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Article type : Original Article

The global epidemiology of *Brucella* infections in terrestrial wildlife: a meta-analysis

Short title: Brucellosis in terrestrial wildlife: a meta-analysis

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ABSTRACT

Brucellosis is a widespread zoonotic disease with serious consequences on human and animal health. *Brucella* infections were reported in many terrestrial wild animals, from subtropical and temperate

regions to arctic regions. In many areas, the epidemiology of brucellosis in wildlife is closely associated with the occurrence of the disease in livestock. Some wild species may contribute to the re-introduction of *Brucella* infections in livestock (spill-back), even in officially brucellosis-free (OBF) regions. Through meta-regression analysis, this study draws a global picture of the prevalence of *Brucella* spp. in terrestrial wild animals, trying to determine most affected subgroups as well as preferential sampling and screening methods. For this purpose, a literature search was carried out among publications published from 1983 to 2019. Different subgroups were compared according to animal species, feeding, gender, age as well as the method used for sampling and for brucellosis diagnostic. To determine heterogeneity of studies, Chi-squared test was used and a random effect model (REM) estimated the pooled prevalence among subgroups. A total of 68 publications, comprising 229 data-reports/studies, were selected. The most reported *Brucella* species in wildlife was *Brucella abortus* and the highest prevalence rate was found in American bison, *Bison bison* (39.9%) followed by Alpine ibex, *Capra ibex* (33%). Serology was the most widely applied diagnostic approach (66%), while PCR appeared to be highly sensitive (36.62% of positive results). The gender of animals showed no significant association with the prevalence of brucellosis ($p > 0.05$). Blood samples and visceral organs constituted the great majority of specimen used for the detection of *Brucella* spp. while lymph nodes showed a high prevalence of positive samples (94.6%). The present study provides insight into the global epidemiology and enzootic potential of brucellosis in wild terrestrial animals worldwide, aiming at helping the appropriate authorities to strengthen prevention, surveillance and control strategies.

Keywords: Brucellosis, Terrestrial wild animals, Prevalence, Diagnostic methods, *Brucella* species, Africa, America, Asia, Europe

1 | INTRODUCTION

Brucellosis is a major zoonotic disease that is widely distributed in humans and animals. The epidemiology of the disease in humans is largely associated with the occurrence of animal brucellosis in livestock and wildlife (Dadar, Alamian, et al., 2019; Jacques Godfroid, Garin-Bastuji, Saegerman,

& Blasco, 2013). About 500,000 new cases of human brucellosis are reported each year, making this disease a major health issue in many regions such as Middle Eastern and South Eastern Asian countries (Dadar, Shahali, & Whatmore, 2018). In animals, *Brucella* infection has deleterious effects on fetal development and reproductive organs, leading to reproductive failure, abortions and infertility (Dadar, Shahali, & Wareth, 2019; Hald et al., 2016). While brucellosis has been successfully eradicated from livestock in several developed countries, the control of this infection in wildlife remains a perpetual challenge worldwide (Jacques Godfroid, 2017). This is especially important as the epidemiological link between livestock and wildlife brucellosis is now well established (Jacques Godfroid, 2018; Jacques Godfroid, Garin-Bastuji, et al., 2013; O'Brien et al., 2017; Simpson et al., 2018). Several studies in Italy and Spain have reported that wild animals who have close contact with other wildlife species and livestock have a higher prevalence of anti-*Brucella* antibodies in comparison with the general wildlife population (De Massis et al., 2019; Muñoz et al., 2010). Hence, animal to animal transmission plays a critical role in the epidemiology of *Brucella* infections and stress the need to determine the contribution of different wildlife species to the risk of spread of this zoonosis. In this respect, it is important to differentiate between a spill-over of infection in wildlife contracted from livestock and a sustainable infection, in which case, *Brucella* spp. maintains itself in wildlife without a source of infection in livestock or another wildlife species. This kind of information could assist control and prevention programmers knowing whether wild species may potentially contribute to the re-introduction of *Brucella* infections in livestock (spill-back), particularly in regions where brucellosis has been officially eradicated (Jacques Godfroid, 2018). However, reports regarding *Brucella* prevalence and geographical distribution in wildlife are disparate and mainly limited to specific regions, following strict surveillance and eradication programs in livestock.

Despite this substantial number of studies and reports worldwide, there are still no accurate estimates about the global prevalence of brucellosis in terrestrial wildlife. The Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), as well as the Office International des Epizootics (OIE) consider brucellosis as one of the most important neglected zoonotic diseases in the world (Corbel, 1997; Franc, Krecek, Häsler, & Arenas-Gamboa, 2018; Hosein, Rouby, Menshawy, & Ghazy, 2016; McLeod, 2011; Musallam, Abo-Shehada, Hegazy, Holt, & Guitian, 2016). The species that most often infect humans is *Brucella melitensis*, followed by *B.*

abortus, *B. suis*, *B. canis* (Dadar et al., 2018; Whatmore, 2009) and *B. inopinata* (De et al., 2008; Holger C Scholz et al., 2010). It is difficult to measure the global impact of *Brucella* infections in wildlife on the (re)-emergence of brucellosis in livestock and human as bacterial transmission is rarely described and poorly known. Thus, understanding the transmission patterns of *Brucella* infections 'from and to wildlife' (spill over and spill back) is crucial to design appropriate control and eradication strategies. Over the last decade, the concept of 'One Health' has been introduced as an integrative and multifaceted approach for brucellosis control and prevention at the wildlife/livestock/human interface, taking into account the complex eco-epidemiological aspects of this zoonosis and the common interface between human, livestock and wildlife (Jacques Godfroid, 2017; Plumb, Olsen, & Buttke, 2013). This approach requires close cross-discipline collaborations and the implication of different sectors involved in the management, surveillance, diagnosis and treatment of the disease. To date, there are a few studies providing epidemiological data of brucellosis in wildlife and no comprehensive study giving a global picture of the *Brucella* epidemiology in terrestrial wildlife. Most studies dealing with wildlife brucellosis are performed on a sporadic basis, with the exception of some protected areas such as the Greater Yellowstone Area (GYA), where a strict control and surveillance program is followed for decades (Rayl et al., 2019). The present meta-analysis aimed at synthesizing reported data regarding *Brucella* infections in terrestrial wildlife species in order to determine the most affected subgroups as well as preferential sampling methods and conditions. This would help to understand the measures needed to improve and strengthen prevention and control strategies in order to reduce the enzootic potential of brucellosis in wild animals.

2 | MATERIALS AND METHODS

2.1 | Search strategy

The current meta-analysis was conducted following Cochrane protocols (Higgins & Green, 2011). The selection and extraction of studies were performed according to PRISMA protocols (Figure 1) (Liberati et al., 2009). A literature search was carried out among publications available in Web of Science, PubMed, Scopus and Embase databases to retrieve papers reporting *Brucella* infection in

wildlife population from 1 January 1983 to 20 October 2019. The following keywords were used to search databases: "Wild" OR "wildlife"OR “wildlife population” AND "prevalence" OR "occurrence" AND "Microbe" OR "bacteria" OR "*Brucella* spp.*″ OR* "*B. abortus*" OR "*Brucella*" OR "*B. melitensis*" OR *"B. ovis* OR *B. vulpis* OR *B. microti* OR *B. inopinata* OR *B. papionis* OR *B. canis*. The reference list of extracted articles was further screened to obtain additional relevant articles.

2.2 | Inclusion/exclusion criteria

The criteria for inclusion were 1) English language full text; 2) cross-sectional and descriptive studies; 3) including both positive and total sample sizes, 4) studies performed on wild animals (not held captive). Books, workshops, clinical trial and thesis have been excluded because of the lack of peer review (Fakhri et al., 2019; Khaneghah, Fakhri, Raeisi, Armoon, & Sant'Ana, 2018).

2.3 | Data extraction

The following data were extracted from all relevant articles: The year of the study, first author, country, data of study, animal, feeding condition (herbivorous, carnivorous, omnivorous), total sample size, positive sample size, method, type of sample, species, sampling from dead or live animal, gender, age and the method used for catching animals (capture, hunting, found dead) were extracted.

2.4 | Meta-analysis of data

In this work, the prevalence of *Brucella* spp. infections in wildlife was estimated as a ratio of positive samples to the total sample size (Keramati et al., 2018; Mousavi , Fakhri, Raeisi, Armoon, & Sant'Ana, 2018). To determine heterogeneity of studies, a Chi-squared test and I^2 index were used. If the $I^2 > 50\%$, heterogeneity was considered (Higgins. & Thompson, 2002). A random effect model (REM) was used to calculate pooled prevalence of *Brucella* infections in different wildlife subgroups. A meta-regression analysis was performed to determine the prevalence of *Brucella* spp. in wildlife population over time (Jackson, Bowden, & Baker, 2015; Stanley & Jarrell, 1989). STATA software, version 12.0 (STATA Corp, College Station, TX, USA) was used for statistical analyses and *P*-value < 0.05 was considered as significant.

3 | RESULTS

3.2 | Literature search

As depicted in Figure 1, 1943 papers were identified within international databases including Web of Science (n = 343), Scopus (n = 754), PubMed (n = 454) and Embase (n = 392) from 1 January 1983 to 20 October 2019. Among them, 1747 duplicates were identified and removed using Endnote 7x software (Philadelphia, PA, USA). A total of 196 articles remained after exclusion of redundancies, of which 74 were excluded given the lack of relevance of their title, not falling under any of the above-mentioned inclusion criteria. The 122 remaining articles were then screened carefully, leading to the exclusion of 26 additional articles after reading their abstracts. The full text of the 96 remaining articles were revised and 28 articles were excluded due to the lack of essential data such as positive sample size or total sample size, lack of English language and interventional studies. Finally, only 68 articles, comprising 229 data-reports and/or studies, were considered appropriate for the purpose of this meta-analysis (Figure1).

3.2 | Distribution of studies and prevalence trends over time

A total of 68 articles investigating the occurrence of brucellosis in wildlife have been reported since 1982, with the highest annual number (n=14) reached in 1999. Since then, the annual number of articles on the occurrence of brucellosis in wildlife decreased in number and regained an upward trend in 2007 (n=10), reaching 12 articles in 2010 and 11 articles in 2016. The results of meta-regression analyses of all the retained studies showed that the prevalence of *Brucella* spp. in wildlife showed marginally-insignificant increase over time (Coefficient = 0.002 and *p value* = 0.06).

3.3 | Geographical distribution of studies

The rank order of countries based on the number of wildlife brucellosis studies (Table 1) was Italy (38) ~ USA (38) > Botswana (21) > Spain (20) > South Korea (14) ~ France (14) > Zimbabwe (13) >Brazil (8) ~ South Africa (8) > Zambia (7) > Austria (6) > Australia (5) ~ Hungary (5) ~ Pakistan (5) > Canada (4) ~ Japan (4) ~ UK (4) > China (3) ~ Germany (3) > Croatia (2) ~ Kenya (2) ~ Mozambique (2) > Mexico (1) ~ Tanzania (1) ~ Argentina (1).

3.4 | Sampling methods and prevalence rates according to the type of samples

1. In most studies, wildlife was sampled after being captured alive (in 159 out of 220 studies). However, the prevalence rate of brucellosis was higher in samples derived from animals that were found dead prior to sampling (23.47%) and lower prevalence rates were observed among hunted (11.52%) or captured (5.11%) animals (Table 2). The biological specimen used for analyses comprised mainly blood samples (n=170 studies), visceral organs (n=37 studies) and lymph nodes (n=13 studies), while other samples such as skin lesions, abscesses, liver, bone marrow and genital swabs were occasionally used (Table 2). As expected, lymph nodes showed a higher prevalence rate of *Brucella* spp. (94.63%) when compared to those obtained for visceral organs (23.65%) and blood samples (6.32%). In chronically-infected animals, genitalia and the lymph node become predilection sites for *Brucell*a spp. survival until the next wave of the disease.

3.5 | Methods for diagnosing brucellosis in terrestrial wildlife

As listed in Table 3, the prominent methods used for diagnosing brucellosis in wildlife were in decreasing order, bacterial culture (n=48), Rose Bengal test (RBT, n=46), complement fixation test (CFT, n=30), conventional PCR (n=26), competitive ELISA (n=25), indirect ELISA (n=24), serum agglutination test (SAT) (n=15) and fluorescence polarization assay (FPA) (n=11). These results are in line with the recommendations of the OIE, suggesting to replace the SAT with other more sensitive and specific screening tests for the diagnosis of brucellosis in animals (Greiner, Verloo, & de Massis, 2009; Ragan, Vroegindewey, & Babcock, 2013). Likewise, the CFT method has been gradually replaced by the indirect ELISA and, more recently, by the FPA methods. However, the majority of these serological methods still need to be validated and standardized for their actual application to the management of wildlife brucellosis (Jacques Godfroid, Nielsen, & Saegerman, 2010).

Among the above-mentioned methods, the highest and lowest prevalence rates of positive samples were obtained by PCR (36.62%) and RBT (2.27%), respectively (Table 3). Other diagnostic

approaches including analytical profile index (API), Real Time PCR, buffered acidified plate antigen (BAPA) and rivanol tests were marginally employed (Table 3).

3.6 | Most studied wild terrestrial animals

Most research articles dealing with *Brucella* infections in terrestrial animals (Figure 1S) concerned wild boar, *Sus scrofa*, with the highest numbers of studies (n = 37) followed by American bison, *Bison bison* (n =25), African buffalo, *Syncerus caffer* (n = 21) and chamois, *Rupicapra rupicapra* (n = 11). Thus, wild boar was by far the most studied wild species around the world with a total of 19852 samples analyzed between 1983 and 2017, of which 2265 were positive (an overall prevalence rate of 11.4%).

3.7 | Brucellosis in Bovidae

Among wild Bovidae, the highest prevalence rate of brucellosis was found in American bison, where 468 out of 1185 analyzed samples (39.5%) were positive. The prevalence of brucellosis was also considerable in other Bovidae such as Alpine ibex, *Capra ibex* (33%), Kafue lechwe, *Kobus leche kafuensis* (31%), muskoxen, *Ovibos moschatus* (23.3%) Blue wildebeest, *Connochaetes taurinus* (18.8%), African buffalo (17.5%), goral, *Naemorhedus griseus arnouxianus* (15%), wild yaks, *Bos mutus* (9%), moose, *Alces alces* (4.7%), Eland antelope, *Taurotragus oryx* (1.4%), and chamois (0.6%). The prevalence of brucellosis in impala (*Aepyceros melampus*) and kudu (*Tragelaphus strepsiceros*), among wild African bovid, as well as in wild caprine species such as Barbary sheep (*Ammotragus lervia*), Iberian ibex (*Capra pyrenaica*) and mouflon (*Ovis orientalis*) was virtually zero.

3.8 | Brucellosis in Cervidae

Elk, *Cervus elaphus canadensis* showed the highest prevalence (22%) of *Brucella* infections among Cervidae, followed by the Sika deer, *Cervus Nippon* (12.9%), Chinese water deer, *Hydropotes inermis* (8%) and the Spanish red deer, *Cervus elaphus hispanicus* (1.3%), respectively. A nil or low prevalence was found in other Cervidae including roe deer, *Capreolus capreolus* (0%), fallow deer, *Ceruus dama* (0.2%), mule deer, *Odocoileus hemionus* (0.2%) and white-tailed deer, *Odocoileus virginianus* (0.3%).

3.9 | Brucellosis in small- and medium-sized terrestrial mammals

Few investigations, performed in Austria and Brazil, have assessed the occurrence of brucellosis among different fox species (da Silva Batista et al., 2019; Holger Christian Scholz et al., 2009).

According to resulting data, the highest and lowest prevalence rates of brucellosis among different fox species were found in red fox, *Vulpes vulpes* (100%) and hoary fox, *Lycalopex vetulus* (9%), respectively. Two *Brucella* species i.e *Brucella vulpis* and *Brucella microti* have been isolated and identified in red foxes from Austria (Holger Christian Scholz et al., 2009; Holger C Scholz, Revilla-Fernández, et al., 2016). Common vole from South Moravia (Czech Republic) was the first wildlife population that proved to be infected by *B. microti* (Hubálek et al., 2007). This *Brucella* species is able to survive in the environment for a long period and soil may act as a potential source of infection (Holger C Scholz et al., 2008).

In South America, a study performed on free-ranging armadillos (*Chaetophractus villosus*) from La Pampa (Argentina) showed that 24 out of 150 tested animals (16%) were seropositive to *Brucella* spp. Two bacterial isolates were recovered from the liver and spleen of infected armadillos and were identified as *B. suis* biovar 1(Kin, Fort, de Echaide, & Casanave, 2014). Likewise, the white-eared opossums (*Didelphis albiventris*) originating from Brazil were subjected to *Brucella* infections with a prevalence rate reaching 2.1% (da Silva Batista et al., 2019). In felids kept in captivity in Cuiabá (Brazil), positive serology and PCR results to *B. abortus* and *B. canis* were reported among jaguars (*Panthera onca*), puma (*Puma concolor*) and ocelot (*Leopardus pardalis*), stressing the need to further investigate the prevalence rate of *Brucella* infections among wild Felidae (Almeida et al., 2013).

In Korea, the seroprevalence of *Brucella* infections in stray dogs (*Canis lupus*) and raccoons (*Procyon lotor*) were 34.7% and 6%, respectively (L. Q. Truong et al., 2011; Q. L. Truong et al., 2016). Rats also showed a high prevalence rate of *Brucella* infection as all studied rats in Australia including allied rat (*Rattus assimilis*) (n=4), large climbing rat (*Melomys cervinipes*) (n=2) and small climbing rat (*Melomys lutillus*) (n=1) were infected (Tiller et al., 2010).

3.10 | Brucellosis in large terrestrial mammals

The seroprevalence of brucellosis among 232 tested giraffes, *Giraffa camelopardalis* was 1.7% (n=4). Among large bovines, the American bison was the most studied (25 studies, 1185 samples), notably within the framework of control and surveillance programs conducted in the Greater Yellowstone Ecosystem (Northern America). The American bison population showed the highest prevalence of brucellosis among wild bovid, reaching 39.5% (368 positive samples). The most broadly sampled wild bovid was the African buffalo with 1955 analyzed samples (prevalence of 17.5%) through 21 studies performed in 4 African countries (i.e. Botswana, Mozambique, South Africa and Zimbabwe). In Asia, the seroprevalence of *Brucella* infection among wild yaks was estimated at 9% (Xulong et al., 2011).

3.11 | Brucellosis in amphibians

The frogs are other animals, which were studied for the presence of *Brucella* infections worldwide. All samples deriving from African bullfrog (*Pyxicephalus adspersus*) (n=39), Pac-man frog (*Ceratophrys ornate*) (n=2), Indian bullfrog (*Hoplobatrachus tigerinus*) (n=1) and Denny's tree frog (*Polypedates dennysi*) (n=1) were *Brucella* positive, while lower prevalence rates were found in white's tree frog (*Litoria caerulea*) (22.2%) and false tomato frog (*Dyscophus guineti*) (nil), respectively (Ali, Saleem, & Imran, 2018; Kimura et al., 2017; Holger C Scholz, Mühldorfer, et al., 2016; Soler-Lloréns et al., 2016; Whatmore et al., 2015). The overall prevalence rate of brucellosis in frog species reached 84.6%. Likewise, turtles were also infected by *Brucella spp.* and the yellow spotted mud turtle (*Kinosternon flavescens*) showed a seroprevalence of 32.3% (Ali et al., 2018).

3.12 | Brucellosis in wild avian species

A study investigated the occurrence of *Brucella* spp. among birds of the Pattoki region (Pakistan) resulting in the detection of *Brucella* specific antibodies in peafowl (*Pavo cristatus*) and Indian blue rock pigeon (*Columba livia*) with a seroprevalence of 12.5% and 9%, respectively (Ali et al., 2018). *Brucella* spp. have also been detected in several poultry and free-range flocks reflecting the susceptibility of various avian species to *Brucella* infections (recently reviewed by (Wareth, Kheimar, Neubauer, & Melzer, 2020).

3.13 | Overall prevalence rates according to gender and feeding conditions

The present meta-analysis showed no significant difference between the prevalence of brucellosis in male (14.2% among 4159 samples) and female (14.3% in 4449 samples) animals. In most available studies (n=152), the gender of sampled animals was not mentioned (NM). The highest overall prevalence rate was observed in herbivorous animals (7.80 %), followed by carnivorous (6.75%) and omnivorous (4.6%) animals (Table 2).

3.14 | Most prevalent *Brucella* spp. in terrestrial wildlife

B. abortus (n=40), *B. melitensis* (n=21) and *B. suis* (n=19) were the most reported *Brucella* species in retained studies. Among these microbial species, *B. abortus* showed the highest prevalence rate (15.81%) followed by *B. suis* (11.01 %), *B. suis* and *B. melitensis* together (8.40%) and *B. melitensis* (6.03%), respectively (Table 2).

However, in 133 studies, *Brucella* isolates were not characterized at species level (prevalence rate of 5.46%). Other *Brucella* spp. including *B. microti* (n=7), *B. inopinata* (n=5), *B. vulpis* (n=2) and *B. papionis* (n=2) were reported in lesser extent (Table 2).

4 | DISCUSSION

The wildlife reservoir of *Brucella* spp. is considered as a threat for livestock with potential adverse public health and economic consequences (Zheludkov & Tsirelson, 2010). As the diversity of reservoir communities may considerably make the management of brucellosis difficult, the identification of common patterns in the interface between livestock, wildlife and human may help to prevent spillover of *Brucella* infections and to implement appropriate strategies for the control and management of the disease. Hence, the accurate surveillance of brucellosis in wildlife should be done with the primary goal to prevent animal to animal transmission, particularly from wildlife to livestock and *vice versa*. The results of this meta-analysis provide an all-round picture regarding the epidemiology of *Brucella* infection in wildlife over a 35-year period and explore different sampling and diagnostic methods used for the detection of *Brucella* species in wild animals.

It should be emphasized that data presented for different countries are based on works available in the literature and cross-country comparisons should be interpreted with due caution because of substantial variations in the number of studies and methodological differences. The scarcity of representative researches in many countries may generate a misconception about the real prevalence of this zoonosis in wildlife in many areas around the world. In contrast, European countries, particularly Italy, Spain and France (Table 1), exert strict surveillance programs on brucellosis in wildlife. In this regard, Italy and United State of America (USA) had the highest number of studies assessing brucellosis in wildlife. The outcome of studies performed over a 13-year period in Italy showed that the prevalence of brucellosis in human and animals is decreasing (Facciolà et al., 2018). This is the result of strict control and eradication programs in livestock and surveillance programs in wildlife. The first serological diagnosis of *Brucella* infections among wild boar in Italy dates back to 1983 using the CFT method on samples collected from San Rossore protected areas (Giovannini, Cancellotti, Turilli, & Randi, 1988). Since then, wild boar was by far the most studied wild animal species in Italy and neighboring European countries (Bergagna et al., 2009; Cvetnić et al., 2009; De Massis et al., 2012; Di Nicola, Scacchia, & Marruchella, 2015; J Godfroid et al., 1994; Köppel et al., 2007; Leuenberger et al., 2007; Pilo, Addis, Deidda, Tedde, & Liciardi, 2015; Rónai et al., 2015). In French Alps, *B. melitensis* (biovar 3) was first isolated from the visceral organs of a chamois in 1988 (Di Blasio et al., 2015; Ferroglio, Rossi, & Gennero, 2000; Garin-Bastuji, Oudar, Richard, & Gastellu, 1990; Salvadori et al., 2016). More recent studies revealed *Brucella* infections among several Alpine species including Alpine ibex (*Capra ibex*), chamois and red deer (*Cervus elaphus*)(Garin-Bastuji et al., 2014). From 2012 to 2017 in the Bargy Massif (French Alps), bacterial culture was performed on urogenital samples or lymph nodes of 321 Alpine ibexes and *B. melitensis* was isolated from 31% of the tested animals (Lambert et al., 2018). In Spain, *B. melitensis, B. suis* and *B. abortus* were isolated from positive blood cultures related to Iberian chamois, wild boar and red deer, respectively (Muñoz et al., 2010). Apart from multiple reports on infected Spanish red deer in the past decade (Muñoz et al., 2010; San-Miguel Ayanz et al., 2017; Serrano et al., 2011), *Brucella* infections were also reported in other European Cervidae including roe deer (Boadella et al., 2010; Gaffuri et al., 2006), fallow deer (Giovannini et al., 1988) and the maral deer, *Cervus elaphus maral* (Tretiak, 1973).

In North America, *Brucella* infections and positive serology have been mainly reported in bison (Harms et al., 2019; Nymo, Beckmen, & Godfroid, 2016; Scurlock & Edwards, 2010) and elk, called wapiti (National Academies of Sciences & Medicine, 2017). At regional and local levels, substantial epidemiological studies and modeling have been performed among elk and bison living in the GYA, one of the few areas where brucellosis persists in United States of America (Cross, Edwards, Scurlock, Maichak, & Rogerson, 2007; Dobson & Meagher, 1996; J. C. Rhyan et al., 2009; J. C. Rhyan et al., 2001; Roffe et al., 1999; Treanor et al., 2011). In the GYA, *B. abortus* has been transmitted to local wildlife populations from its cattle reservoir in the early 1900s. Brucellosis has been eradicated in cattle several decades ago, however, spill-back in cattle grazing in the GYA have repeatedly been reported since then. Importantly, *B. abortus* has become a sustainable infection in elk as it was in bison. The high prevalence of brucellosis among the bison and elk populations of the GYA led to the implementation of a long term adaptive management plans of the disease to prevent spill back events from wildlife to livestock (J. C. Rhyan et al., 2001). In this respect, it is worth noting that elk has been the main source of spill-back infections to cattle since the end of the last century (National Academies of Sciences & Medicine, 2017). In northern America, other Cervidae such as reindeer (*Rangifer tarandus*), Rocky mountain elk (*Cervus elaphus nelsoni*) and mule deer were found to be susceptible to Brucellosis (Forbes & Tessaro, 1993; (Roug, Swift, Torres, Jones, & Johnson, 2012; Scurlock & Edwards, 2010; Shury, Nishi, Elkin, & Wobeser, 2015). *Brucella* infections were also detected among muskoxen in Canada (Tomaselli et al., 2019) and Alaska (Nymo et al., 2016).

In Africa, brucellosis is an important zoonotic disease and different wildlife populations have been infected by *Brucella* spp., often brought in by domestic animals (Assenga, Matemba, Muller, Malakalinga, & Kazwala, 2015; J. Muma et al., 2011).

Among African countries, the highest number of studies on wildlife brucellosis was performed in southern regions including Botswana (n=21), Zimbabwe (n=13), South Africa (n=8) and Zambia (n=7). African buffalo was the most extensively studied species (Alexander et al., 2012; Gorsich, Bengis, Ezenwa, & Jolles, 2015; Madsen & Anderson, 1995; Motsi, Tichiwangana, Matope, & Mukarati, 2013; Nyirenda, Letlojane, & Syakalima, 2016), and showed the highest prevalence of *Brucella* infection (17.5%) along with the blue wildebeest (18.8% in Kenya). This number of studies

highlights considerable efforts made by Southern African countries to control brucellosis among African wild bovid in protected areas (an important asset for the tourism industry in these countries). In addition, control of brucellosis in wildlife is aiming to limit the transmission and re-introduction (spill-back) of bovine brucellosis to livestock, like in the case of the South African veterinary 2016-2026 strategic plan, bent on implementing effective control programs to prevent bovine brucellosis across the country (Ducrotoy et al., 2017; Makwavarara, 2018). Besides African buffalo, most studies focused on wild African herbivores such as giraffe (Alexander et al., 2012; Madsen & Anderson, 1995), Kafue lechwe (J. Muma et al., 2011; J. B. Muma et al., 2010; Pandey et al., 1999), Eland antelope (Madsen & Anderson, 1995) and impala (Madsen & Anderson, 1995; Motsi et al., 2013).

Although no proof of direct transmission of *Brucella* spp. from wildlife to humans has been reported in sub-Saharan Africa (Jacques Godfroid, 2018), the consumption of wild animal meat, particularly buffalo bush meat, has been suggested as a potential source of human infection (Alexander et al., 2012).

Much of the research conducted in Asia took place in south eastern countries where blood samples from Sika deer (Liu et al., 2018), Chinese water deer (L. Q. Truong et al., 2011; Q. L. Truong et al., 2016), raccoon, *Procyon lotor* (L. Q. Truong et al., 2011; Q. L. Truong et al., 2016), goral (L. Q. Truong et al., 2011; Q. L. Truong et al., 2016) and wild boar (L. Q. Truong et al., 2011; Q. L. Truong et al., 2016) were infected by *Brucella* spp. In Pakistan, several wildlife species were tested using RBT and positive samples were found in pea fowl (*Pavo cristatus*), blue rock pigeon (*Columba livia*), yellow spotted mud turtle (*Kinosternon flavescens*) and Indian bullfrog (*Hoplobatrachus tigerinus*) (Ali et al., 2018). A Japanese study led to the identification of *Brucella* inopita-like bacteria from the bone marrow of the white's tree frog, *Litoria caeruleau,* using PCR-based detection (Kimura et al., 2017).

In Australian wildlife, *Brucella* spp. was identified from the visceral organs of several native rodent species(Tiller et al., 2010) and from blood samples of feral pigs, *Sus scrofa* (Ridoutt et al., 2014).

According to our results, the prevalence of brucellosis varied between herbivorous (7.47%), carnivorous (6.7%) and omnivorous (4.62%) animals ($p < 0.01$). Differences in the proportion of infected animal among different diet-based subgroups were significant and revealed a higher

prevalence rate in herbivorous and carnivorous wild animals when compared to omnivorous species ($p < 0.01$). This could stress the need to further extend the current researches and surveillance programs of brucellosis to small carnivorous wild animals such as rats and frogs showing high prevalence rates of *Brucella* infections.

Statistic comparisons between gender subgroups were performed on the basis of all available data for different species and revealed no significant association between this parameter and the prevalence of brucellosis in wildlife ($p > 0.05$). This is in accordance with the results of previous studies conducted in livestock and wild animals showing that gender and history of abortion had independent effects on individual seroprevalence to *Brucella* spp. (Gomo, de Garine-Wichatitsky, Caron, & Pfukenyi, 2012; Matope et al., 2011; J. Muma et al., 2006; Randi et al., 1985). In the vast majority of studies (n=189), the age of studied animals was not determined or mentioned. In the remaining studies, the age was often not precisely determined, but rather reported by age ranges (e.g. < 1 yr, >1 yr, 1-11 yrs).

Importantly, it has been shown that in deer, seroprevalence declined above the age of ten, with no evidence of disease-induced mortality. The probability of antibody loss was estimated to be 0.70 per year after a five-year period of seropositivity, suggesting that individuals are unlikely to become re-infected. This study highlighted that serological data may introduce a bias if age is not taken into consideration (Benavides et al., 2017).

A variety of diagnostic methods and biological specimen have been used for diagnosing brucellosis in wild animals. Blood samples, visceral organs and lymph nodes constituted the great majority of specimen used for the detection of *Brucella* spp., among which lymph nodes showed a higher prevalence of positive samples (94.6%). Considering the low prevalence of *Brucella* spp. detected from blood samples (6.3%), non-lethal complementary sampling from vaginal swaps and skin lesions/ abscesses can strengthen the diagnostic of brucellosis in wild animals (Table 2). However, further investigations should be done to confirm this issue as the number of studies reporting the presence of *Brucella* spp. in abscesses (n=1) and skin lesions (n=4) remained limited. In animals found dead, the collection of additional samples from lymph nodes and livers could be of great diagnostic value (Table 2).

Among laboratory methods used for the detection of *Brucella* spp., conventional PCR showed the highest prevalence of *Brucella* positive samples (36.62%), while bacterial culture, with a prevalence rate of 13.61%, was most frequently used (Table 3). Real Time PCR and API were also successfully used to identify *Brucella* spp. from different biological matrix and warrant consideration in future studies in this area. However, in some cases the use of the API may lead to misidentification (Fischer et al., 2012). Although bacterial cultures are considered as the 'gold standard' for brucellosis diagnostics, the results of this meta-analysis showed that PCR-based detection resulted to higher prevalence rates of *Brucella* positive samples. For clinical samples, it is now well-documented that better results could be obtained by combining culture method and PCR detections (Hinić et al., 2009; Leyla, Kadri, & Ümran, 2003; Marianelli et al., 2008). However, some limitations exist to the culture of *Brucella spp.* that require optimal storage, handling, biosafety and culture media conditions (Dadar, Shahali, et al., 2019). Furthermore, the bacterial culture is time consuming and represents a substantial risk for laboratory personnel. Thus, a first screening using PCR-based methods completed by a bacterial culture on PCR-positive samples seems to be an appropriate approach for reliable epidemiological screening of *Brucella spp.* infections in wildlife surveillance programs (Jacques Godfroid et al., 2010).

Besides these methods, serology is widely applied for the epidemiological surveillance of wildlife brucellosis worldwide (Figure 2). Although a large number of serological tests are currently used in the diagnosis of brucellosis (Table 3), a renewed effort is needed to implement interlaboratory standards for different wild species as most of the serological tests are not validated for wildlife. Furthermore, cross-reactions between *Brucella* species and other Gram-negative bacteria including *Salmonella urbana* group N, *Francisella tularensis*, *Escherichia coli* O:157, *Yersinia enterocolitica* O:9, *Stenotrophomonas maltophilia*, and *Vibrio cholerae* (Bricker, 2002; Vengust, Valencak, & Bidovec, 2006) should also be taken into account when using LPS-based antigens.

Given the ubiquitous use of serology as a tool for surveillance and epidemiological modeling of wildlife diseases, it is important to consider limitations of serological tests such as cross-reactivity and sometimes non-standardized cut-off values to interpret an antibody-positive results. Worldwide, serological methods have been used in livestock to estimate true prevalence based on seroprevalence taking into account sensitivity and specificity of tests. For wildlife, brucellosis serological tests have

not been validated (this is out of reach) and therefore, there will always be uncertainty estimating true prevalence by serological means for brucellosis in wildlife. Culture is important to confirm brucellosis, even more so in wildlife where uncertainty is inherent to the current serological testing. Indeed, culture (or alternatively detecting the presence of *Brucella* DNA) is the only diagnostic certainty. To the best of our knowledge, there is no published information related to establishing prevalence through culture in wildlife. Actually, the challenge in wildlife is confirming seropositivity by isolation of *Brucella* spp. or PCR-based methods. *Brucella* isolation from wildlife is challenging. Successful pathogen isolation requires sound sampling particularly in remote geographic areas during difficult field work, cold-chain transport of samples and laboratory capacity. Also, infection burdens may be low or the pathogen may be sequestered in organs, thereby requiring lethal sampling, as shown with brucellosis in bison (*Bison bison*) and elk (*Cervus elaphus*)(Baldwin & Roop, 2002). This later fact also represents an important restriction for direct molecular diagnostic and PCR-based methods.

Therefore, it is often recommended that more than one serological test should be used for better reliability. Godfroid et al. have published a paper where a strategy to "confirm" brucellosis by serology is provided: an agglutination test has to be confirmed by ELISA and testing for coherence of the results (Jacques Godfroid, Beckmen, & Helena Nymo, 2016).

According to the present meta-analysis including 229 studies, these limitations have been taken into account in the majority of studies (n=190), where serological results were further confirmed by either another serological test (n=75), bacterial culture on positive samples (n=87) or PCR-based methods (n=28).

The procedures used to collect the samples had also a significant impact on the output of the studies. Interestingly, the prevalence of brucellosis was higher in animals that were found dead prior sampling (20.4%) when compared to live animals (4.6%) (Table 2). The meta-analysis of the prevalence of different *Brucella* spp. in exanimated wildlife population revealed that *B. suis* showed the highest prevalence in wild boar and feral pig with an overall prevalence of 11.01%. *B. microti* was mainly detected in red fox and wild boar, while *B. vulpis* was isolated from red fox. *B. inopinata* was the most prevalent *Brucella* species among tested frogs including white's tree frogs, Pac-man frog, and

Denny's tree frog with an overall prevalence of 91.5%. *B. abortus* had an overall prevalence of 15.81% in infected wild animals including elk, American bison, red deer, horse, Chinese water deer, raccoon, goral and African buffalo. *B. melitensis* showed an overall prevalence of 6% in chamois, Alpine ibex, wild boar and Iberian wild goat. However, recently the reemergence of *B. melitensis* infections among Alpine ibex in the Bargy, French Alps (up to 45% of tested animals) suggested that Alpine wildlife is a potential cause of the bovine brucellosis reemergence and sporadic outbreaks in the French Alp massif since 2012 (Garin-Bastuji et al., 2014).

Over the last few years, the health of wild animals has increasingly become a cause of common tension for various stakeholders such as public administrations, farmers, veterinary services, wildlife conservationists, gamekeepers as well as hunters and civil society at large. The transmission of infectious agents appeared to be in both directions, with livestock being a source of infection for wild animals and wildlife acting as reservoirs of infectious agents affecting domestic animals and humans (Gaffuri et al., 2006). Livestock owners, animal health authorities, as well as wildlife conservationists have a longstanding challenge for controlling brucellosis in wildlife (National Academies of Sciences & Medicine, 2017). This is a major public health issue as an increasing prevalence of brucellosis in livestock and/or wildlife may lead to an elevated incidence of human brucellosis at a local or regional level (Jacques Godfroid, Al Dahouk, et al., 2013). In this regard, identification of *Brucella* spp. in wildlife and livestock reservoirs is critical to control transmission to humans. The recognition of wildlife as a reservoir of brucellosis is increasingly emphasized (Ali et al., 2018; Simpson et al., 2018; Tomaselli et al., 2019; Q. L. Truong et al., 2016; Tyers et al., 2015; Whatmore et al., 2015). Control and cost-effective prevention of brucellosis in wildlife require an international cooperation as well as holistic and interdisciplinary approach. On the other hands, laboratory capability, surveillance, communication, education, training and research are key factors to reduce the risk of brucellosis in both free-ranging/captive wildlife and livestock (Bengis, Kock, & Fischer, 2002; Jacques Godfroid et al., 2011; J. Rhyan, 2013). Changes in wildlife management and agricultural land use practices have also dramatically influenced the wildlife population and their health status (Gortázar, Acevedo, Ruiz-Fons, & Vicente, 2006; National Academies of Sciences & Medicine, 2017). The substantial long experience acquired in the YCA clearly demonstrates that the eradication of brucellosis in livestock could not be sustainable unless it is integrated and linked with broader management processes taking

into account the dynamic of brucellosis in wildlife, socioeconomic conditions and the protection of wild lands, particularly mixed grazing areas and newly converted lands (National Academies of Sciences & Medicine, 2017). In this respect, wildlife able to transmit the infection should be carefully identified and movements/translocation should be strictly controlled. Although serology is often the first tool to detect *Brucella* infections, the presence of anti-*Brucella* antibodies rather reflects exposure to *Brucella* spp. but not necessarily an active infection at the time of sampling (Jacques Godfroid et al., 2010).

Thus, there may often be a bias in the prevalence estimation in wildlife brucellosis. However, with careful study design and interpretation, antibody prevalence can be an invaluable tool for understanding the disease dynamics, even in poorly-studied systems such as wildlife populations. For example in the GYA, although brucellosis in red deer does not have an obvious impact on population dynamics (National Academies of Sciences & Medicine, 2017), this infection can potentially reduce the growth rates of wild populations due to abortion events or even because of lower survival and pregnancy rates as shown for elk in the GYA (Cotterill et al., 2018; Cross et al., 2015) or for the African buffalo population of the Kruger National Park in South Africa (Gorsich, Ezenwa, Cross, Bengis, & Jolles, 2015). These changes may remain unnoticed without longitudinal and sustainable monitoring programs. It is thus important to investigate whether infection could persist in a population and a particular wildlife species could transmit the disease to other wildlife species (like elk transmitting *B. abortus* to bison in the GYA), livestock and humans. The present study provides an overall picture of the epidemiology of brucellosis in wildlife around the word, helping to respond adequately to the epidemic threats for wildlife and to better control brucellosis transmission "to and from" wildlife.

AKNOWLEDGEMENTS

This work was supported by the Razi Vaccine and Serum Research Institute (RVSRI); Agricultural Research, Education and Extension Organization (AREEO) [grant number 2-18-18-033-950404-3].

CONFLICT OF INTEREST

The authors have no competing interests to declare regarding this study.

ETHICAL STATEMENT

The authors confirm that the ethical policies of the journal have been adhered to and Ethics approval was not required for this study.

DATA AVAILABILITY STATEMENT

Data available on request from the authors

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Table 1. A country-based meta-regression analyses on the selected studies exanimating the prevalence of brucellosis in terrestrial wildlife.

Country	Number study	ES[1]	Lower	Upper	Weightof ES (%)[2]	Heterogeneity statistic[3]	Degrees of freedom	p value	I^2 (%)[4]
Italy	38	6.07	2.66	10.35	19.07	2109.34	37.00	<0.001	98.00
Kenya	2	18.81	13.28	25.01	1.12	.	1.00	.	.
Tanzania	1	17.45	13.68	21.77	0.59	.	0.00	.	.
France	14	25.45	17.65	47.54	3.92	7.54	13	0.01	74.00
South Africa	8	13.41	4.35	18.99	3.89	.	2.00	.	.
USA	38	27.54	19.24	33.58	17.01	560.42	37.00	<0.001	95.00
Argentina	1	16.52	14.32	20.22	0.45	.	0.00	.	.
Zambia	7	22.44	11.89	35.03	3.74	63.15	6.00	<0.001	91.00
Zimbabwe	13	0.42	0.00	3.54	6.51	168.02	12.00	<0.001	93.00
Botswana	21	0.00	0.00	0.00	7.33	34.32	20.00	0.02	42.00
Spain	20	0.04	0.00	1.16	11.06	1537.17	19.00	<0.001	99.00
Japan	4	8.89	0.00	46.50	1.15	7.30	3.00	0.06	59.00
Austria	6	100.00	77.39	100.00	0.88	0.07	5.00	1.00	0.00
Canada	4	0.00	0.00	0.00	1.74	21.48	3.00	<0.001	86.00
Croatia	2	19.77	16.43	23.35	1.16	.	1.00	.	.
Mexico	1	0.43	0.01	2.39	0.59	.	0.00	.	.
China	3	11.49	7.94	15.59	1.77	.	2.00	.	.
Mozambique	2	27.42	19.83	35.70	1.10	.	1.00	.	.
South Korea	14	6.33	3.61	9.64	7.39	41.50	13.00	<0.001	69.00
Germany	3	100.00	99.99	100.00	1.15	.	2.00	.	.

UK	**4**	100.00	99.99	100.00	0.63	0.00	3.00	1	
Hungary	5	100.00	56.03	100.00	0.56	0.00	4.00	1.00	
Australia	5	17.31	0.00	48.97	1.70	57.02	4.00	<0.001	93.00
Brazil	8	1.73	0.19	4.24	3.84	12.17	7.00	0.10	42.00
Pakistan	5	20.62	5.52	39.88	1.65	5.41	4.00	0.25	26.00
Overall	**229**	**7.21**	**5.22**	**9.59**	**100.00**	**10744.41**	**228**	**<0.001**	**93.00**

[1] Effect size (ES): prevalence of *Brucella spp* (ratio of positive samples / total samples).
[2] Weight of ES that is related to the total sample size of individual studies.
[3] Variation in study outcomes between studies.
[4] Heterogeneity across studies (I^2)

Table 2. Statistical and meta-regression analyses regarding the prevalence of *Brucella* infections in wildlife according to the following subgroups: feeding, type of sample, microbial species, living conditions and gender.

Groups	Subgroups	Number study	ES[1]	Lower	Upper	Weight of ES (%)[2]	Heterogeneity statistic[3]	degrees of freedom	*p* value	I^2 (%)
Feeding	Herbivorous	155	7.80	5.40	10.50	69.90	7230.1	153	0	97.90
	Omnivorous	66	4.80	2.00	8.20	27.30	2725	65	0	97.60
	Carnivorous	8	6.75	3.80	10.00	2.20	241.7	7	0	97.10
Sampling kind	Blood	170	6.32	4.65	8.17	82.87	8632.36	169	0	98.04
	Viceral organs	37	23.65	11.99	36.98	12.58	1513.44	36	0	97.62
	Aborted fetus	1	42.86	9.90	81.59	0.31	.	0	.	.
	Lymph nodes	13	94.63	63.83	100.00	2.07	24.29	12	0.02	50.60
	Skin lesions	4	100.00	100.00	100.00	1.22	1.13	3	0.77	0.00
	Abscess	1	100.00	2.50	100.00	0.11	.	0	.	.
	Genital swab	1	30.84	25.83	36.21	0.57	.	0	.	.
	Bone marrow	1	50.00	1.26	98.74	0.16	.	0	.	.
	Liver	1	100.00	2.50	100.00	0.11	.	0	.	.
Microbial species	*Brucella* spp.	133	5.46	3.47	7.74	58.94	3449.49	132	0	96.17
	B. melitensis	21	6.03	1.65	12.06	9.97	761.26	20	0	97.37

	B. abortus	40	15.81	9.59	22.99	19.46	4088.62	39	0	99.05
	B. suis	19	11.01	4.56	19.12	9.74	2574.99	18	0	99.30
	B. microti	7	100.00	65.46	100.00	0.76	0	6	1	0.00
	B. vulpis	2	100.00	55.56	100.00	0.32	.	1	.	.
	B. inopinata	5	91.53	40.18	100.00	0.59	1.09	4	0.9	0.00
	B. papionis	2	100.00	21.26	100.00	0.22	.	1	.	.
Living conditions	Live animal	173	4.60	2.75	6.75	75.55	8731.71	172	0	98.03
	Dead animals	44	20.44	13.40	28.25	18.52	1839.09	43	0	97.66
	NM[5]	12	16.89	6.71	30.10	5.94	188.02	11	0	94.15
Gender	Male	22	14.2%	2.76	12.88	9.25	256.34	21	0	91.81
	Female	55	14.3%	5.39	15.83	23.57	630.69	54	0	91.44
	NM	152	4.19	2.45	6.24	68.18	8225.3	151	0	98.16

[1] Effect size (ES): prevalence of *Brucella spp* (ratio of positive samples / total samples).
[2] Weight of ES that is related to the sample size of individual studies.
[3] Variation in study outcomes between studies.
[4] Heterogeneity across studies (I^2)
[5] Not mentioned (NM)

Table 3. Statistical and meta-regression analysis on the prevalence of *Brucella* infections in terrestrial wildlife based on animal conditions prior to sampling and the diagnostic method. CFT indicates complement fixation test; RBT, the Rose Bengal test; I-ELISA, indirect ELISA; BAPA, buffered acidified plate antigen; C-ELISA, competitive ELISA; SAT, serum agglutination test; FPA, fluorescence polarization assay and API; analytical profile index.

Groups	Subgroups	Number study	ES[1]	Lower	Upper	Weight of ES (%)[2]	Heterogeneity statistic[3]	degrees of freedom	*p* value	I^2 (%)
Animal conditions prior to sampling	Captured	156	5.11	2.97	7.63	68.72	8288.07	156	0	98.12
	Found dead	22	23.47	2.47	52.61	8.40	897.55	21	0	97.66
	NM[5]	1	11.93	7.94	16.99	0.57	.	0	.	.
	Hunted	49	11.52	8.00	15.44	22.31	1620.21	48	0	97.04
Method	CFT	30	10.46	6.15	15.62	15.64	1666.81	29	0	98.26
	RBT	46	2.27	0.28	5.48	19.12	702.27	45	0	93.59
	I-ELISA	24	4.16	1.61	7.56	12.35	1393.65	23	0	98.35
	Culture	48	12.74	6.42	20.22	18.28	2988.76	47	0	98.43
	BAPA	2	0.24	0.06	0.50	1.14	.	1	.	.
	Wright	11	9.75	4.03	17.31	5.67	277.15	10	0	96.39
	C-ELISA	25	7.14	2.55	13.25	12.86	945.49	24	0	97.46
	FPA	11	35.15	19.46	52.54	5.21	153.88	10	0	93.50
	PCR	26	36.62	16.43	58.73	7.75	424.66	25	0	94.11
	SAT	15	4.275	1.805	7.525	7.65	184.47	14	0	94.04
	Real time PCR	2	6.45	1.39	8.20	0.64	1650.68	1	.	.%
	Flow Cytometry	1	100.00	2.50	100.00	0.11	.	0	.	.%
	API	1	100.00	2.50	100.00	0.11	.	0	.	.%
	Rivanol test	1	14.00	7.87	22.37	0.55	.	0	.	.%

[1] Effect size (ES): prevalence of *Brucella spp* (ratio of positive samples / total samples).
[2] Weight of ES that is related to the sample size of individual studies.
[3] Variation in study outcomes between studies.
[4] Heterogeneity across studies (I^2)
[5] Not mentioned (NM)

FIGURE LEGENDS

Figure 1. Flow chart of the article selection process (PRISMA flow diagram)

Figure 2. Frequency of different methodological approaches used for the screening of *Brucella* infections in terrestrial wild animals.

Figure 1S. Prevalence of *Brucella* spp. in wild terrestrial animals according to their feeding conditions (herbivorous, carnivorous and omnivorous) and the frequency of relevant studies according to infected species.

Figure 1

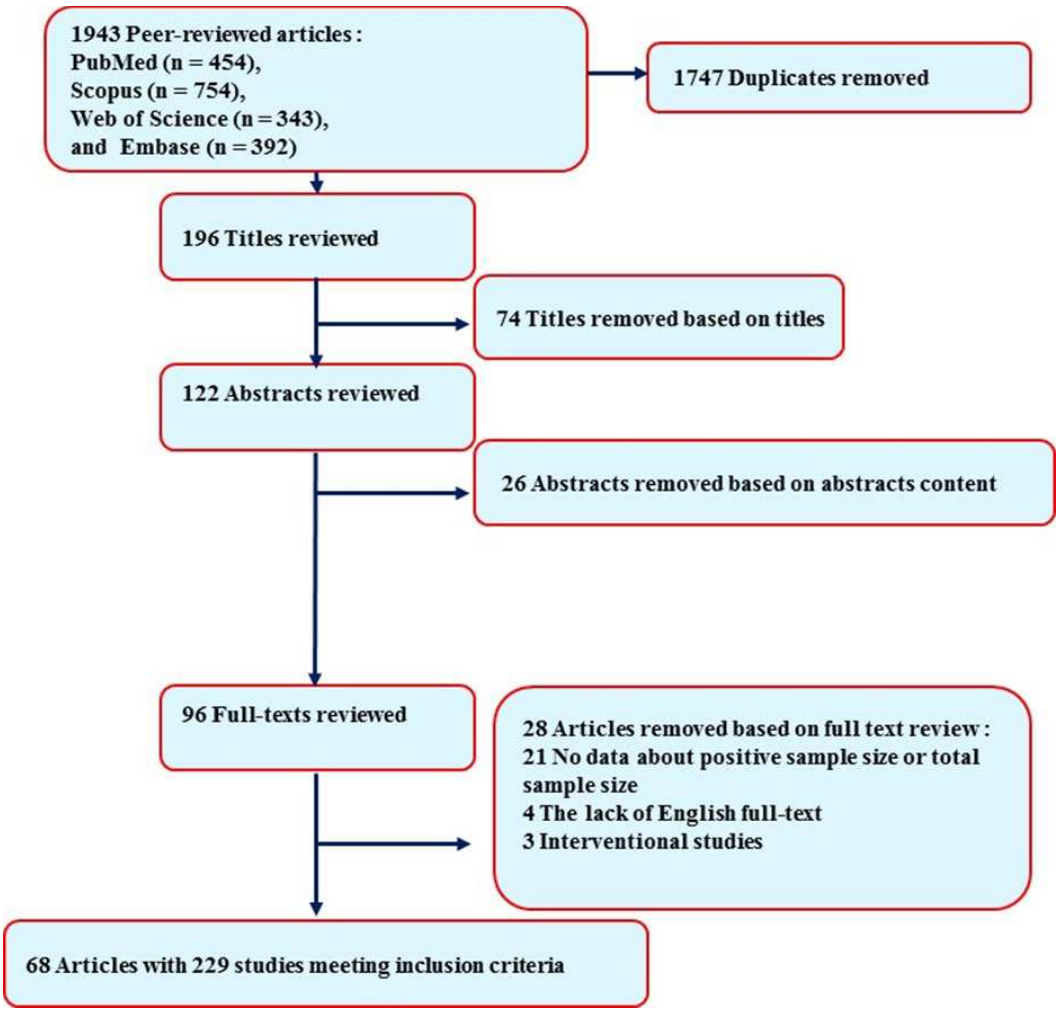

Figure 2