of the points developed here may also apply to wildlife research more generally. The review is organized into the following sections:

- (1) Generalizability and comparability of prevalence estimates in the wild bird literature
- (2) Methods and the interpretation of prevalence estimates in the wild bird literature:
 - (a) Type of sample (e.g. cloacal swabs versus fecal swabs)
 - (b) Microbiological methods
- (3) Epidemiological and statistical considerations for the interpretation of prevalence estimates in studies of wild birds:
 - (a) Study design
 - (b) Bias
 - (c) Sample size and confidence intervals
 - (d) Defining prevalence outcomes and prevalence estimate parameters
 - (e) Accounting for clustering

This review focuses on the methodological and epidemiological underpinnings of prevalence estimates. Ecological factors, such as season and location, known to influence the carriage of certain microorganisms in wild birds, will only be addressed in passing in the context of study design ('Study design'). Other factors that only determine the true prevalence of microorganisms in wild birds such as age, sex, and immunity (individual-level factors) will not be discussed in this review, but are addressed in a review by Benskin *et al.* (2009). Where possible, confidence intervals, test

statistics, and *P*-values are taken directly from publications cited in this review, and confidence intervals calculated using data reported within publications are indicated by an asterisk (*). In relation to the topics outlined above, a comprehensive review of the literature on *Campylobacter*, *Salmonella*, and *E. coli* in wild birds was undertaken, where applicable. The literature search was performed using PubMed, ISI Web of Science, and Google Scholar in March 2018 without date restrictions, using search terms associated with the review subject (e.g. wild birds, foodborne illness, *Salmonella*, *Campylobacter*, and *E. coli*). To identify potentially relevant publications that may not have appeared in our database searches, reference list checking was performed on all relevant articles obtained through these searches.

Generalizability and comparability of prevalence estimates in the wild bird literature

Generalizing a prevalence estimate to a target population and comparing prevalence estimates between different studies are two important ways in which prevalence estimates are used. One significant obstacle to generalizing prevalence estimates arises when there is no clearly defined target population (i.e. the population to which the study prevalence would be extrapolated). Without such guidance, users of the literature may need to determine an appropriate target population, based on the information provided by a given prevalence study. Challenges and common pitfalls in generalizing from prevalence estimates in the wild bird literature are discussed in relation to study design and other epidemiological considerations, in the third section (below), in 'Epidemiological and statistical considerations for the interpretation of prevalence estimates in studies of wild birds.'

Besides generalizing or extrapolating prevalence estimates, comparing the prevalence estimates of different studies in the wild bird literature raises a number of important issues and challenges. Beyond straight comparisons of prevalence, researchers and public health officials may be interested in generating hypotheses from these comparisons about which ecological or perhaps individual-level factors are primarily responsible for measured differences in prevalence. This assumes, however, that the methodological and epidemiological inputs to the different prevalence estimates do not vary to such an extent as to preclude the generation of such hypotheses altogether. Moreover, apparently minor differences in methodological and epidemiological inputs to prevalence estimates from different studies may constrain valid comparisons between them, as explored (below) in 'Methods and the interpretation of prevalence estimates in the wild bird literature' and in the third section (below), 'Epidemiological and statistical considerations for the interpretation of prevalence estimates in studies of wild birds.'

Methods and the interpretation of prevalence estimates in the wild bird literature

Prevalence studies in wild birds rely on a variety of methods for collecting and processing samples. There are often a number of considerations that are relevant to determining the method of sample collection to be used. Impact to the wild bird species, cost, labor, and relative ease of obtaining samples, are core practical considerations for this process. The effectiveness of the sample collection method itself, however, is obviously a fundamental consideration as well. Similarly, the effectiveness and accuracy of microbiological techniques used to isolate and identify microorganisms is a further consideration. Because differences in

effectiveness have important implications for the interpretation of prevalence estimates in the wild bird literature, we review research that has specifically assessed the relative effectiveness of different methods for the detection of *Campylobacter*, *Salmonella*, and *E. coli* in wild birds in the subsections that follow.

Type of sample (e.g. cloacal swabs versus fecal swabs)

The type of sample collected (e.g. cloacal swab versus fecal swab) to assess the prevalence of microorganisms can influence research findings, but relatively few studies have specifically examined this issue in the literature on *Campylobacter*, *Salmonella*, and *E. coli* in wild birds (Luechtefeld *et al.*, 1980; Girdwood *et al.*, 1985; Morishita *et al.*, 1999; Waldenström *et al.*, 2007). In a comparison of different sampling techniques of 64 wild ducks in Colorado, Luechtefeld *et al.* (1980) found that isolation of *C. jejuni* was significantly higher in caecal specimens compared to cloacal swabs ($n=9/64$ caecal and cloacal, $n=11$ caecal only, $n=1$ cloacal only, $P<0.001$ based on McNemar's test, additional statistics not available). Work by Girdwood *et al.* (1985) demonstrated differences in isolation rates depending on the type of sample obtained from several species of gulls, although the confidence intervals were overlapping; a higher prevalence of *Salmonella* was obtained from culture of the entire gut (15%, $n=71/456$, 95% CI: 12–19%*) compared to a cloacal swab (10%, $n=44/456$, 95% CI: 7–13%*). This same study found that *Salmonella* was isolated from a greater proportion of cloacal lavage samples (11%, $n=90/847$, 95% CI: 9–13%*) versus a cloacal swab only (8%, $n=69/847$, 95% CI: 6–10%*; Girdwood *et al.*, 1985), again, with overlapping confidence intervals. Based on one study, the oral cavity of birds (the choana) does not appear to be an effective sampling site for the recovery of *Salmonella* or *E. coli* from live birds; Morishita *et al.* (1999) did not recover any isolates from passerines by swabbing this area (0%, $n=1709$, one-sided 97.5% CI: 0–0.2%*). The effectiveness of fecal versus cloacal swabs is perhaps of most interest and relevance in this research field, as both of these techniques are commonly used to collect samples from wild birds. Although we did not find any research assessing the relative effectiveness of these sample collection methods for specific microorganisms, recent work examining the microbiomes of different gut regions (ileum, caecum, colon) in relation to cloacal swabs versus fecal samples suggests that there may be differences in effectiveness (Videvall *et al.*, 2018). Finally, the same terminology may be used to describe different types of samples. For example, a 'fecal sample' may refer to a fecal swab, or a whole or partial fecal deposit, and may be collected fresh or after becoming desiccated. The choice of technique will depend, in part, on research intent to sample live, euthanized, or deceased birds, as well as the research objective. For instance, compared to other more sensitive sampling methods, fecal samples may be considered a more appropriate sample type for the assessment of the shedding of microorganisms into the environment by wildlife, since fecal material is in direct contact with the environment (Allen *et al.*, 2013).

Microbiological methods

Based on our comprehensive review of the literature on *Campylobacter*, *Salmonella*, and *E. coli* literature in wild birds, we identified only one study examining the impact of time to sample processing on isolation rates from wild birds. This study examined the impact of transportation time on the recovery

of Campylobacterales, and found that isolation rates of *Helicobacter canadensis* in Barnacle geese (*Branta leucopsis*; $n=116$) were significantly lower 2 days after initial sampling ($\chi^2=11.06$, d.f. = 2, $P=0.004$; Waldenström *et al.*, 2007). In the same study, however, no difference was observed in the isolation rates of *Campylobacter lari* from redshank (*Tringa tetanus*; $n=122$), even though the time from sampling to culture varied from 0 to 5 days (likelihood ratio $\chi^2=9.05$, d.f. = 5, $P=0.11$; Waldenström *et al.*, 2007). Further research is needed to assess the importance of variability in time to sample processing on culturability of *Campylobacter*, *Salmonella*, and *E. coli* samples from wild birds. The sensitivity and specificity of culture techniques, along with other microbial detection methods, should be considered as another potential source of variation for prevalence estimates in the literature, especially given the fastidious nature of organisms such as *Campylobacter* (Mi'kanatha *et al.*, 2012). Poor test sensitivity and specificity can distort the accuracy of prevalence estimates (Martin, 1984). The sensitivity and specificity of different isolation methods for foodborne pathogens are discussed recently by the following authors: Love and Rostagno (2008), Priyanka *et al.* (2016), and Rodgers *et al.* (2017). Likewise, the methods used in the detection of AMR (e.g. susceptibility breakpoints used) and of molecular subtypes of pathogens are important considerations when comparing prevalence estimates. Further discussion of these topics can be found with Jorgensen and Ferraro (2009), Hombach *et al.* (2012), Priyanka *et al.* (2016), and Anjum *et al.* (2017). In summary, heterogeneity among methods of sample collection and processing is not merely an abstract concern, but a concrete issue in the methods underlying prevalence estimates in the wild bird literature. It is plausible, of course, that certain differences in the methods used by different prevalence studies do not undermine their comparability. When there is evidence supporting a marked difference in the relative effectiveness of certain methods, however, it may be problematic to compare prevalence studies that utilize these different methods. For instance, our review of the relevant wild bird literature suggests that, other things being equal, caecal specimens are more effective for the isolation of *Salmonella* compared to cloacal swabs (Luechtefeld *et al.*, 1980). Thus, a comparison of wild bird studies, one using caecal specimens, the other, cloacal swabs, is not straightforward, and may prove problematic.

Epidemiological and statistical considerations for the interpretation of prevalence estimates in studies of wild birds

Study design

Prevalence studies of wild birds typically employ a cross-sectional or repeat cross-sectional study design. Repeat cross-sectional studies performed across multiple seasons, while holding the methods and location(s) fixed, allow for broader generalizability in comparison with data obtained from birds in a single or limited number of seasons. Given the evidence of seasonal variation in the carriage of a number of pathogenic organisms by wild birds (*Campylobacter*, *Salmonella*, and *E. coli* and associated AMR; Girdwood *et al.*, 1985; Morabito *et al.*, 2001; Broman *et al.*, 2002; Kirk *et al.*, 2002; Colles *et al.*, 2009; Hughes *et al.*, 2009; Hald *et al.*, 2016; Vogt *et al.*, 2018, 2019), it may be problematic to compare prevalence estimates from studies which sampled birds in different seasons. Data from repeat cross-sectional studies that represent multiple seasons may be of use in assessing the

seasonality of the carriage of certain microorganisms and can thereby support or undermine the comparability of certain prevalence estimates from different seasons. The above considerations about the generalizability and comparability of studies examining limited seasons also apply to individual-level and ecological characteristics, such as age and location type, respectively (Kapperud and Rosef, 1983; Ito *et al.*, 1988; Cole *et al.*, 2005; Benskin *et al.*, 2009; Guenther *et al.*, 2010; Hald *et al.*, 2016).

Bias

While there are many types of bias, the only relevant type of bias in the context of prevalence estimates is selection bias. In order to avoid a selection bias, each animal in the source population must have an equal opportunity to be identified and sampled, otherwise the study population used may not be representative of the source population (Wobeser, 2007; Dohoo *et al.*, 2014). Unfortunately, this is not always possible in the context of wild bird studies or wildlife studies more generally. Wild bird studies rely heavily on opportunistic sample collection, with the consequence that some birds are more likely to be sampled than others (Wobeser, 2007; Nusser *et al.*, 2008). Opportunistic sample collection, then, is a type of non-probability sampling. Some examples of convenience-based sample sources include samples obtained from: hunters, diagnostic centers, pest control agencies, roadkill, and wildlife rehabilitation centers (Anderson, 2001; Wobeser, 2007; Nusser *et al.*, 2008). Clearly, such sample sources can bias the results of a prevalence study. For instance, the use of samples from wildlife rehabilitation centers in a prevalence study is likely to result in a selection bias, as animals with human-induced diseases are more likely to be admitted (Spalding and Forrester, 1993; Kelly and Sleeman, 2003). To take a concrete example, the use of samples from birds at a wildlife rehabilitation center might overestimate the prevalence of AMR in the general population, as animals admitted to rehabilitation centers may be more likely to live in anthropogenic environments and have more opportunities to acquire resistant bacteria. Finally, sampling of trap-happy wildlife and tame wildlife may also represent cases of non-probability sampling, as behavioral differences might reflect biological differences in those animals (Michelangeli *et al.*, 2015).

Overall, generalizing results from studies utilizing non-probability sampling to an appropriate target population is problematic but unavoidable when limited data are available. In some cases, mitigation strategies can be used to assess, at least in part, the possible influence of a given sampling source on study results. In our previous research examining the prevalence of resistant *E. coli* in Canada geese, for example, the records of diagnostic samples originally obtained from a wildlife rehabilitation center were examined to verify that none of the birds had a record of being treated with antimicrobials, as this could have directly impacted the AMR prevalence data (Vogt *et al.*, 2018). Some prevalence studies of wild birds obtain samples from a variety of sources that may not be biased to the same degree, or at all, as with probability sampling techniques. Studies with a selection bias arising from different convenience-based sampling sources may be of limited comparability, as the degree of bias exhibited in the different prevalence estimates cannot always be determined and corrected for (Wobeser, 2007; Dohoo *et al.*, 2014; Conn *et al.*, 2017).

Probability sampling in wildlife research, unfortunately, seems at some level removed from true probability sampling. In human research, for example, it is often possible to establish a clear sampling frame (i.e. complete listing of all eligible study subjects),

from which study subjects may then be randomly selected. In contrast, wildlife populations are challenging to delimit (Wobeser, 2007; Bowler *et al.*, 2019). The characterization of any wildlife sample as having been obtained in a truly random manner will likely be open to serious debate, as most sampling techniques may plausibly select for animals which are tame or otherwise amenable to capture or restraint. For instance, in our previous research examining free-living Canada geese, one source of samples were fecal swabs obtained from live birds immediately following defecation (Vogt *et al.*, 2018). This required field technicians to remain in close proximity to the geese, in order to collect fresh samples from unique birds within the flock, without resampling. It may be argued that the population of geese sampled in this way represented a subpopulation of Canada geese in the region, namely urban birds with a tolerance to being in close proximity to human beings. Overall, sampling wild birds in the field, although not problem-free, is preferable to opportunistic sampling, as birds obtained from sources such as wildlife rehabilitation centers, or diagnostic centers (found deceased), may often represent a biologically salient subpopulation.

Sample sizes and confidence intervals

Depending on the species sampled, it can be a challenge to obtain a large number of samples from wild birds. As a result, sample sizes used in these studies are often quite small. Needless to say, perhaps, the smaller the sample size, the more limited the generalizability of prevalence estimate. On the practical side, reporting of associated confidence intervals improves interpretability of prevalence estimates (Hazra, 2017). Prevalence estimates may appear markedly different from one another without a statistically significant difference between them. When prevalence estimates from different studies are significantly different from one another, their associated confidence intervals can provide a simple means of ascertaining this difference; confidence intervals which do not overlap indicate a statistically significant difference between the prevalences. Overlapping confidence intervals indicate that there may or may not be a statistically significant difference between estimates; further statistics are then needed to resolve the issue (Hayek and Buzas, 1997; Steidl *et al.*, 1997; McGarigal *et al.*, 2000; Hazra, 2017).

Defining prevalence outcomes and prevalence estimate parameters

Comparing prevalence estimates from different studies is sometimes complicated by differences in the definition of prevalence outcomes such as multi-drug resistance (MDR), and definitions of certain prevalence estimate parameters, such as season. Where researchers report prevalence estimates from different seasons, the categorization of season may vary, depending on months represented in the dataset, according to biological relevance, or for other reasons. Hald *et al.* (2016) categorized season based on available data, with winter samples collected in January and February and summer samples collected in August and September. Another study categorized season based on biological relevance, and considered a mild-weather reproductive season (March–September) and a cold-weather non-reproductive season (October–February; Morabito *et al.*, 2001). In our previous research, when samples were collected between May and October, we used three different categories of season (early summer, late summer, fall) in order to equally distribute our

observations among those categories and to facilitate subsequent statistical analyses (Vogt *et al.*, 2019).

Regarding prevalence outcomes, definitions are typically focused on the presence or absence of a certain microorganism, thus differences in definition arise infrequently. Multi-drug resistance, however, is a prevalence outcome that is often defined in a number of different ways. As wild birds are often studied in the context of AMR, this definitional issue, though not unique to the wild bird literature (Medalla *et al.*, 2013), figures prominently in this research field. In what follows, select studies are used to illustrate the diversity of AMR definitions used in the relevant wild bird literature. A comprehensive review of AMR in wildlife is available from Wang *et al.* (2017).

One major difference in definitions of MDR concerns which classification metric is used. Isolates may be classified as multi-drug resistant on the basis of resistance to a certain number of individual drugs, drug classes, or drug categories, and the use of different metrics in the literature results in different working definitions of MDR. Furthermore, a recent study highlights the importance of these different MDR classification metrics; changes in the metric alone produced changes in prevalence values (MacKinnon *et al.*, 2018). In the relevant wild bird literature, some studies utilized resistance to individual drugs as a metric (Guenther *et al.*, 2010; Shobrak and Abo-Amer, 2014; Carroll *et al.*, 2015; Pinto *et al.*, 2015; Vidal *et al.*, 2017), while others utilized drug classes (Jurado-Tarifa *et al.*, 2016; Moré *et al.*, 2017; Thapaliya *et al.*, 2017). Another important feature of MDR definitions concerns the minimum number of individual drugs, drug classes, or drug categories required for an isolate to be considered multi-drug resistant. Some studies in the wild bird literature have used two (Carroll *et al.*, 2015) or three (Guenther *et al.*, 2010; Shobrak and Abo-Amer, 2014; Jurado-Tarifa *et al.*, 2016; Moré *et al.*, 2017; Thapaliya *et al.*, 2017; Vidal *et al.*, 2017) as the minimum number. A final complication for MDR definitions concerns the existence of isolates with intermediate susceptibility. Sometimes, such isolates are retained as intermediates, but they may also be reclassified into either resistant or susceptible categories. Of the sample of articles discussed in this subsection, a small number of studies reported how isolates with intermediate susceptibility were handled. Two studies retained such isolates as intermediates (Shobrak and Abo-Amer, 2014; Vidal *et al.*, 2017), another reclassified the isolates as susceptible (Pinto *et al.*, 2015), and in our previous work we classified all intermediates as resistant (Vogt *et al.*, 2018, 2019). Recognition of the various factors that influence MDR definitions has resulted in recent efforts to develop a standard definition of MDR that is internationally recognized, in order to facilitate comparisons between studies (Magiorakos *et al.*, 2012).

Accounting for clustering

Although the focus of this paper has been on those features of prevalence estimates that are relevant to their interpretation (most often, their generalizability and comparability), we have occasionally noted certain factors that may affect the accuracy of prevalence estimates themselves. In this subsection, we discuss a potential shortcoming of many prevalence estimates in the wild bird literature that can affect their accuracy: failure to account for clustering. We discuss this issue here as it has received little attention in the wild bird literature and represents a fairly straightforward way in which future work in this area can be improved. At the time of writing this review, few studies were found that

controlled for clustering in the relevant wild bird literature (Hughes *et al.*, 2009; Vogt *et al.*, 2018, 2019).

In wild bird studies, there are generally two relevant hierarchical levels at which clustering of samples may occur: location of sampling, and flock. It is plausible that samples obtained from birds within the same flock are more similar to one another than they are to samples obtained from birds belonging to different flocks. Similarly, when birds are sampled from multiple locations within a study and data from the different locations are used to inform a single prevalence estimate (e.g. numerous parks within a city), it is, again, plausible that samples obtained from the same location are more similar to one another than they are to samples obtained from birds at different locations. Control of clustering allows researchers to account for potential correlation between samples obtained from the same locations and/or flocks. When clustering is present, the absence of statistical control may result in inaccuracies in prevalence estimates and their associated confidence intervals, because the presence of clustering decreases the effective sample size (Dohoo *et al.*, 2014). Several methods can be used to control for clustering, and further information can be found in Dohoo *et al.* (2014).

Conclusion

In this paper, we reviewed a number of methodological and epidemiological inputs to prevalence estimates in the wild bird literature that are pertinent to their interpretation. Our main focus was the impact of these inputs on the generalizability and comparability of prevalence estimates. Given the opportunistic nature of much sample collection in wildlife research, the generalizability of prevalence estimates found in the literature is often rather limited. Furthermore, the diversity of methodological and epidemiological inputs to prevalence estimates often makes comparisons between studies challenging. Through our examination of these inputs, it is hoped that some of the pitfalls for the interpretation of prevalence estimates in the wild bird literature can be avoided. It is also hoped that our discussion may be of some use to future prevalence studies as well. Our examination of bias and study design suggests that future work would benefit from utilizing, when possible, a probability sampling approach, or as close to this approach as can be managed in wildlife studies. Additionally, our discussion of clustering suggests that future studies obtaining samples from multiple birds within different flocks, and/or from multiple locations, would benefit from statistical control of clustering. Finally, clear reporting of the methodological and epidemiological details of prevalence studies will continue to be an indispensable part of future work in this field.

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