Tick-borne zoonotic flaviviruses and *Borrelia* infections in wildlife hosts: what have field studies contributed?

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Abstract

Tick-borne flaviviruses and *Borrelia* spp. are globally spread pathogens of zoonotic potential which are maintained by a transmission cycle at the interface between ticks and vertebrate hosts, mainly wild animals. We reviewed how those infections have been studied in wildlife species in the field to discuss how collected data provided relevant epidemiological information and identify needs for further studies. The literature was screened for populational studies on direct or antibody detection for tick-borne *Borrelia* spp. and flaviviruses in animals, in the wild. Overall, *Borrelia* spp. were more studied (73% of case studies, representing 297 host species) than flaviviruses (27% of case studies, representing 114 host species). Studies on both *Borrelia* spp. and flaviviruses focused mainly on the same species (namely bank vole and yellow-necked mouse). Species with potential to act as reservoir hosts were overlooked, notably passerine birds. Most studies were order-specific and cross-sectional. We highlight the necessity of collecting both demographics and infection data in wildlife studies, and to consider communities of species, to better estimate zoonotic risk potential.

Keywords

Tick-borne diseases, wildlife, *Borrelia*, flavivirus, reservoir, sentinel, host.

Introduction

Vector-borne and zoonotic infections are emerging threats to public health[1]. Tick-borne pathogens (TBP) are maintained by multi-hosts and complex epidemiological cycles at the interface between ticks and animal hosts[2]. Transmission to humans occur on specific occasions, and understanding and quantifying it in a One Health framework is necessary to prevent and control disease. Persistence of tick-borne and zoonotic pathogens is allowed at the interface between the tick vector and a large variety of animal hosts, allowing ticks to feed and complete their life cycles. In those zoonotic tick-host systems, the early tick stages (larvae and nymph) usually feed on small mammals and birds, while mature tick stages mainly feed on larger animals (such as deer, wild boar, cattle)[3].

Beyond their feeding role for the tick, animal species can act as pathogen reservoir or sentinel hosts. Infection and exposure data from both types of hosts give precious information on the dynamics of the studied TBP[4]. Whilst reservoir hosts allow the pathogen to multiply and transmit it further, sentinel species are characterized by their ability to reflect an underlying epidemiological phenomenon and by being of easy access[5]. For instance, for zoonotic *Toxoplasma gondii*, wild red and Arctic foxes have been suggested as sentinels for human and animal toxoplasma risk in Northern Canada, based on serology and direct detection of the pathogen on carcasses[6]. Also, animal ecology can drive variations in risk of transmission to human[7]. Therefore, because understanding pathogens' transmission and distribution in wildlife is prerequisite to addressing the risk of transmission to humans, quantitively tracking infection and exposure to pathogens in those hosts is necessary.

Tick-borne *Borreliaceae* and flaviviruses are common tick-borne pathogens of zoonotic potential which are the most prevalent in the temperate regions of the world[8] and are thus of interest for both human and veterinary public health. Tick-borne encephalitis virus (TBEV), causative agent for a disease known from the 18th century, has first been described in Austria in 1931[9] and then isolated in Far Eastern Russia in 1937[10]. It is the most common tick-borne Flavivirus (TBFV) in Europe, with several endemic foci in Europe and Asia and growing number of cases in human populations[11,12]. Its ecology and epidemiology have early been studied in Europe, especially in Czech Republic[13]. Other TBFV have later been isolated from wild animals and their ticks such as Meaban virus (MEAV) in Brittany, France[14] or from human cases such as Powassan virus (POWV) in Powassan town, Canada[15]. Vectors for TBFV are hard ticks *Ixodes ricinus* in Western and Central Europe and *I. persulcatus* in Eastern Europe as well as local tick species in other parts of the world, such as *I. scapularis* and *I. cookei* in North America[16–18]. Similarly transmitted bacterial genospecies from the *Borrelia burgdorferi* sensu lato (Bbsl) complex are the causative agents for Lyme disease, one of the most common TBD in the Northern Hemisphere. Other tick-borne *Borrelia* spp. outside of this complex are identified to cause relapsing fever in humans[19,20].

Recent reviews on tick-borne *Borrelia* spp. and TBFV have focused on getting information on the biology of infection in vertebrate hosts[21–28], reporting prevalence and clinical cases in human[29–33] and discussing diagnostic methodologies[34–37], the role of non-vector transmissions for TBFV[38–40], or on the importance of modelling TBP regarding climate change consequences[41,42]. Some reviews have focused on wildlife hosts for TBP but only at a national or continental scale[43–46], or on reservoirs associated with the flavivirus genus in general, with little focus on TBFV[47]. Finally, a lot of research effort has focused on studying the infectious agents in the tick vectors[48–51].

Despite their major importance in transmission, no study has so far reviewed and analysed the body of evidence in wildlife hosts from field data at a global scale. Yet, data on the level of infection or exposure

in sampled free ranging populations are critical to model the dynamics of infectious diseases[52]. Strong inference about the transmission processes underlying those dynamics often require a combination of approaches, notably experiments to ascertain the role of reservoirs[53], but quantitative data on levels of infection and exposure of potential hosts are a prerequisite in most cases. When it comes to wildlife species, a strong heterogeneity in the type of data gathered is nevertheless expected, from local cross-sectional sampling of particular host species, to broad spatial surveys or surveillance data over series of years. Estimating epidemiological parameters may also require to account for potential biases in detection probabilities and host population parameters[54,55], which may require specific study designs.

The aim of this review was thus to investigate how tick-borne *Borrelia* spp. and flaviviruses infection burden has been studied in free-ranging wildlife species (that is, excluding the tick vector) to discuss how collected data provided relevant epidemiological information and to identify perspectives and needs for further studies. We predicted that a strong heterogeneity would appear in the types of hosts investigated, with a high number of studies conducted on common species sharing landscape use with human in the Northern Hemisphere. We expected few studies including whole communities of local potential hosts and studies providing estimates of TBP burden to allow exploring host, temporal and spatial variation while accounting for uncertainty. We anticipated that monitoring of wildlife diseases would have been difficult and that knowledge of what is precisely measured would have been uncertain.

Studied hosts and their potential role in transmission cycle

It was possible to reconstruct 947 “cases studies” (unique combinations of species-pathogen-area-study period) studying overall 347 host species, from the 314 articles matching the selection criteria. Detailed metrics on the results of the literature search are available in SuppInfo1. Figure 1 links the order of tested species and the investigated pathogen. Species from Rodentia order were the more studied for both *Borrelia* spp. and TBFV, followed by Artiodactyla order for TBFV and Passeriformes for *Borrelia* spp. (Figure 1).

Historical papers shaped research on Rodents as reservoir hosts

In our review, we identified that Rodentia was the most investigated order with more than half (363/689) of the *Borrelia* cases and more than a third (91/258) of the TBFV study cases. The white-footed mouse (*Peromyscus leucopus*) has been regarded as the most common reservoir host in wildlife in Northern America for tick-borne *Borrelia* spp. at least since the eighties[56,57], and therefore was investigated in the first published studies from Northern America. Concerning TBFV, bank vole (*Myodes glareolus*) and yellow-necked mouse (*Apodemus flavicollis*) were recognized as reservoirs for TBEV in Europe[58]. Historical aspect is important to explain realized works. Taking rodents as example, studies included first the *Peromyscus* family, only present in Northern America, and secondly the *Microtus* and *Mus* families. Even earlier, in the sixties in Canada, a series of studies from the same authors detected POWV in *Tamias*, *Urocitellus* and *Callospermophilus* chipmunks and *Tamiasciurus* and *Sciurus* squirrels, with an average seroprevalence of 15%[59–67]. Groundhogs (*Marmota monax*) sampled in the same studies, reported the highest prevalence of anti-POWV antibodies (around 43%)[63–65]. Publications investigated as well striped skunk (*Mephitis mephitis*) and snowshoe hare (*Lepus americanus*) with respectively 41% (121/299) and 11% (143/1301) of seropositive animals[64,66]. Rodents as squirrels and chipmunks were then presented as primary reservoirs for POWV on the basis of these studies. Several studies on the eco-epidemiology of TBFV were parallelly conducted in the USSR but were not published in English (see limitations in SuppInfo1). Rodents were later sampled on other continents and TBFV detections were several times successful in *Apodemus*, *Microtus*,

Myodes families in Europe, and in these families as well as in *Micromys*, *Mus* and *Sciurus* families for *Borrelia*. In addition, Bbsl has been detected in *Tamias*, *Tamiasciurus* and *Neotoma* spp.. Furthermore, *Gerbillus*, *Mastomys*, *Praomys* and *Apodemus*, *Cricetulus*, *Eothenomys*, *Meriones*, *Mus*, *Niviventer* and *Rattus* spp. bore relapsing fever causative *Borrelia spp.* respectively in Africa and in Asia. It is notable that Eastern grey squirrels (*Sciurus carolinensis*) similarly carried *Borrelia* DNA, both in their native countries and in Europe where they are invasive, as raccoon (*Procyon lotor*) and raccoon dog (*Nyctereutes procyonoides*) do.

Overall, in all studied areas, rodent species evidenced similar levels and ranges of pathogen or serological detection. For case studies testing over 50 individuals, the median proportion of pathogen detection was lower than the proportion of seropositivity, yet both of these values were higher for *Borrelia* than TBFV. Specifically, the median proportion of *Borrelia* detection was 5.30% (range 0.2%-76%) and seropositivity was three times higher (median at 15%, range 0.7%-89%), whilst the median value for TBFV detection was 1.8% (range 0.8%-62%) with seropositivity median value at 4.95% (range 0.4 - 44%). Variations were remarked along study sites and periods, regardless of taxonomy. Beyond that such TBP might be widespread, this could be explained by the ecology broadly shared by rodents, living close to the ground where foraging infected ticks can be encountered[68], and therefore being similarly exposed. Variations in detection rate across studies were observed, notably for a same species, such as for Bbsl prevalence in yellow-necked mouse tissues varying from a proportion of 5.8% in Hungary in 2014-2015[69] to 23% in Germany in 2012-2014[70]. No studies looking at Kyanasur Forest Disease virus (KFDV), MEAV, Saumarez Reef Encephalitis virus (SREV), Tyuleni virus (TYUV) and Yamaguchi virus (YV) in rodents were recorded. Whilst KFDV and YV have terrestrial hosts[71], MEAV, SREV, TYUV are known to be seabird viruses.

Deer, broadly studied as sentinel hosts

Other important investigated hosts were Artiodactyls. This second most important order by the number of tested individuals was mainly represented by the *Cervidae* family as in terms of number of tested animals (82%, 26,090/31,862) and in terms of number of species investigated (56%, 9/16) (Figure 2B and 2D). The first fieldwork setting targeting *Borrelia* in deer identified in this review dates back from 1971. Deer are also considered as incompetent reservoir for *Borrelia* spp. by some authors because of borreliacidal properties of their sera[72]. Yet, studies showed that the complement borreliacidal activity depends on the strain and also exists on other *Borrelia* strains for other vertebrates including rodents[73,74]. Despite their poor competence as reservoir, deer are frequently met all across the world and can therefore be infected by feeding adult ticks they commonly host[75]. *Cervidae* was widespread in this review, with locally present species from America, Europe and Asia seeming to show similar level of TBP contact (with an average seroprevalence of 18%). For *Borrelia*, average proportion was of 15.6% and average seroprevalence of 27.3%, and were respectively of 1.1% and 14.4% for TBFV. As such, deer could help identifying endemic or emerging risk areas and give information on underlying processes. In addition, monitoring displayed restraint home range sizes of no more than 10km² among the different deer species[76–79], which allowed studying the infection process at a rather small spatial scale. Moreover, surveillance activities and pathogen detection may be facilitated through deer hunting activities, since such reviewed studies allowed planning widespread sampling program for reasonable costs and working effort on a yearly or at least regular basis[80–83]. Furthermore, because of that, deer were repeatedly considered as potential sentinels of tick-borne diseases in the wild. Other game Artiodactyls identified in this review such as wild boar (*Sus scrofa*) offer the same kind of possibilities for such sentinel surveying approaches[84].

Birds, reservoir hosts, have been overlooked

Birds were also reported in the literature as reservoirs[85]. Contrary to rodents, birds may have been overlooked. In our review, the average seroprevalence of anti-TBFV in birds was 35%, corresponding to an overall 24% (783/3273) of anti-TBFV positive individuals all cases taken together (Figure 2C). We hypothesized that the limited availability of data on birds might be due to the logistical difficulties related to blood sampling in the wild (logistical difficulties, training and legal requirements, ethical concerns for endangered species). However, it is notable that antibodies against Gadgets Gully and Saumarez Reef viruses were extensively studied in a collection of Australian seabirds' sera[86]. This constitutes a unique occurrence of rare TBFV in the reviewed articles, but sadly no clarification was made on which species of the collection retrieved seropositivity.

Although birds represented about 19% (129/689) of the *Borrelia* cases, they originated from only 12% (28/228) of the selected publications. In those, *Borrelia* spp. presence was often screened in a banding study configuration. For instance, Newman *et al.* caught birds on the campus of the University of California in 2003 and 2004[87]. Their wide-range study allowed to detect Bbsl in 23 species for which at least two individuals were caught and one positive, out of 53 caught species. Mistnetting increases the possibility of capturing more birds and therefore more potential reservoirs, and underlines the interest of also surveying birds. However, this kind of study design is mostly favourable for Passeriformes, and passerines were the most studied avian order to get insight on the potential of reservoir for TBP they have. In addition, for migratory species or those exploiting very large areas during their life cycle, the infection status might not always reflect the underlying local infection processes. However, combined with movement information, they can unravel critical information on pathogen dissemination over long distances and between various ecosystems[88,89].

Least studied species with recent growing interest

Other vertebrate species of interest have been less studied. Recently however new species of tick-borne *Borrelia* spp. have been sought for and encountered in animals from other orders. For example, although quite unusual, Squamates as host for *Borrelia* were investigated in America, Africa and Europe, with 18 species associated with either *B. burgdorferi* sensu lato or *B. lusitaniae*, the latter being also found in rodents in Mediterranean Europe[90,91]. Primates interestingly were found positive to KFDV antibody in India, a TBFV member of the mammal group close to POWV and DTV[92]. In the UK, an isolated clinical case of lethal borreliosis was found in a *Pipistrellus* spp. bat[93]. Neotropical bats have been identified to carry particular strains of relapsing fever *Borrelia* spp. and Bbsl in Central and Southern America. Studies in Central America have been conducted as a follow-back of a paper published in 1968 by Marinkelle & Grose[94]. Actually, some areas are currently investigated more in depth or monitored. For instance, Columbian cave mentioned above has been resampled for neotropical bats, and Tribeč mountain region in Czech Republic has been screened for TBEV since the seventies and a research presence on TBP is still maintained there.

Infection data in host species

The average *Borrelia* spp. proportion of detection, all species confounded, tended to uniformize between 15% and 30% as the number of tested individuals increased (Figure 3A). For TBFV, average prevalence ranged from 8% for MEAV to 51% for TYUV (Figure 3B).

207 For *Borrelia*, 297 wild animal species (including mammals, birds and lizards) were investigated. Pathogen
208 detection proportions in studies with over 50 tested individuals ranged from 0.1% for *B. lusitaniae* in the
209 Eastern grey squirrel *Sciurus carolinensis* to 76% for Bbsl in the white-footed mouse *Peromyscus*
210 *leucopus*[95], and from 0.2% for relapsing fever causing species in the house mouse *Mus musculus*[96] to 50%
211 for *B. miyamotoi* in the wild turkey *Meleagris gallopavo*[97]. Mammals were more studied than birds, and
212 for those mammalian species with over 100 specimens tested, Artiodactyls, Rodents and Carnivores
213 showed level of prevalence between 20 and 25%. Chiropters, little studied, presented both seropositivity
214 (36%) and pathogen prevalence (25%) of rather high level (Figure 2A). In birds, Columbiformes,
215 Passeriformes, Charadriiforms and Galliformes were the most studied in terms of number of tested
216 individuals, with Charadriiforms and Passeriformes reaching 29% of seroprevalence (Figure 2B).

217 TBFV or antibodies were searched for and detected in 114 terrestrial mammal and bird species (Figure 2C
218 and 2D). Serological detection proportions in studies with more than 50 tested individuals ranged from
219 0.07% for MEAV in the red deer *Cervus elaphus*[98] to 68% for TYUV/MEAV/SREV-like in the European herring
220 gull *Larus argentatus*[99], and for mammalian TBFV, from 0,2% for TBEV/LIV in the roe deer *Capreolus*
221 *capreolus*[100] to 63% for TBEV in the European bison *Bison bonasus*[101]. Most studied avian orders on TBFV
222 were Passeriformes, Charadriiforms, Galliformes and Procellariforms, with highest percentage of
223 seropositivity for Passeriformes (about 40%), followed by Charadriiforms (27%) and Galliformes (21%)
224 (Figure 2C and 2D) when Procellariforms tended to have less seropositive or viremic individuals. In
225 mammals, Eulipothyphla were well-studied, only by PCR techniques, and showed high rates of viremia (of
226 about 50%). Rodents, Artiodactyls, Lagomorphs were also well investigated but had lower seropositivity
227 rates. For Pelecaniformes, 80% of European shag *Phalacrocorax aristotelis* had antibodies to TYUV, but
228 only 8 birds were tested, and on a single location in the eighties[99].

229 Diagnostic tests: current versus past infections

230 Direct and indirect detection methods were at different use according to the type of pathogens. Whilst
231 most Borrelia studies (71%) looked for the genome of the pathogen and used molecular techniques to
232 identify an ongoing infection, 89% of flaviviruses studies used serological detection methods based on
233 antibody detection. Long-lasting in the organisms *Borrelia* spp., were often sought for and found by PCR
234 (60% of case studies overall). Skin and blood were materials used for spirochetes PCR detection, allowing
235 positive samples to be sequenced and species identified, although the use of blood to directly detect
236 *Borrelia* is discussed[102]. The determination of Borrelia subspecies was used in understanding the role of
237 hosts in disease cycles[103]. Different *Borrelia* spp. have been preferentially detected in different hosts, such
238 as *B. lusitaniae* which was the only Bbsl genospecies found in lizards in Europe[104–107].

239 In contrast, the short viremia noted after flaviviruses infection as demonstrated by experimental
240 infections[108,109] makes viral detection challenging, especially in wildlife. Flaviviruses detection by PCR was
241 therefore rarely used (only 11% of TBFV studies used PCR), but allowed sequencing of TBEV subtypes that
242 clustered with TBEV-Eur (European) in Croatia, Slovakia and in the Netherlands[110–112], TBEV-Eur and TBEV-
243 Sib (Siberian) in Finland[113], TBEV-Eur, TBEV-Sib and TBEV-FE (Far Eastern) in Russia[114] and TBEV-Sib in
244 China[115]. Bakhvalova *et al.* evidenced co-infection of several subtypes in a same individual[114]. Dai *et al.*
245 called the strain they studied in China TBEV-Him (Himalayan)[116]. Whilst identifying precise strains will help
246 understanding further the ecology and dynamics of pathogens, it can also assist disease surveillance, with
247 phylogenetics providing information on circulation and evolution[117]. In addition, viral identification is also
248 an asset for public health management. For example, TBEV, a burden for human health, could be

249 sometimes mistaken for Looping Ill virus (LIV), causing severe conditions mainly in sheep[82]. Bai *et al.*, were
250 led to reconsider high values of TBEV seroprevalence because of the discovery of Karshi virus' circulation
251 in China[118].

252 Flaviviruses infections induce an immune response with neutralizing antibodies that could be detected in
253 blood samples lifelong in human[119] and up to 168 days after experimental infection in bank voles[120]. The
254 use of serological approaches was preferred through ELISA techniques (89% of flaviviruses cases, and 38%
255 of overall cases). Serological information, in combination with metadata (such as host age) can provide
256 precious information on pathogen transmission[121–123]. Yet, cross-reactivity in wide-range immunoassays
257 may hamper the interpretation of data[124]. For instance, MEAV antibodies were detected in *Cervidae*[98]. This
258 detection is particularly surprising because MEAV is part of the Seabird TBFV group in phylogeny[125]. As
259 unusual, antibodies against LIV have been identified in seabirds *Fratercula arctica* and *Hydrobates*
260 *leucorhous*[126]. Both of these identifications however might be misclassification or cross-reactions, which is
261 known as a possible weakness of serological studies[127]. Other issue with immunoassays is the choice of
262 cut-off in data analysis, which is generally reproduced from the manufacturer. However some authors
263 proposed other methodologies for cut-off determination for ELISA results, such as mathematical fit on
264 observed data[128]. Paillard *et al.* even chose to present raw ELISA results as a signal of contact with TBFV
265 and therefore not to indicate cut-off, nor prevalence[129].

266 Publications about TBFV outside of TBEV and POWV were marginal and specific detection for these TBFV
267 antibodies was not always commercially available. Arnal *et al.* found MEAV-seropositivity in *Larus*
268 *michaellis michaellis* egg yolks in France using flavivirus-ELISA cross-reactivity properties, with the ID-Vet
269 ID Screen® kit designed for the mosquito-borne flavivirus West Nile virus (WNV)[130]. Similarly, through both
270 COMPAC® WNV-ELISA and an exclusion via serum neutralization tests (SNT), Jurado-Tarifa *et al.*
271 investigated flaviviruses in Spanish birds of prey and detected evidence of exposure to flaviviruses
272 different from the mosquito-borne WNV and Usutu (USUV) viruses and from MEAV in *Circus pygargus*,
273 *Falco tinnunculus*, *Hieraaetus pennatus* and *Tyto alba*[131]. This relies on lack of specificity of commercially
274 available anti-WNV antibodies detection kits. Some commercial TBEV ELISA have also been compared for
275 their reliability according to the TBEV-strain used and for the at-risk cross-reactivity[132]. In the UK, Holding
276 *et al.* recorded similar LIV and TBEV seropositivity results in cervids[82] although TBEV was though not to be
277 present on the British Isles[133]. Holding et *al.* used the FSME/TBE All-Species Progen® ELISA kit and a
278 hemagglutination assay specific to LIV. For confirmation, Holding *et al.* sequenced TBEV in a tick itself.
279 Ytrehus *et al.* also showed presence of both LIV and TBEV antibodies in Southern Norway in *Alces alces*
280 and *Capreolus capreolus* using ELISA kits for TBEV and hemagglutination for LIV as well as SNT for
281 verification[134]. However, their results appeared differentiable for the two pathogens. On the opposite,
282 Bournez *et al.* chose to deliver their final TBEV micro-neutralization test serology results under the
283 TBEV/LIV label, after a first antibodies screening in *Capreolus capreolus* and *Sus scrofa* with the ID-Vet ID
284 Screen® WNV-ELISA[100]. TBEV and LIV as well as the Turkish Sheep Encephalomyelitis virus (TSEV) and the
285 Spanish Sheep Encephalomyelitis virus (SSEV) belong to the TBEV serocomplex[92], hence can particularly
286 easily be mistaken in serology.

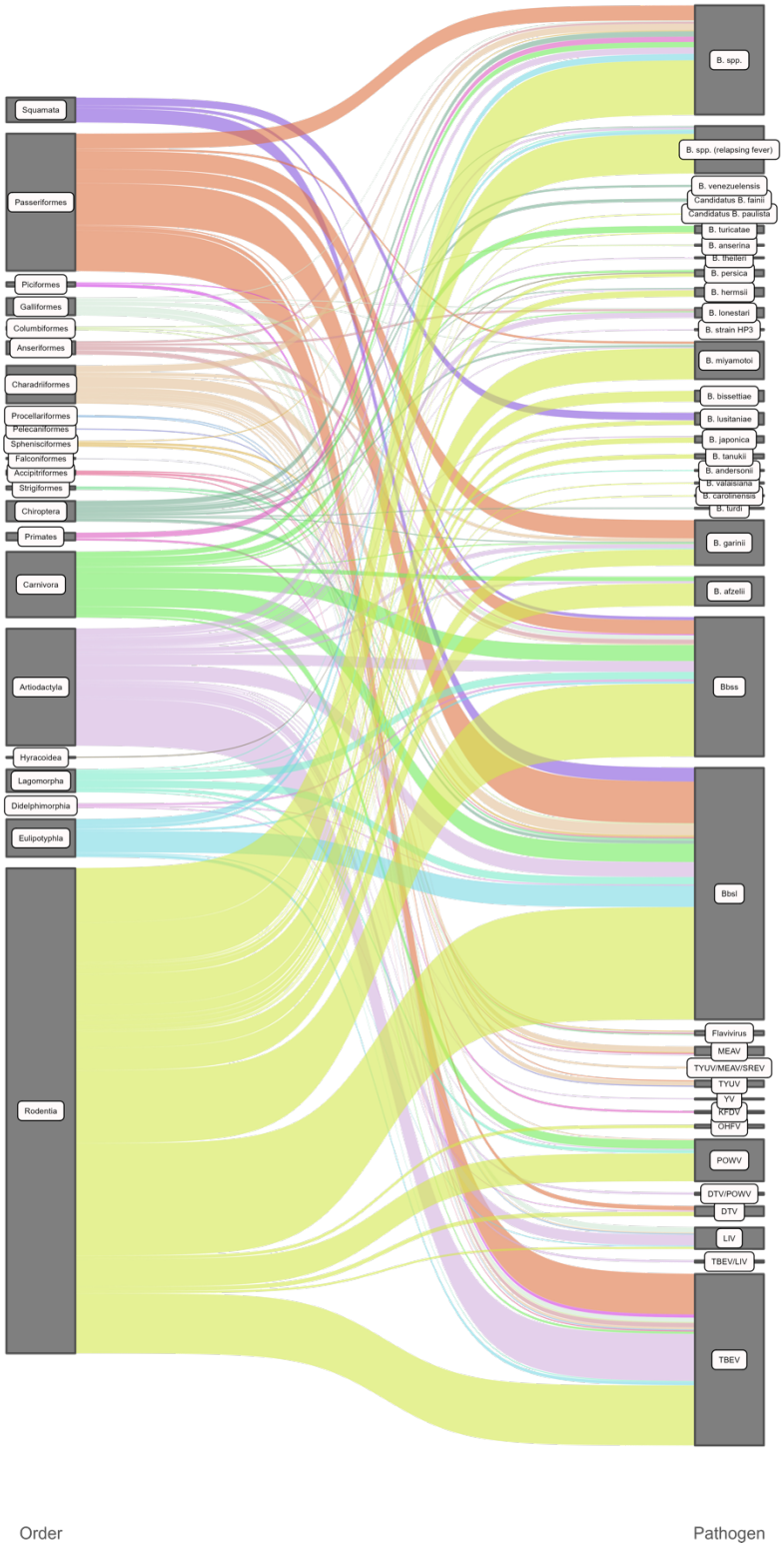

Figure 1: **Sankey diagram pairing study cases by pathogen and order.** Width of the ribbon is proportional to the number of cases included in this order-pathogen couple. Data are available in Supplementary Dataset 1.

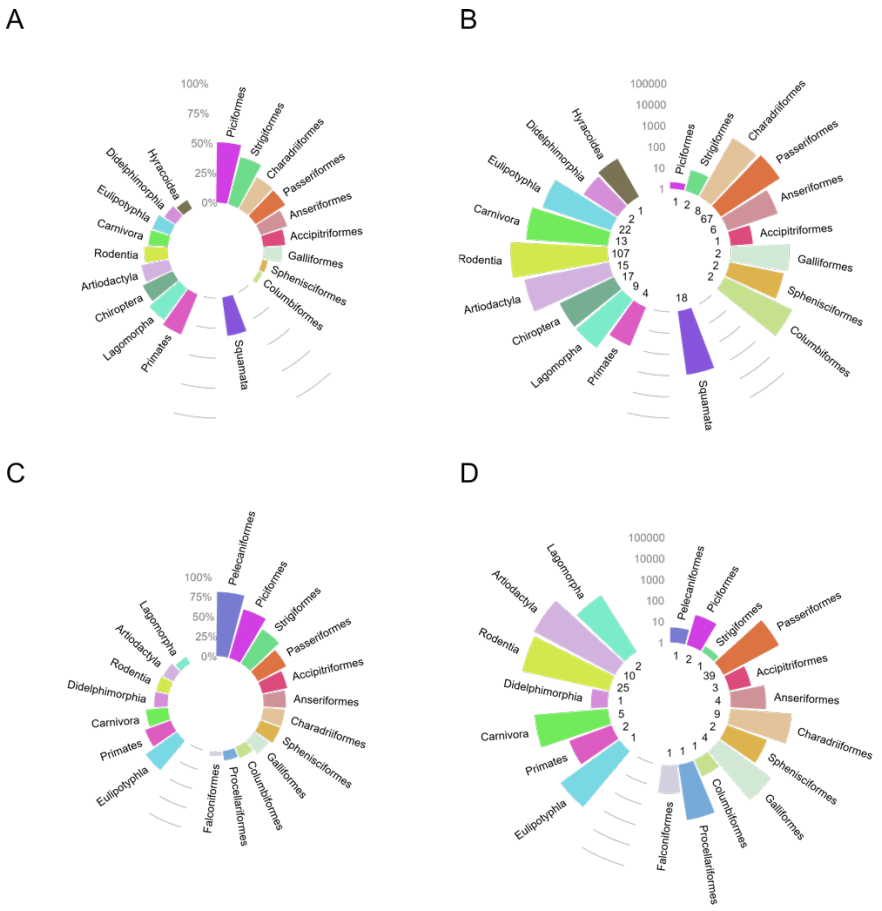

Figure 2A-D: **Investigation efforts for TBP by Orders.** Top row shows for *Borrelia* spp. **(A)** the percentage of animals positive to tick-borne by order, **(B)** the number of tested animals in each order. Bottom row shows for TBFV **(C)** the percentage of animals positive by order, **(D)** the number of tested animals in each order. Most publications regarding TBFV used an indirect methodology (antibody detection), direct detection was used in only 11% of TBFV cases. **(B)** and **(D)** plots shows the number of tested individuals on a log10 scale as well as the number of different host species in each order on the inside. Orders are grouped by class, Mammalia are shown to the left, Aves to the right, and for *Borrelia* spp., Lepidosauria at the bottom. Data are available in Supplementary Dataset 1.

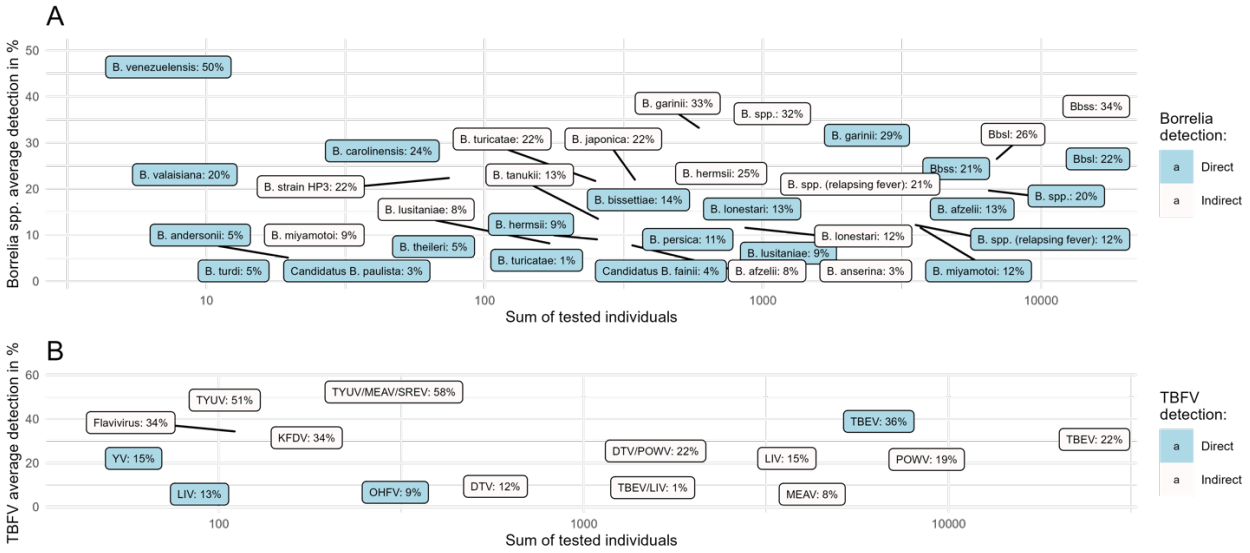

Figure 3A-B: **Average detection rates of pathogens and antibodies in the tested individuals, rounded to 0 digit.** The x-axis represents the log10 of the sum of individuals that have been tested for **(A)** ***Borrelia* spp.** and **(B) Flaviviruses** in the selected publications, the y-axis represents the % of average detection. DTV in oldest publications might have been detected under the name of POWV, because DTV formerly corresponded to POWV lineage II. Data are available in Supplementary Dataset 1.

Study designs and eco-epidemiological parameters

Proposed study designs varied in spatial and temporal sampling, resulting in varying quality in data acquisition. Most studies were cross-sectional (180), and 20 did not inform a study period. A total of 142 fieldworks were reported on two years or more (average study period = 2.9 years, median = 1 year) and few studies (N = 56) were conducted over a 5-year time. Some studies conducted repeated sampling without taking into consideration the period of sampling in data presentation, providing limited epidemiological information. The longest studies recorded lasted 34 years to detect TBEV in striped field mouse (*Apodemus agrarius*) and northern red-backed vole (*Myodes rutilus*) in Russia[114] and 30 years to detect anti-POWV antibodies in white-tailed deer (*Odocoileus virginianus*)[135]. The spatial resolution varied from locality to country scale, with more often province chosen as sampling location. Fieldwork areas were described with environmental characteristic or administrative nomenclature. Inconstance came from different focus either on a local targeted area or on over a broader-scale exposure to the pathogens. Sizes of datasets were often small. The median sample size was 40 and the maximum sample size was 3186 rock doves (*Columbia livia*) from an urban setting in Italy for *B. anserina* detection[136].

A total of 41 publications tagged captured individuals. But tags were not always specific and trustable, some authors simply relying on scars made by biopsy or on feather clipping. The aim of marking was sometimes only to avoid resampling, with immediate release in case of recapture. Only 24 publications used individual tags and repeated capture occasions, and analysed for pathogen detection. In addition, capture-mark-recapture data was not systematically analysed using relevant techniques. Capture-mark-recapture is however an important specific sampling method to try to account for detection uncertainty. For instance, Lambert *et al.* used seroprevalence and capture-mark-recapture data to model the force of infection of brucellosis in the Alpine ibex (*Capra ibex*)[137]. Yet, in studies with no recapture, necropsies were possible, allowing to search for the pathogen in specific organs. Infection and exposure can be evidenced differently depending on tested biological material[138,139].

Moreover, metadata informing basic demographics such as sex and age were lacking in almost every case. Whilst there could be a variability in pathogen susceptibility depending on age, these data are key in understanding pathogens' transmission processes[140]. Mammals and birds or squamates were not studied together in same areas. Designs in the selected publications tended to investigate a single order by setting field material only fit for this order, whilst theorical definitions of reservoir and sentinel suggest that reservoirs are communities rather than species[53]. The underlying population of hosts is seldomly discussed and considered. Furthermore, although convenient, getting material from hunted, road-killed or rescued animals implies bias in detection that should be accounted for. Monitoring in the wild should integrate demographics. Indeed, recommendations of Yoccoz *et al.* is to use information on spatial scale and hosts detectability to estimate unbiased demographic parameters[141]. Thereby, acquiring infection and exposure data in wildlife should be designed using longitudinal studies targeting community of species with capture-marking-recapture or patch-occupancy modelling.

Preceding remarks lead to interrogate the way species are targeted. Dogs have already been suggested as possible sentinels for *Borrelia burgdorferi* and TBEV for humans[142,143]. Although easier to study from a logistical perspective, these domestic species do not capture the information on the early dynamics of transmission, mainly occurring in wildlife for tick-borne pathogens[75]. In addition, one gap in literature is that for many investigated TBP, pathogenicity in vertebrate hosts is disregarded. For instance, while *Coxellia burnetii* causes human Q fever, others *Coxellia*-like spp. are symbiotic in ticks and are not pathogenic in vertebrates[144]. Therefore, combining data on pathogenicity from the field and the laboratory is still needed.

The complementarity of laboratory and tick data

In the literature, models and experimental infections have attempted to characterize reservoir competence[145–147]. Especially, xenodiagnoses are used to detect reservoir capacities in experimental laboratory settings[148]. This consists in feeding non-infected ticks on a TBP laboratory-infected host, and then record infection rates on uninfected hosts brought in contact with the ticks. In this way, vector competence of ticks can also be assayed. Such experimental methodologies however do not account for the infection in their ecosystems. Furthermore, ticks from the field have extensively been searched for pathogens. For instance, from the 827 articles matching our literature search, 224 publications were directly looking into ticks for pathogens with methods ranging from PCR to metagenomics[149,150]. Concerning birds, a literature review reported that most field studies were on host-attached ticks rather than on samples from the birds themselves[151]. Studies on ticks can be complementary to data from hosts insofar as they analyse tick-host interactions. Their deployment is less invasive on hosts than blood or tissue sampling. They need less expertise in the field and are subject to less strict ethical protocols. In addition, identification of ticks' life stages allows to infer ticks' contamination capacity, although other ways of transmission such as vertical transmission in vertebrate hosts have been reported for some agents[152]. It is also faster to collect a large number of individuals. Although it can be logistically easier to collect ticks in the field to get an insight of TBP circulation and diversity, accounting for the whole local host community and the infection status remains as important.

Conclusion

TBP are present in a wide range of vertebrate species, and rodents and artiodactyls were the most investigated. Some infectious agents, rare TBFV or particular strains of *Borrelia* spp., were little studied and information on their infecting capacities are still needed. Diagnostic detection methods varied with

the targeted pathogens. Selected publications showed that study designs, both in data collection and in data analysis, were heterogenous. Key information on host age and precise location was often unavailable; 58% (200/342) of the studies conducted fieldwork at one time point, and very few studies used quantitative methods to analyse the collected data. In combination with experimental data, quantifying transmission needs to gather empirical infection data from populational studies, including hosts demographics and allowing to identify what species act as reservoir in the local host community, and which one can be used as sentinel. Whilst we acknowledge that gathering all this information is difficult, this review highlights the necessity of including eco-epidemiological parameters in wildlife infection studies. The subject discussed in this review concerns a multidisciplinary scientific community (namely ecology, epidemiology, microbiology, parasitology, modelling, public health), emphasizing that getting and interpreting zoonotic infection data from wild animals in a rigorous way is important to build workable One Health programs.

Data availability

The data that support this study are available as Supplementary Dataset 1. Source data are provided with this paper.

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Supplementary information

Literature metrics

A total of 827 articles were retrieved from the literature search, and 314 articles matched the inclusion criteria (Figure S1). The reference of the article, the study area, the study period, the pathogen, the species, the testing material and methodologies were recorded, as well as the prevalence of the pathogens and the number of tested individuals (see Supplementary Dataset 1). Those 314 articles were published between 1959 and 2022, with 2006 and 2008 as median start and end dates for fieldwork efforts in year-referenced publications (Figure S-2A).

Case studies were conducted over a 63-years span (1959-2022), with almost three-quarters (71%, N = 678) done in Europe and Northern America. 73% looked at *Borrelia* and 27% at flaviviruses. Finally, from the 347 vertebrate species investigated, 86% (N = 297) were searched for *Borrelia* spp., 32% (N = 114) for flaviviruses, and 7% (N = 64) were investigated for both.

From these, a total of 947 unique combinations (or "case studies") were identified, defined by one species, studied for one infectious agent, on a defined study area, and over a specific time period. 52 countries were represented in the working dataset. Most case studies were reporting fieldwork data from Europe (37%, N = 352) and Northern America (34%, N = 326), whilst 21% were conducted in Asia (N = 201), 5% in Central and Southern America (N = 50), 2% in Africa (N = 17) and less than 1% in Oceania (N = 1) (Figure S-2B). 69% of case studies (N = 689) looked at *Borrelia* spp. and 31% (N = 258) looked at TBFV. Out of 314 publications, only 3 looked at both kinds of pathogen. Over two-third of Borrelia studies (71%, N = 490) searched for the pathogen itself, whilst the others searched for antibodies. In contrast, the large majority of TBFV studies (89%, N = 230) performed antibody detection, whilst the remaining 11% (N = 28) searched directly for the pathogen.

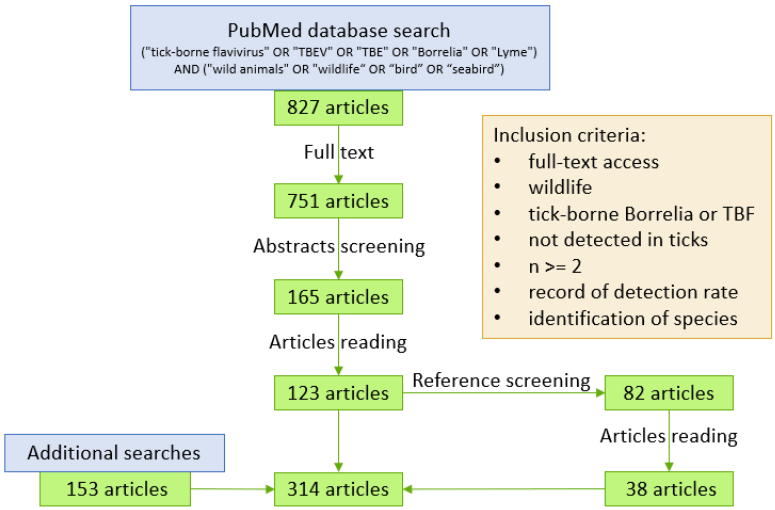
Among tick-borne *Borrelia* spp., the most searched pathogens were *B. burgdorferi* sensu lato (Bbsl), but a large diversity of species and genospecies were studied. They included, ordered from greatest to smallest amount of tested individuals, *B. burgdorferi* sensu stricto (Bbss), unclassified tick-borne *B.* spp., relapsing fever group tick-borne *B.* spp., *B. afzelii*, *B. miyamotoi*, *B. anserina*, *B. garinii*, *B. lonestari*, *B. hermsii*, *B. lusitaniae*, *B. persica*, *B. turicatae*, *B. bissettiae*, *B. japonica*, *Candidatus* B. fainii, *B. tanukii*, *B. theileri*, *B.* strain HP3, *Candidatus* B. paulista, *B. carolinensis*, *B. turdi*, *B. andersonii*, *B. valaisiana* and *B. venezuelensis*. More than half of TBFV cases looked at antibody detection against Tick-Borne Encephalitis virus (TBEV) or Powassan virus (POWV). TBEV and POWV were well-investigated for some localities, with large datasets: out of the 12 largest datasets (i.e. with more than 1000 tested individuals), 8 were collected for anti-TBEV or anti-POWV antibodies detection and 2 for TBEV-targeted PCR. Yet, the diversity of TBF investigated also included Meaban virus (MEAV), Louping Ill virus (LIV), Deer Tick virus (DTV), Omsk Haemorrhagic Fever virus (OHFV), Tyuleniy virus (TYUV), Kyansanur Forest Disease virus (KFDV), Yamaguchi virus (YV) and Saumarez Reef virus (SREV).

About 40% of the studies used a form of PCR (such as nested-PCR, rt-PCR, q-PCR) for pathogen detection and about 20% ELISA to get seroprevalence (Figure S2C). Detection methods changed with time and availability of new techniques. Oldest articles used mainly microscopy on biological material cultivation on Barbour-Stoenner-Kelly medium or blood smears, hemagglutination assay and immunofluorescence assay on blood instead of PCR on nucleic acid extracted from blood and other tissues and ELISA on whole blood or serum. Inoculation of serum in mice in order to survey infection used to be applied for detection. Spirochetes cultivation used to be central in identifying *Borrelia* spp. and progressively was validated and

759 then substituted by the use of PCR. PCR, like the use of ELISA detection before a validation with serum
760 neutralization test, is now frequently used as a screening tool to optimize sequencing use.

761 Limitations due to paper access

762 Effectiveness in TBFV investigation can also particularly be questioned, with some flaviviruses having a
763 status of neglected diseases making difficult to find relevant work on them (Bhatia *et al.* 2020). In addition,
764 some publications on rare TBFV were not available online (without abstract and not in English). TYUV for
765 instance is a Siberian seabird TBFV that has been known for a long time. During the seventies, publications
766 started to clarify which species could host the virus but today traces of them are lacking. Effort in virus
767 investigation has also later been less intensive, before a new surge of interest today.



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769 *Figure S1:* **Diagram of inclusion and exclusion in database screening.**

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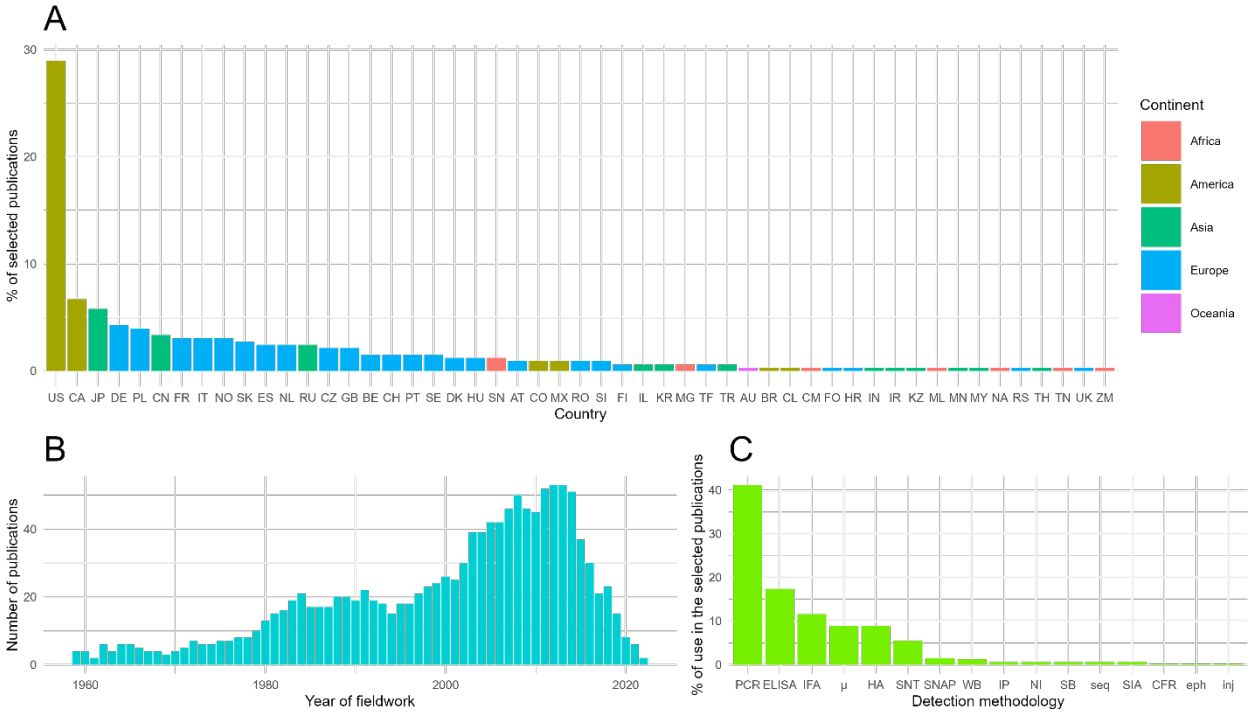

Figure S2A-C: **Location, period and method in selected publications. (A) Country location of selected publications' study area, categorized by continent**. About a third of the publications (34%) were from the US or Canada, 37% related to European countries, and 21% to Asia. AU = Australia; AT =Austria; BE = Belgium; CA = Canada; CH = Switzerland; CN = China; CO = Colombia; CZ = Czech Republic; DE = Germany; DK = Denmark; ES = Spain; FI = Finland; FO = Faeroe Islands; FR = France; GB = Great Britain and Northern Ireland; HU = Hungary; IL =Israel; IS = Iceland; IT = Italy; JP = Japan; KR = Republic of Korea; KZ = Kazakhstan; MA = Morocco; MG = Madagascar; ML = Mali; MN = Mongolia; MR = Mauritania; MX = Mexico; MY = Malaysia; NA = Namibia; NL = the Netherlands; NO = Norway; PL = Poland; PT = Portugal; SI = Slovenia; SK = Slovakia; TF = French Southern Territory; TH = Thailand; TN = Tunisia; TR = Turkey; RO = Romania; RU = Russia; US = United States of America; ZM = Zambia. **(B) Distribution of the study years of fieldwork effort.** From data on start and end dates, a count of publications putting fieldwork effort was implemented by year. Unfortunately, 20 publications did not inform their time period of fieldwork. **(C) Detection methodologies.** PCR = Polymerase in Chain Reaction; ELISA = Enzyme-Linked Immunosorbent Assay; IFA = Immunofluorescence Assay; µ = microscopy; HA = Hemagglutination Assay; SNT = Serum Neutralisation Titer; SNAP = commercial veterinary Ig detection kit; WB = Western Blot; IP = Indirect Immunoperoxidase; NI = Not Informed; SB = Southern Blot; seq = Sequencing; SIA = Strip Immunoblot Assay; CFR = Complement Fixation Reaction; eph = electrophoresis; inj = Injection in mice. Data are available in Supplementary Dataset 1.