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Research Article

The significance of viral, bacterial and protozoan infections in zebra: a systematic review and meta-analysis of prevalence

Carlo Andrea Cossu[1,2,*], Raksha Vasantrai Bhoora[1], Rudi Cassini[2], Henriette van Heerden[1]

[1] University of Pretoria, Faculty of Veterinary Science, Department of Veterinary Tropical Diseases, Onderstepoort, South Africa
[2] University of Padova, Faculty of Veterinary Medicine, Department of Animal Medicine, Production and Health, Legnaro, Padova, Italy

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Abstract

Wild equids can harbour multi-host infectious agents that are able to affect other wildlife species, but also domestic animals and humans. The direct and indirect contact between wild and domestic equids is constantly increasing due to global movement of horses and equine products, the depletion of natural areas and climate and land-usage changes, which could result in burdensome epidemics. Nevertheless, currently there is a lack of adequate epidemiological data from zebra.

Three electronic databases were searched from 10 to 20 March 2021 for publications reporting bacterial, viral and protozoan infections in zebra. Data for a total of 12 relevant variables were extracted from reviewed papers to undergo a qualitative analysis. Prevalence-reporting studies were subjected to meta-analysis for estimating the pooled prevalence and seroprevalence of microbials in wild zebra populations.

We identified 30 pathogen species and the most represented were equine Herpesvirus 1 and 9, *Bacillus anthracis*, African horse sickness virus and *Theileria equi*. They were reported from all the three zebra species, both in captivity and wilderness. Pooled seroprevalences were estimated for the equine Orbiviruses AHSV (70%; 95% CI: 35–96%) and EEV (21%; 95% CI: 8–38%) and for the equine α-Herpesviruses EHV-1 (72%; 95% CI: 43–93%), EHV-4 (40%; 95% CI: 0–100%) and EHV-9 (58%; 95% CI: 9-98%), and pooled prevalences for the equine piroplasms *T. equi* (100%; 95% CI: 94–100%) and *B. caballi* (8%; 95% CI: 0–28%).

Zebra is most probably a component of the reservoir from which AHSV, EHV-1 and *T. equi* can be directly or indirectly transmitted to horse populations, potentially causing disastrous epidemics. Zebra can also harbour zoonotic pathogens like *B. anthracis*, *Brucella* spp., *A. phagocytophylum*, CCHFV and *T. brucei*. Other agents like EHV-9, BPV-1 and BPV-2 have the potential to spread from zebra to other wild endangered animal species. We conclude that zebra is an important host of multiple and dangerous pathogens for both animals and humans. Comprehensive studies focused on the prevalence of infectious agents present in zebra populations and the associated risk factors are required.

Introduction

Wildlife associated microbial pathogens have an impact, either directly or indirectly on both animal and human health (Rhyan and Spraker, 2010). Approximately 60% of all emerging infectious diseases in humans are zoonoses, with nearly 70% being linked with a wildlife origin (Jones et al., 2008). Viral pathogens (such as avian influenza virus, Hendra virus, Nipah virus, Hantavirus, Ebolavirus and Marburg virus), bacterial pathogens (like *Borrelia burgdoferi* causing Lyme disease and *Leptospira* spp. causing leptospirosis) and protozoan pathogens (such as *Trypanosoma* spp.) infecting humans have been demonstrated to originate from wildlife reservoirs (Cleaveland et al., 2005; Jones et al., 2008). In addition to the more recent severe acute respiratory syndrome — coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV) (Markotter et al., 2020) and severe acute respiratory syndrome — coronavirus 2 (SARS-CoV-2) (Zhou et al., 2020), human immunodeficiency virus 1 (HIV-1) (Gao et al., 1999) and 2 (HIV-2) (Hirsch et al., 1989) are also thought to have derived from wildlife hosts. Emerging wildlife diseases like foot and mouth disease (FMD), African swine fever, theileriosis, brucellosis and bovine tuberculosis (BTB), have a serious economic impact due to imposed national and international trade restrictions of livestock (Cleaveland et al., 2005). Furthermore, wildlife infectious diseases may elicit a decline in endangered wildlife populations, exacerbating their conservation status (van de Bildt, 2002; Grogan et al., 2014).

The rate of wildlife-emerging infectious diseases is increasing globally due to environmental, climate and land usage changes, human encroachment into natural habitats, domestication of wildlife species and the movement of animals and people worldwide (King et al., 2006; Jones et al., 2008). The horse industry in particular, has grown exponentially over the past decades and horses travel internationally more than any other animal species to compete in sporting events (racing, dressage, endurance riding, show jumping etc) or for breeding purposes (Timoney, 2000a). Relevant infectious diseases, such as African horse sickness (AHS) outbreaks in Spain and Portugal between 1987 and 1991; Equine influenza A outbreak in South Africa in 1986; equine viral arteritis outbreaks in South Africa in 1994–1998, could influence the international trade of equids and equid semen (Timoney, 2000b). For instance, zebra is the prime suspected source of infection for the devastating AHS outbreaks in Spain that later spread in Portugal through midges (Rodriguez et al., 1992). These epidemics killed thousands of horses, and required massive eradication efforts (≈40000 horses vaccinated in Spain and 170000 in Portugal) with costs, estimated around US $ 1.9 million (Portas et al., 1999). Therefore, it is of par-

*Corresponding author
Email address: ca.cossu@tuks.co.za (Carlo Andrea Cossu)

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ticular interest to determine the pathogens that are able to infect zebra and consequently develop a carrier state from which either vector transmission or direct contact can lead to further transmission to domestic equids.

Zebras are members of the African equids/horse family (genus *Equus*, family Equidae, order Perissodactyla) identified by their distinctive black-and-white striped coats and, unlike their closest relatives', horses and donkeys, zebras have never been domesticated. The taxonomy of zebra, proposed by Groves and Bell (Groves and Bell, 2004) and later revised by Moodley and Harley (Moodley and Harley, 2005), describes three species of zebra that all live in sub-Saharan Africa: Grevy's zebra (*Equus grevy*, subgenus *Dolichohippus*), the plains or Burchell's zebra (*E. quagga*, subgenus *Hippotigris*), and the Mountain zebra (*E. zebra*, subgenus *Hippotigris*) that includes the subspecies Cape Mountain zebra (*E. zebra zebra*) and Hartmann's zebra (*E. zebra hartmannae*). Grevy's zebra (≈2000 mature individuals) can be found in arid and semiarid grasslands and shrublands of northern Kenya and southern Ethiopia (Rubenstein et al., 2016). The plains zebra (150000–250000 mature individuals) (King and Moehlman, 2016) is distributed in Somalia, Tanzania, Zambia, Mozambique, southern Angola, Katanga Province of Zaire and South Africa (Barnard, 1998). The Mountain zebra population consists of nearly 35000 mature individuals: Hartmann's zebra subpopulation has a distribution range in Namibia and Angola (Gosling et al., 2019), while the Cape mountain zebra subspecies lives in the Eastern and Western Cape Province of South Africa (van Dyk et al., 2009) (Fig. 1). The World Conservation Union (IUCN) Red List classifies Mountain zebra as vulnerable and Grevy's zebra as endangered. The Convention on International Trade in Endangered Species (CITES) lists these species in Appendix II and I, respectively. All three species of zebra play similar roles in their respective habitats. They survive on a diet of rough vegetation with relatively low nutritional value, which their hindgut fermentation process allows them to digest. Because they cut back old growth and consume lower quality plant matter, they increase the overall quality of vegetation in areas they inhabit, which is beneficial to other herbivores. They are also an important source of food for many of Africa's carnivores, with as many as 30% killed by lions and hyenas (Rubenstein, 2010).

Currently, there is a lack of adequate epidemiological data of infectious agents from wildlife. The difficulty in conducting studies on wild animals (e.g. zebra) arises with challenges in acquiring the appropriate permits, identifying suitably qualified wildlife veterinarians to conduct the darting and sample collections and most importantly the lack of sufficient funding (Vrbova et al., 2010; Grogan et al., 2014; Onyiche et al., 2019).

Figure 1 – Geographic distribution of wild zebra populations of the Grevy's zebra (*Equus grevy*), plains or Burchell's zebra (*E. quagga*), and the mountain zebra (*E. zebra*) in central and southern Africa, modified from (Gosling et al., 2019; King et al., 2006; Rubenstein et al., 2016).

Made with MapMaker NatGeo interactive:

https://mapmakerclassic.nationalgeographic.org/dJQMXpxywvTPGlW8w82YMQ/?edit=grBhEZt0kESLYPoWGmdql

In this paper, we systematically review the infectious pathogens detected in zebra, analysing their characteristics in a host-oriented epidemiological investigation. Where possible, random-effects meta-analysis was performed to estimate the prevalence of viral, bacterial and protozoan infections in wild zebra populations. The final aim is to unravel the epidemiological role of zebra for different pathogens, which is here discussed and interpreted by the authors.

Materials and methods

Search strategy

This systematic review and meta-analysis is registered with PROSPERO (code `CRD42021267534`) (Cossu et al., 2021) and is reported following the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA) guidelines (Moher et al., 2009). The PRISMA checklist on reporting and the extension for abstracts are provided in Tab. S1 in Supplemental Information. An additional checklist, based on a systematic review of systematic reviews and meta-analyses of prevalence (Borges Migliavaca et al., 2020), is provided in Tab. S2 in Supplemental Information. The PICO (population-intervention-comparison-outcome) model was adopted to formulate the research questions hence the search strategy (Straus et al., 2018): The "Population" (P) of interest here are all the species of zebra. The "Intervention" (I) was biological sampling and laboratory detection/diagnosis. No "Comparison" (C) was set because only laboratory detection was the intervention of interest. The "Outcome" (O) of interest was the presence of viruses, bacteria and/or protozoans in biological samples. The research was based on one background question and two foreground questions. The background question was "What viruses, bacteria and/or protozoans are able to infect zebra?". The foreground questions included (a) "What is the prevalence of viruses, bacteria and/or protozoans or related antibodies in wild zebra populations?" and (b) "What is the possible role of zebra in the epidemiology of the infectious agents considered? For instance, is zebra just an incidental host or can it represent a source of infection to other animals? Could the detected viruses, bacteria and/or protozoans persist within zebra populations?"

Three bibliographic databases (Web of Science, ScienceDirect, and PubMed) were searched to retrieve and review articles (both peer-reviewed and pre-prints) published in English, Spanish and Italian. Additional hand searched documents from the authors' collections were also included. No temporal limit was posed. Homonimes of zebra not referred to the population considered (eg. "zebra mussel", "zebra finch", "zebra protein" of Epstein-Barr disease) were excluded. The last search was conducted on March 20, 2021. Table 1 shows the comprehensive search strategy used for each database. Our search strategy was adapted on the database searched and the results obtained from each. A maximum of 8 boolean operators (i.e. number of "`AND`" or "`OR`" or "`NOT`") is allowed to enter the advanced search of Science Direct. Therefore, we could not proceed with the same search strategy utilized for the other databases, where a more selective approach was needed, and we had to select 8 keywords that could give us the most significant papers. Web of Science (WOS) provided us with far less results than PubMed, even though more significant. We therefore proceeded adding more synonyms in the WOS search strategy to maximize the number of papers that could be obtained from this database. In PubMed, our search strategy retrieved almost 2000 papers. We then decided to select the 50% most applicable papers basing on the "Best match" display option provided by this database. This option is based on a weighted term frequency algorithm which includes machine learning to re-rank the top articles returned. It is therefore a non-biased method to select articles according to the key-words entered. The resulting references from all databases were exported to a reference manager software (Mendeley

Table 1 – Search strategy and criteria used to retrieve papers of interest.

Database: Web of Science [all databases]	
	`TOPIC: (Zebra infectious disease/OR Zebra pathogen/OR zebra virus/OR zebra bacterium/OR zebra bacteria/OR zebra parasite/OR zebra zoonosis/OR zebra zoonoses) AND (sample/OR samples/OR sampling/OR serum/OR sera/OR tissue/OR spleen/OR liver/OR lymph node) AND (lab/OR laboratory/OR diagnosis/OR diagnostics/OR PCR/OR serology/OR ELISA/OR RLB/OR antibody/OR antibodies) NOT ("zebra fish"/OR "zebra finch"/OR "zebra finches"/OR "zebra mussel"/OR "zebra caterpillar"/OR "zebra shark"/OR "zebra protein"/OR "Epstein-Barr")`
	Refined by: `LANGUAGES: (English OR Spanish OR unspecified) AND [excluding] RESEARCH AREAS: (agriculture OR plant sciences OR entomology OR pharmacology pharmacy OR nutrition dietetics) AND [excluding] DOCUMENT TYPES: (meeting OR editorial material)`
	Timespan: all years
	Databases: WOS Core Collection, BCI, CCC, DRCI, DIIDW, KJD, MEDLINE, RSCI, SCIELO, ZOOREC
	Number of results: 123
Database: ScienceDirect	
	`TOPIC: zebra pathogen AND sample AND (antibodies OR diagnosis) NOT ("zebra finches" OR "zebra mussel" OR "zebra shark" OR "Epstein-Barr")`
	Refined by: `ARTICLE TYPE: (Review articles OR Research articles) AND SUBJECT AREAS (Immunology and Microbiology OR veterinary science and veterinary medicine OR biochemistry, genetics and molecular biology)`
	Number of results: 391
Database: PubMed	
	`(Zebra infectious disease OR Zebra pathogen OR zebra virus OR zebra bacterium OR zebra bacteria OR zebra zoonosis OR zebra zoonoses) AND (sample OR samples OR sampling) AND (diagnosis OR PCR OR ELISA OR RLB OR antibody OR antibodies) NOT (fish OR finch OR finches OR mussel OR caterpillar OR shark OR "zebra protein" OR Epstein-Barr OR chip OR potato OR tomato)`
	Refined by: `ARTICLE TYPE: (Review OR Journal articles OR systematic review) AND LANGUAGE: (English OR Italian OR Spanish)`
	Number of results: 1951
	Sorted by: best match and imported the first 1000 documents.

WOS = Web Of Science core collection; BCI = BIOSIS Citation Index; CCC = Current Contents Connect; DRCI = Data Citation Index; DIIDW = Derwent Innovations Index; KJD = Korean Journal Database; RSCI = Russian Science Citation Index; ZOOREC = Zoological Record.

Desktop, version 1.19.4; ©2008–2019 Mendeley Ltd.) and duplicate records were removed.

Screening, eligibility and inclusion criteria

References were initially screened by title and abstract. References were excluded: (i) if zebra were not included in the title and/or abstract; (ii) if the research field differed from our study (aquaculture, agriculture, plant sciences, cancer research, etc.); (iii) if pathogen taxa other than viruses, bacteria and protozoa were examined and (iv) if the abstract and PDF were not available.

Full-text examination was finally undergone. Publications were screened and selected based on the following inclusion criteria: (i) contained data on any positive diagnostic test result for a pathogen in zebra species; (ii) contained data on incidence, prevalence and distribution of a pathogen in any infected zebra species; (iii) contained data on experimental transmission among zebra and other animals; (iv) contained data on pathogenesis, clinical signs and lesions of an infectious pathogen in zebra; (v) contained data on genotype/serotype characterization for pathogens detected in zebra; and (vi) contained data on correlation between pathogen prevalence and influencing factors in zebra. Full text publications were excluded for one or more of the following reasons: (i) review/dissertation/OIE manuals; (ii) population did not include zebra; (iii) intervention information such as sampling and/or laboratory diagnosis not mentioned; (iv) pathogen or antibody detection outcome missing.

Exclusion criteria for the meta-analyis included case reports, case series, experimental studies, studies with samples collected after an outbreak or following an experimental infection or from clinically sick animals or captive animals. Additionally, studies that reported unrepresentative samples or unclear reports of sample size (not clear numerator or denominator, respectively) were excluded from the meta-analysis.

Quality assessment of individual studies

To critically assess included studies, the study design for each paper that passed the inclusion criteria was determined and the papers were categorized according to the individual study designs. They were classified as: prevalence-reporting studies, cohort studies, case reports, case series, and quasi-experimental studies (non-experimental trials). Each paper was then critically assessed according to the Joanna Briggs Insitute (JBI) standardized checklists (Moola et al., 2020). Depending on the study design, checklists with between 8 and 11 questions, were used to determine the potential risk of bias. The questions could be answered with "yes", "no", "unclear" or "non applicable". The risk of bias of individual studies was determined with the following cut-offs: low risk of bias if 70% of answers scored yes, moderate risk if 50 to 69% questions scored yes and high risk of bias if yes scores were below 50% (Melo et al., 2018; Goplen et al., 2019). All the authors made their quality assessment of included studies. Any disagreement was discussed and resolved by consensus. JBI checklists and our individual critical appraisals are reported in Tab. S3 in Supplemental Information.

Data extraction and preparation

Data from eligible articles were extracted for a total of fifteen variables. These include: twelve nominal variables (i.e. reference, continent, country, location, zebra species, zebra origin (wild or captive), sample type, pathogen, diagnostic method, genotypes/serotypes, clinical signs and influencing factors), and three discrete random variables (i.e. publication year, animals tested and animals positive). Values obtained were entered onto a pre-defined Excel spreadsheet (Microsoft Corp., Redmond, Washington, USA).

For meta-analysis, seven variables were retained (i.e. pathogen species, reference, diagnostic method, zebra species, country, tested animals and positive animals). According to OIE terrestrial manuals (World Organisation for Animal Health, 2017a,b, 2018), diagnostic tests were combined into two categories that included: (i) agent detection tests; (ii) antibody detection tests (serological tests). In order to avoid double-counting of the sampled population, results from individual studies using more than one diagnostic test of the same category were merged and the mean of positive animals calculated. Each row of the spreadsheet corresponded to one dataset, having a unique combination of the variables selected. Our meta-analysis data are publicly available on Mendeley Data with doi:10.17632/dgxx92xjyf.4.

Analysis

A qualitative analysis was conducted using descriptive statistics in Excel. To determine where studies were conducted globally, the percentage frequency distribution of the "Country" variable was graphically represented with a choropleth. On the other hand, the frequency distributions of the variables "publication year" and "pathogen species" — later stratified per the variables "Zebra origin (wild or captive)" and "Zebra species" — were graphically represented using bar plots. Spe-

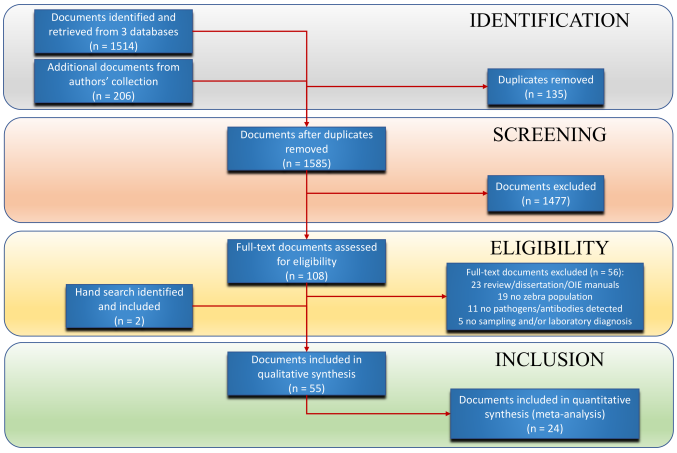


Figure 2 – Flow-chart representing the selection of studies for inclusion in the systematic review and meta-analysis following the PRISMA guidelines.

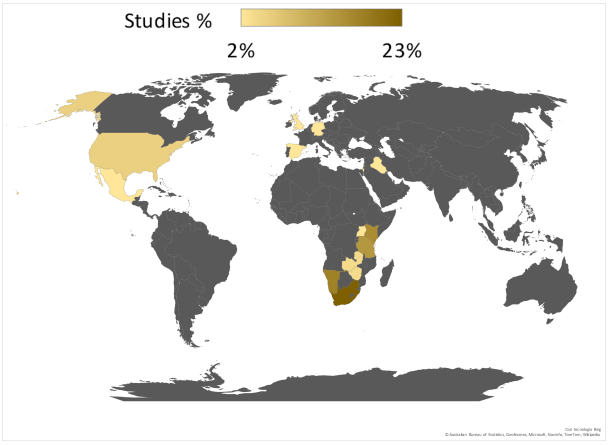


Figure 3 – Geographic distribution and abundance of studies reporting infectious agents from zebra.

cific details extracted for the other categorical variables (i.e. diagnostic method, genotypes/serotypes, clinical signs and influencing factors) were arranged in summary tables.

A quantitative analysis was conducted to estimate the pooled prevalence and seroprevalence of infectious agents in wild zebra. Prevalence and seroprevalence are here interpreted as the probability that a member of the population tests positive to an agent detection test or an antibody detection test, respectively, at a point in time. Therefore, prevalence/seroprevalence is the sample proportion of events $\hat{p} = \frac{x}{n}$, that is an unbiased estimator of the parameter p of a binomial distribution $B(n, p)$, where n=number of trials (i.e. tested animals), x=number of successes (i.e. positive animals), p=success probability for each trial, and $q = 1 - p$=failure probability for each trial (Elston, 1969). On these premises, we developed a custom program for our meta-analysis of event rates using the packages `dmetar` (Harrer et al., 2019), `metafor` (Viechtbauer, 2010) and `meta` (Balduzzi et al., 2019) of R language (R Core Team, 2021) in RStudio software (RStudio Team, 2021). Codes and functions utilized can be retrieved from the first author GitHub website using the URL: https://github.com/CarlVet/Zebra-infectious-agents.git.

Details on our meta-analytical method are here listed and briefly commented:

- Random effects model. In our meta-analysis, the effect size is represented by the prevalence of infectious agents in wild zebra populations, which may differ considerably for different zebra species and country. Our goal was therefore not to estimate the one true prevalence of all studies, but the mean of the distribution of the true prevalences;
- Sidik-Jonkman variance estimator with Hartung-Knapp adjustment method leading to results more conservative than the common DerSimonian-Laird method, indicated by wider confidence intervals (Inthout et al., 2014);
- Clopper-Pearson confidence interval for individual studies. Mainly, two different methods exist for obtaining a Confidence Interval (CI) for the binomial parameter p: the normal theory method (Wald method) and the exact method (Clopper-Pearson method). The normal theory method is appropriate when $npq \geqslant 5$ (Elston, 1969). In our case, npq was often <5 because of very small sample sizes hence we opted for the more conservative Clopper-Pearson method.
- Freeman-Tukey double arcsin transformation. In a meta-analysis of prevalence (i.e. proportions), when the estimate for a study is close to 0% or 100%, the variance for that study tends to zero and thus its weight is overestimated. Therefore, prevalence estimates (raw proportions) were transformed using the Freeman-Tukey double arcsin method. The final pooled result and 95% CIs were backtransformed to a proportion.
- Methods used for assessing between-study heterogeneity: Higgins & Thompson's I^2 statistic, Q-Test of Heterogeneity and Prediction Interval (PI). The I^2 statistic is defined as the percentage of variability in the effect sizes that is not caused by sampling error. Low heterogeneity is represented by I=25%, values of 50% indicates moderate heterogeneity while substantial heterogeneity is represented by $I \geqslant 75\%$. The Q-Test of Heterogeneity is a hypothesis test where the null hypothesis (μ_0=no heterogeneity, and the alternative hypothesis μ_1=significant heterogeneity. The null hypothesis is rejected if p-value<0.05, which is indicative of statistically significant heterogeneity. Finally, the Prediction Interval can give us a range into which we can expect the effects of future studies to fall based on present evidence (Harrer et al., 2021).
- Forest plot: our meta-analysis was visualized in forest plots. Such plots provide a graphical display of the observed effect, confidence interval and the weight of each study (based on the inverse-variance method). The pooled effect we have calculated can be seen as a diamond at the bottom of the plot.
- Publication bias: small study effects method, assuming that only small studies with a very high effect size are published, while others remain in the file drawer (Egger et al., 1997; Begg and Mazumdar, 1994). This method relies on the evaluation of funnel plot asymmetry, assessed either qualitatively (visual inspection of the funnel plot) and quantitatively, using Egger's regression test. For this test, a p-value<0.05 was interpreted as presence of significant asymmetry in the funnel plot. Where applicable, Duval & Tweedie Trim and Fill Method was then used to adjust for funnel plot asymmetry (Duval and Tweedie, 2000).

Quality assessment of the body of evidence

Prevalence is a useful measure for the development of guidelines or other decision-making tasks, and for addressing future research. To avoid errors or mispractice, judgements and recommendations based on prevalence data should be weighted on the quality of evidence (QoE). To ensure appropriate methodologic consistency, we evaluated the QoE for our prevalence estimates using a GRADE (Grading of Recommendations Assessment, Development, and Evaluation) system (Atkins et al., 2004). This method rates the QoE as high, moderate, low or very low which reflects our certainty/confidence that the study outcomes are representative of the true effects. The rating process is as follows:

- Initial QoE based on the study design. In our case, the effect of interest is the prevalence of pathogens/antibodies in a wild population, which can only be reported by observational studies (prevalence-reporting surveys or cross-sectional studies) (Thrusfield, 2005) As a consequence, the study design does not impact the quality of evidence of our prevalence estimates and the initial QoE is therefore excluded from our rating process.
- Five domains can downgrade the initial QoE: risk of bias, inconsistency, indirectness, imprecision and publication bias. Risk of bias was evaluated using the JBI checklists (see above), inconsistency relies on the similarity of point estimates and the overlap of

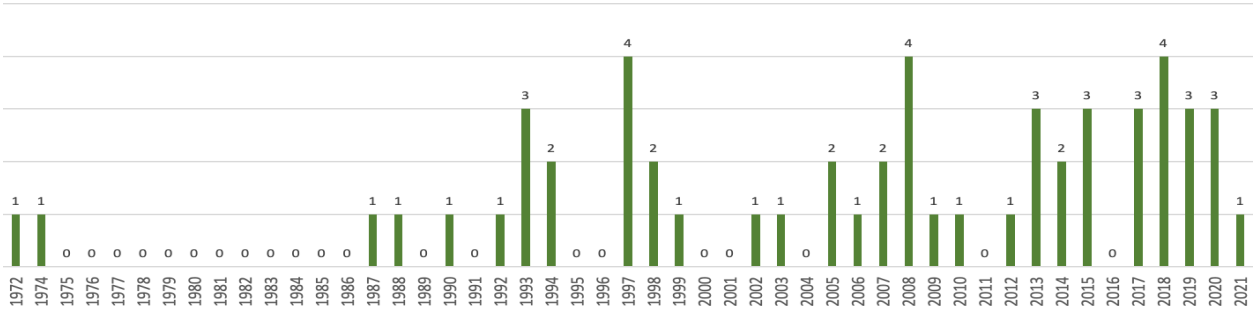


Figure 4 – Temporal distribution of the studies reporting infectious agents from zebra.

their confidence intervals, imprecision focuses on the 95% confidence interval around the best estimate of the absolute effect, and publication bias was assessed with the small study method (see above). Studies showing indirectness were already excluded from meta-analysis during the eligibility process.

- Three domains can upgrade the QoE: large-effect, dose-response gradient and if residual confounding would only decrease the magnitude of effect.
- Final QoE: subjective pooling/merging of the different rating domains.

Case definition

A critical comparison of the findings of our study is produced narratively and interpreted in a broader context thanks to the support of additional references. The possible role of zebra is investigated for each pathogen. In order to avoid confusing and misleading assessments, we provide the following definitions:

Infectious agent: microparasite (i.e. bacterium, virus or protozoan) or macroparasite (i.e. helminth or arthropod) that is able to enter and multiply in one or more organisms, namely hosts (Thrusfield, 2005);

Susceptible host: a host in which the infectious agent can enter and multiply and/or produce toxin/toxins, resulting in either clinically apparent or inapparent disease (Thrusfield, 2005);

Natural host: a host that is susceptible to the infectious agent under natural conditions (Haydon et al., 2002);

Source of infection: any substance (e.g. soil) or organism from which an infectious agent can potentially spread to susceptible hosts (Thrusfield, 2005);

Dead-end or incidental host: a host from which an infectious agent usually cannot spread to other susceptible hosts (Thrusfield, 2005);

Carrier host: a host with inapparent (i.e. without obvious illness) or covert (i.e. latent) infection that can potentially spread the infectious agent to other susceptible hosts (Thrusfield, 2005);

Amplifying or amplifier host: a susceptible host or vector that contributes greatly to the transmission dynamics of an infectious agent due to high relative abundance/density of the host/vector (i.e. super-abundance) and/or high prevalence of the infectious agent in the host/vector (i.e. super-infection) (Streicker et al., 2013; Wardrop, 2016) and/or high infectiousness (i.e. super-shedding) (Matthews et al., 2006; Brown et al., 2008; Streicker et al., 2013) and/or behavioural or genetic differences within host species that can act as exceptional transmitters (i.e. super-dispersal) (Streicker et al., 2013) and/or provision of a direct epidemiological link to other susceptible host species (i.e. spill-over) (Karesh et al., 2012; Jones et al., 2008);

Maintenance host: a host species in which the infectious agent persists even in the complete absence of transmission from other host species (Haydon et al., 2002; Rhyan and Spraker, 2010);

Maintenance host community/complex: one or more epidemiologically connected populations or environments in which the infectious agent can be permanently maintained (Haydon et al., 2002);

Target host: the population of concern for the observer (Haydon et al., 2002);

Reservoir host: maintenance host or maintenance host community/-complex from which the infectious agent can be transmitted to the defined target population (Haydon et al., 2002).

Results

Qualitative analysis

A total of 1720 (1514 from databases, 206 from hand-search) potentially relevant citations were initially identified from the searches based on reference titles (Fig. 2). After screening the titles and abstracts, and removal of duplicates, 108 references remained, which were subjected to a full text search. A total of 55 publications that satisfied our inclusion criteria for the systematic review were identified (Fig. 2) and included in the qualitative analysis.

The spatial and temporal distribution of included articles are represented in Fig. 3 and 4, respectively. The majority of the studies were conducted in the African continent (81%) (the continent where wild zebra lives) with most studies originating from South Africa (23%) followed by Kenya (18%), Namibia (18%), and Tanzania (14%) (Fig. 2).

The oldest document dates to 1974. Following a 12-year gap, between 1974 and 1987, publications on zebra pathogens started emerging with only 16 papers published between 1987 and 2001. From 2002, publications on zebra pathogens increased and a total of 39 references were included with an average of 2.1 per year between 2002 and 2020 (Fig. 4).

Pathogens demonstrated to infect zebra include 17 viruses, 7 bacteria and 6 protozoans listed in Fig. 5. The most reported pathogens were equine Herpesvirus 1 (EHV-1) and *Bacillus anthracis* (10 publications each), African horse sickness virus (AHSV) (9 publications), equine Herpesvirus 9 (EHV-9) (8 publications), and *Theileria equi* (5 publications) (Fig. 6A). While most of the pathogens were found in wild zebra, only three pathogens were reported exclusively from captive zebra (Fig. 6B). Plains zebra was the most affected/represented zebra species (55%), following mountain zebra (29%) and Grevy's zebra (12%) (Fig. 6C). Various laboratory detection methods for pathogens identified in zebra are reported in Tab. 2. Information regarding the categorical variables "clinical signs", "influencing factors" and "genotypes/serotypes" are provided in Tab. S4 in Supplemental Information.

Quantitative analysis

A total of 24 studies (representing 52 datasets) were included in the quantitative analysis of prevalence (Fig. 2). The datasets represented 33 antibody-based detection, 16 molecular detection and 3 pathogen identification methods. A meta-analysis could be performed for each pathogen reported from at least two different studies with the same diagnostic test group: the equine Orbiviruses AHSV (5 studies, 5 datasets) and equine encephalosis virus (EEV) (2 studies, 3 datasets), the equine α-Herpesviruses EHV-1 (6 studies, 7 datasets), EHV-4 (3 studies, 3 datasets) and EHV-9 (4 studies, 4 datasets), and the equine piroplasms *T. equi* (5 studies, 7 datasets) and *B. caballi* (3 studies, 4 datasets). Results are graphically represented with forest and funnel plots in Fig. 7. The prevalence and two-sided Clopper-Pearson 95% CIs of pathogens reported in wild zebra from less than two different studies are listed in Tab. 3.

Table 2 – Laboratory strategies used for the detection of viruses, bacteria and protozoa in zebra.

Pathogen species	Diagnostic method	References
Viruses		
African horse sickness virus	MNT	Hamblin et al. (1990); Barnard (1993, 1997); Barnard et al. (1994); Lord et al. (1997)
	SNT	Binepal et al. (1992)
	ELISA	Barnard (1993, 1997); Williams et al. (1993); Becker et al. (2018)
	CI-ELISA	Hamblin et al. (1990)
	AGID	(Barnard, 1993; Lord et al., 1997)
	CI ELISA	(Hamblin et al., 1990)
	Virus isolation in cell cultures	Hamblin et al. (1990); Barnard et al. (1994); Becker et al. (2018)
	RT-PCR	Becker et al. (2018)
Akbane virus	CFT	Barnard (1997)
Asinine herpesvirus	Nested PCR, qPCR and sequencing	Seeber et al. (2019)
Bovine papillomavirus 1	PCR, ISH, and DNA sequencing	Löhr et al. (2005)
	Real-time PCR	van Dyk et al. (2009)
Bovine papillomavirus 1	PCR, ISH, and DNA sequencing	van Dyk et al. (2009)
Crimean-Congo haemorrhagic fever virus	IFAT	Shepherd et al. (1987)
	RPHI	Shepherd et al. (1987)
Equine arteritis virus	SNT	Borchers et al. (2005)
Equine encephalosis virus	ELISA	Williams et al. (1993); Barnard (1997)
	MNT	Barnard and Paweska (1993)
	SNT	Williams et al. (1993)
Equid herpesvirus 1	Peptide-based ELISA	Abdelgawad et al. (2015); Guevara et al. (2018); Seeber et al. (2019)
	ELISA	Barnard (1997); Flanders et al. (2018)
	MNT	Barnard and Paweska (1993)
	SNT	Borchers and Frölich (1997); Borchers et al. (2005); Abdelgawad et al. (2015)
	CFT	Blunden et al. (1998)
	IFAT	Borchers and Frölich (1997)
	IPA	Seeber et al. (2017)
	Virus isolation	Blunden et al. (1998)
	qPCR	Flanders et al. (2018)
	PCR	Seeber et al. (2020)
	pan-herpes PCR and further genotyping	Seeber et al. (2017)
Equid herpesvirus 2	IFAT, SNT	Borchers and Frölich (1997)
	PCR	Ehlers et al. (1999)
Equid herpesvirus 4	IFAT	Borchers and Frölich (1997)
	MNT	Barnard and Paweska (1993)
	SNT	Borchers and Frölich (1997); Borchers et al. (2005)
Equid herpesvirus 5	Nested PCR, qPCR and sequentiation of gB	Seeber et al. (2019)
	PCR	Ehlers et al. (1999)
Equid herpesvirus 7	PCR	Seeber et al. (2019)
	pan-herpes PCR and further genotyping	Seeber et al. (2017)
Equid herpesvirus 9	Peptide-based ELISA	Abdelgawad et al. (2015); Guevara et al. (2018); Seeber et al. (2020)
	ELISA	(Flanders et al., 2018)
	SNT	Borchers et al. (2005, 2008); Abdelgawad et al. (2015)
	PCR and DNA sequencing	Borchers et al. (2008); Schrenzel et al. (2008); Seeber et al. (2020)
	qPCR	Flanders et al. (2018); Moeller et al. (2018)
	Virus isolation	Moeller et al. (2018)
Equid hepatitis B virus	PCR and antibody-based test	Rasche et al. (2021)
Wild ass herpes virus	Nested PCR, qPCR and sequentiation of gB	Seeber et al. (2019)
	pan-herpes PCR and further genotyping	Seeber et al. (2017)
Wesselsbron virus	MNT	Barnard (1997)

MNT = Microneutralization Test; SNT = Serum Neutralization TestPCR = Polymerase Chain Reaction; qPCR = quantitative Polymerase Chain Reaction; ITS = Internal Transcribed Spacer; gB = glycoprotein B; IFAT = Indirect Fluorescent Antibody Test; ELISA = Enzyme-Linked ImmunoSorbent Assay; EP = Equine Piroplasmosis; BIIT = Blood Incubation Infectivity Test; NAT = N.caninum Agglutination Test; IPA = Indirect immunoPeroxidase Assay; RPHI = Reversed Passive Hemagglutination Inhibition; ISH = In-Situ Hybridization; MLVA = Multiple Locus Variable-number tandem repeats Analysis; Anti-PA ELISA= Anti-Protective Antigen ELISA; CI-ELISA = Competitive Indirect ELISA; AGID = Agar Gel ImmunoDiffusion assay; CFT = Complement Fixation Test; CER cells = Chicken Embryo-Related cells; BHK cells = Baby Hamster Kidney cells, MGBTM = Minor Groove Binding TaqMan probes Quantitative analysis (meta-analysis); SAT = Serum Agglutination Test.

Table 2 – *(continued)* Laboratory strategies used for the detection of viruses, bacteria and protozoa in zebra.

Bacteria

Pathogen species	Diagnostic method	References
Anaplasma spp.	CI-ELISA	Ngeranwa et al. (2008)
Bacillus anthracis	Anti-PA ELISA	Cizauskas et al. (2014, 2015)
	Microscopy	Lindeque et al. (1994); Clegg et al. (2007); Muoria et al. (2007); Wafula et al. (2008); Turner et al. (2013); Hassim et al. (2017); Gachohi et al. (2019)
	Bacterial culture	Lindeque et al. (1994); Muoria et al. (2007); Wafula et al. (2008); Turner et al. (2013); Hassim et al. (2017)
	Mice inoculation	Wafula et al. (2008)
	PCR	Wafula et al. (2008)
	Genotyping with 31-marker MLVA	Beyer and Turnbull (2013)
Brucella spp.	SAT	Condy and Vickers (1972)
Clostridium difficile	Microbial culture	Álvarez-Pérez et al. (2014)
Escherichia coli	Bacterial culture and agglutination test	Hamzah et al. (2013)
Spotted fever group *Rickettsia* spp.	PCR	Ndeereh et al. (2017)

Protozoa

Pathogen species	Diagnostic method	References
Babesia caballi	IFAT	Zweygarth et al. (2002)
	MGBTM qPCR	Bhoora et al. (2020)
	Multiplex EP qPCR	Smith et al. (2019)
	Microscopy and in vitro culture	Zweygarth et al. (2002)
Cryptosporidium spp.	Microscopy and further anti-*Cryptosporidium* monoclonal antibody technique	Mtambo et al. (1997)
Klossiella equi	Microscopy	Suedmeyer et al. (2006)
Neospora caninum	NAT	Ferroglio et al. (2003)
Theileria equi	IFAT	Zweygarth et al. (2002)
	Multiplex-EP qPCR	Smith et al. (2019); Bhoora et al. (2020)
	T. equi specific real-time qPCR	Bhoora et al. (2020)
	qPCR	Tirosh-Levy and Rothschild (2020)
	Nested PCR	Hawkins et al. (2015)
	In vitro culture	Zweygarth et al. (2002)
	Microscopy	Zweygarth et al. (2002); Hawkins et al. (2015)
Trypanosoma spp.	ITS PCR	Auty et al. (2012)
	Inoculation into Wistar rats, microscopy and characterized by the standard BIIT	Mulla and Rickman (1988)

MNT = Microneutralization Test; SNT = Serum Neutralization TestPCR = Polymerase Chain Reaction; qPCR = quantitative Polymerase Chain Reaction; ITS = Internal Transcribed Spacer; gB = glycoprotein B; IFAT = Indirect Fluorescent Antibody Test; ELISA = Enzyme-Linked ImmunoSorbent Assay; EP = Equine Piroplasmosis; BIIT = Blood Incubation Infectivity Test; NAT = N.caninum Agglutination Test; IPA = Indirect immunoPeroxidase Assay; RPHI = Reversed Passive Hemagglutination Inhibition; ISH = In-Situ Hybridization; MLVA = Multiple Locus Variable-number tandem repeats Analysis; Anti-PA ELISA= Anti-Protective Antigen ELISA; CI-ELISA = Competitive Indirect ELISA; AGID = Agar Gel ImmunoDiffusion assay; CFT = Complement Fixation Test; CER cells = Chicken Embryo-Related cells; BHK cells = Baby Hamster Kidney cells, MGBTM = Minor Groove Binding TaqMan probes Quantitative analysis (meta-analysis); SAT = Serum Agglutination Test.

Table 3 – Prevalence estimates of the viruses, bacteria and protozoa reported from less than two different studies per diagnostic test group.

Viruses			
Pathogen	**Diagnostic method**	**Prevalence (95% CI)**	**References**
African horse sickness virus	Agent detection test	0.26 (0.12–0.45)	Becker et al. (2018)
Akabane virus	Antibody detection test	0.23 (0.0–0.54)	Barnard (1997)
Bovine Papillomavirus 1	Agent detection test	0.34 (0.00–1.00)	van Dyk et al. (2009)
Bovine Papillomavirus 2	Agent detection test	0.09 (0.00–0.30)	van Dyk et al. (2009)
Crimean-Congo hemorrhagic fever virus	Antibody detection test	0.17 (0.10–0.26)	Shepherd et al. (1987)
Equine arteritis virus	Antibody detection test	0.24 (0.13–0.37)	Borchers et al. (2005)
Equine herpesvirus 2	Antibody detection test	0.41 (0.00–1.00)	Borchers and Frölich (1997); Ehlers et al. (1999)
	Agent detection test	0.30 (0.12–0.54)	Ehlers et al. (1999)
Wesselbron virus	Antibody detection test	0.27 (0.18–0.37)	Barnard (1997)
Bacteria			
Pathogen	**Diagnostic method**	**Prevalence (95% CI)**	**References**
Anaplasma spp.	Antibody detection test	0.73 (0.39–0.94)	Ngeranwa et al. (2008)
Brucella spp.	Antibody detection test	0.24 (0.13–0.38)	Condy and Vickers (1972)
Spotted fever group *Rickettsia* spp.	Agent detection test	0.03 (0.00–0.13)	Ndeereh et al. (2017)
Protozoa			
Pathogen	**Diagnostic method**	**Prevalence (95% CI)**	**References**
Babesia caballi	Antibody detection test	0.20 (0.06–0.44)	Zweygarth et al. (2002)
Cryptosporidium spp.	Agent detection test	0.28 (0.12–0.49)	Mtambo et al. (1997)
Neospora caninum	Antibody detection test	0.71 (0.54–0.84)	Ferroglio et al. (2003)
Theileria equi	Antibody detection test	1.00 (0.83–1.00)	Zweygarth et al. (2002)

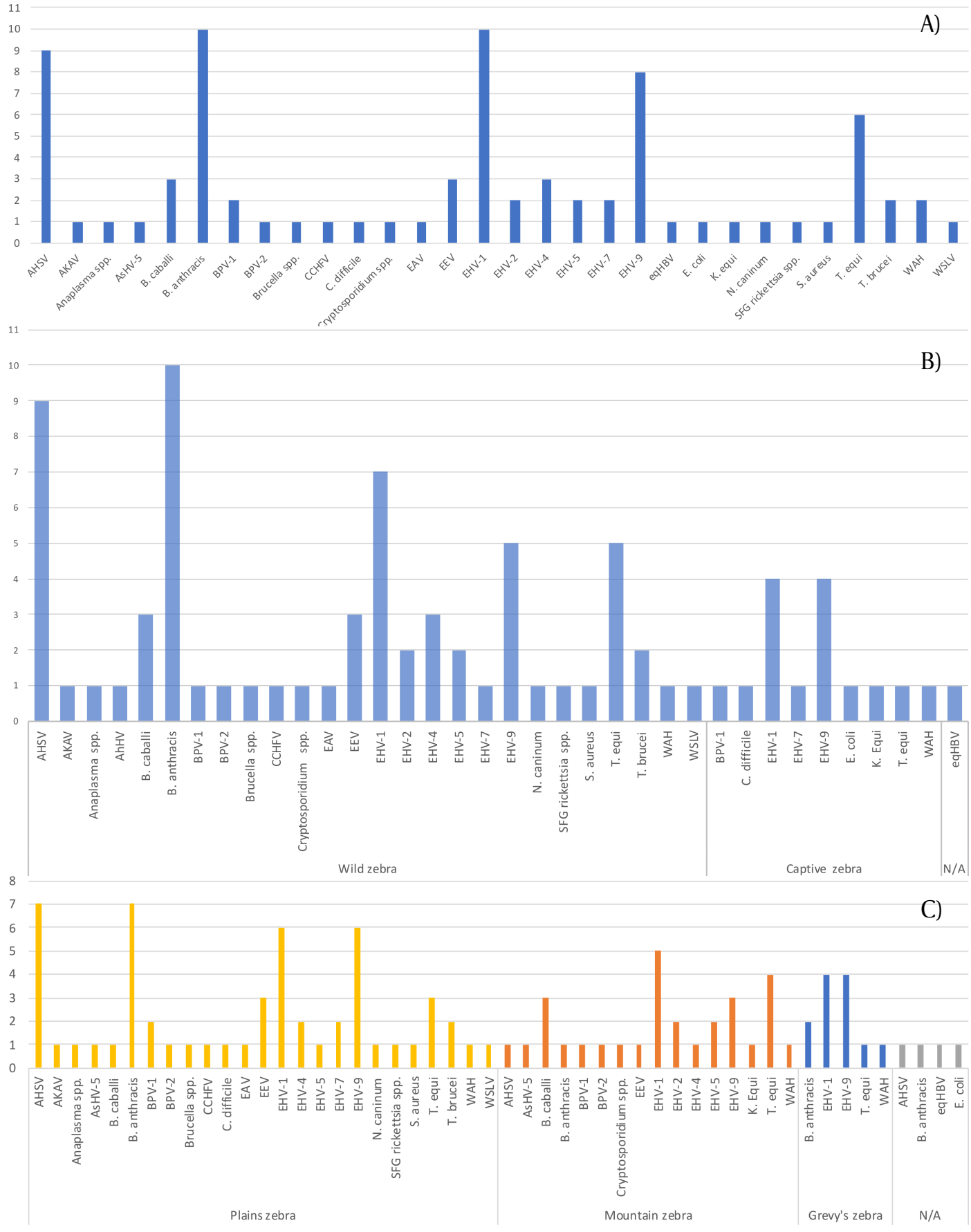


Figure 6 – Frequency distribution of the variable "Pathogen species" (A); frequency distribution of the variable "Pathogen species" stratified per the variables "Zebra origin" (B); frequency distribution of the variable "Pathogen species" stratified per the variable "Zebra species" (C);. N/A = Not Available.

A high level of heterogeneity ($I^2 \geqslant 75\%$; $p<0.01$; $0 \leqslant PI \leqslant 1$) was generally present. This was true except for the EEV analysis, which I^2 value=0%, and p-value=0.48. This result means that the variability in the effect estimate is mainly due to sampling error (chance) rather than heterogeneity (Higgins et al., 2003). Interestingly, the prediction interval (*PI*) for *T. equi*=82–100% and for *B. caballi*=0–52%, meaning that 95% of future values will fall within these intervals.

Studies in the top part of the funnel plots (those with low standard errors) generally cluster together, and not too far away from the pooled effect size. In the lower part of the plot, with increasing standard errors, effect sizes are scattered more heavily to the left and right of the pooled effect. The data points in the funnel plots have a rough symmetrical distribution. This condition is supported by the Eggers' test, which does not indicate the presence of funnel plot asymmetry. However, R warns that Egger's test may lack the statistical power to detect bias when the number of studies is small (i.e., <10). In the funnel plots of AHSV and *B. caballi*, we cannot see data points in the left quadrant that balance the studies on the right quadrant, which means that small studies with low effect size are likely to be missed. This result is indicative of the presence of publication bias, which is statistically not significant but biologically significant. Trim-and-fill method was therefore used to adjust the pooled estimates obtained for AHSV and *B. caballi*. Prevalences resulted then 17% and 6% lower for AHSV and *B. caballi*, respectively (Tab. 4).

Quality assessment of pooled estimates

Considering the overall body of evidence, we can't be highly confident in our pooled estimates (Tab. 4). Our GRADE rating was mainly hampered by high risk of bias, and three over seven pathogens' pooled prevalence estimates have a very low level of evidence. This means that the mean of the distribution of the true prevalences is likely to be substantially different from the estimated prevalences. We only rated the prevalence estimate for *T. equi* as "high", which means that we are really confident that the true effect is similar to our effect estimate. The application of the trim and fill method allowed us to upgrade the AHSV and *B. caballi* estimates of one grade (from low to moderate) because of the resolution of publication bias.

Discussion

The epidemiological relationship among zebra and each of the pathogens for which a good amount of information was available in the literature — i.e. the equine Orbiviruses AHSV and EEV, the equine α-Herpesviruses EHV-1, EHV-4 and EHV-9, the equine piroplasms *T. equi* and *B. caballi*, and *Bacillus anthracis* — is here discussed. The limited literature available on the other pathogens has been discussed in the final section.

Role of zebra for the equine Orbiviruses

Evidence of AHSV infection was reported in free-ranging plains and mountain zebra (Fig. 6C) from 4 African countries i.e. Kenya, Namibia, Tanzania and South Africa. AHSV was isolated in cell cultures, or alternatively detected with RT-PCR. Group-specific antibodies were found using several ab-based techniques (AGID, CF, ELISA tests) and the detection of antibodies against all nine serotypes were reported in most studies using SNT and MNT (Tab. 2). Thus, zebra is susceptible to infection with AHSV, which circulate extensively in all wild zebra species. Indeed, our AHSV seroprevalence estimate in wild zebra populations is 70% (95% CI: 35–96%), even though the underlying true seroprevalence might be markedly different from the estimated seroprevalence due to the presence of publication bias and overall low QoE (Tab. 4). Wild zebra is thus considered a super-infected host which can potentially spread AHSV to non-infected vectors (i.e. biting midges) that remain in contact with and feed on other susceptible equids. The mortality rates in domestic equids is undeniably high (50–95%) and no efficient treatment is currently available (World Organisation for Animal Health, 2017b). For this reason, restrictions on the movement of equids and equid products are currently in place and control measures include stamping out of infected individuals (Council Directive 92/35/EEC, World Organisation for Animal Health, 2019a). Serological surveillance of AHSV in captive and wild zebra is strongly suggested in both disease-free zones in order to confirm the absence of AHSV serotypes and in infected zones to identify changes in the boundary of the zone and the circulating AHSV types. Virological surveillance can then be used to confirm the serological results (World Organisation for Animal Health, 2019b).

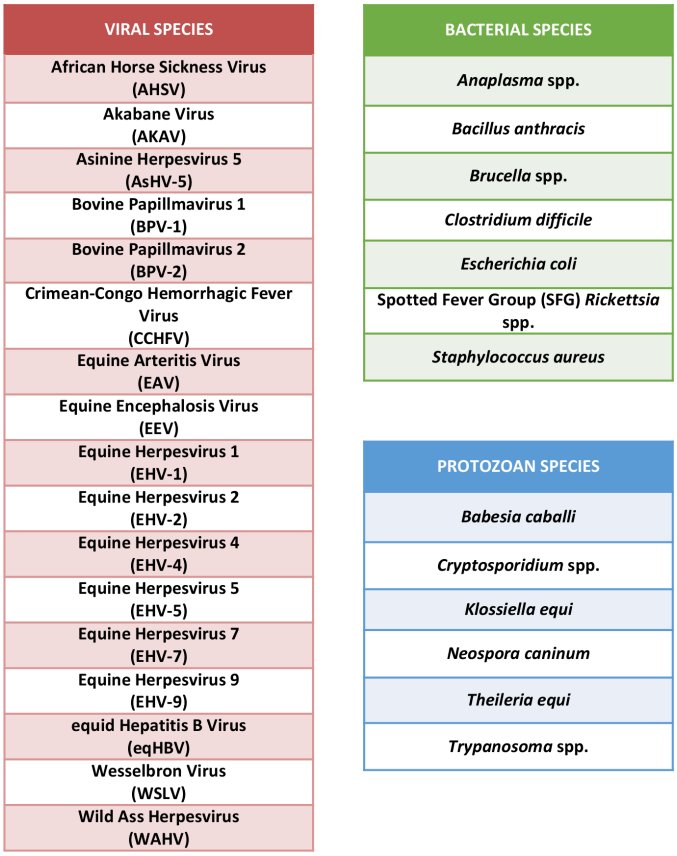


Figure 5 – Infectious agents associated with zebra.

A total of three studies reported EEV antibodies in zebra. All of them were conducted in the same country (South Africa), location (Kruger National Park), sampling period (1991–1996), and zebra species (plains zebra), although Barnard (1997) also investigated infections in Cape mountain zebras from different vegetation zones other than KNP. Meta-analysis indicated homogeneity between studies (I^2=0%), with a pooled antibody tests prevalence estimated to be 21% (95% CI: 8–38%; Fig. 7A). EEV-neutralizing antibodies were evidenced throughout the year against all 7 serotypes suggesting that despite a decrease in *Culicoides* activity, sufficient numbers remain to ensure infection of zebra in KNP (Barnard and Paweska, 1993). To summarise, these studies demonstrated a moderate presence of EEV group and specific antibodies in every season of the year, with medium pooled prevalence among zebra populations, which implies that zebra may have a role in the epidemiology of EEV but with a pattern and vectors different than that of AHSV (Barnard, 1997). Further studies might be considered to corroborate these observations (Williams et al., 1993).

Role of zebra for the equine α-Herpesviruses

Susceptibility of zebra to EHV-1 has been proven in all zebra species, both captive and wild, (Fig. 6B and 6C) with a wide range of diagnostic methods including virus isolation, PCR techniques and ab-based tests (ELISA and SNT) (Tab. 2) and in three different continents (North America, Europe, and Africa). Infection can be acquired through inhalation, either directly or indirectly by contact of the animal host with nasal discharge (main route of infection), aborted foetuses and placenta (World Organisation for Animal Health, 2017a).

Table 4 – GRADE system for rating the quality of evidence of our pooled estimates. QoE: Quality of Evidence level.

Effect	Pooled estimate % (95% CI)	Reasons to downgrade	Reasons to upgrade	Resulting GRADE QoE
AHSV seroprevalence	70 (35–96)	Moderate to high risk of bias Publication bias	Large effect	Low ⊕⊕
AHSV seroprevalence [trimmed & filled]	53 (16–88)	Moderate to high risk of bias	Large effect	Moderate ⊕⊕⊕
Anti-EEV antibodies	21 (8–38)	High risk of bias	None	Very low ⊕
Anti-EHV-1 antibodies	72 (43–93)	Moderate to high risk of bias	Large effect	Moderate ⊕⊕⊕
Anti-EHV-4 antibodies	40 (0–100)	Moderate to high risk of bias Inconsistency Imprecision	Large effect	Very low ⊕
Anti-EHV-9 antibodies	58 (9–98)	High risk of bias Inconsistency Imprecision	Large effect	Very low ⊕
T. equi prevalence	100 (94–100)	None	Large effect	High ⊕⊕⊕⊕
B. caballi prevalence	8 (0–28)	Publication bias	None	Low ⊕⊕
B. caballi prevalence [trimmed & filled]	2 (0–18)	None	None	Moderate ⊕⊕⊕

Moreover, Seeber et al. (2019) suggested that transmission via faeces may occur as equids frequently sniff the feces of conspecifics, and coprophagy is not unusual. EHV-1 host range includes both equid species (horse, donkey, zebra, onager) (Abdelgawad et al., 2015) and non-equid species like Indian tapirs (*Tapir indicus*), black rhinoceros (*Diceros bicornis*) and white rhinoceros (*Ceratotherium simum*) (Borchers et al., 2005). Indeed, transmission of EHV-1 from zebra to polar bears and exotic equids in zoological gardens has been suggested by Flanders et al. (2018), although it remains unclear if the bears became exposed from exotic equids or from other unknown sources. EHV-1 was isolated from tissue samples of a captive Grevy's zebra with severe clinical signs and a post-mortem serum sample had positive EHV-1 CF titres (Blunden et al., 1998). However, antibodies were detected also in healthy wild Grevy's zebras (Guevara et al., 2018). Such results can be justified with the ability of EHV-1 to persist latent in the host, already shown in horses, and the virus can be reactivated as a result of stress or pregnancy causing clinical disease (World Organisation for Animal Health, 2017a). These findings, together with the abundance of EHV-1 antibodies in free-ranging zebra populations (72% with moderate QoE; 95% CI: 43–93%; Fig. 7B), suggest that zebra may act as a source of infection and a super-infected host that may provide spill-over infections to several other animal species, thus amplifying the spread of EHV-1. Extensive use of vaccines has not eliminated EHV-1 infections, and the world-wide annual financial impact from this equine pathogen is immense (World Organisation for Animal Health, 2017a). Thus, further studies are encouraged for clarifying the role of zebra in maintening and spreading EHV-1.

EHV-4 is known to circulate in wild plains and mountain zebra populations (Fig. 6C) from different african countries (South Africa, Namibia, and Tanzania) with a very low evidence of medium-high antibody prevalence (40%; 95% CI: 0–100%; Fig. 7B). Studies focusing on EHV-4 were far less than EHV-1 (3 vs. 10, respectively), and important details that include clinical signs and influencing factors were not reported. However, as an enzootic disease present in most countries where EHV-1 also occurs, causing economic damage to the horse industry worldwide, further studies are needed to clarify the role and impact of zebra in spreading the EHV-4.

EHV-9 is an emerging Alphaherpesvirus that is raising concerns due to its virulence and wide host range, not yet fully understood. Indeed, all zebra species are susceptible to EHV-9, which can become latent in trigeminal ganglia (Borchers et al., 2008) and later reactivate causing mild to serious clinical signs, eventually killing the host (Tab. S4). Both captive and wild zebra were found antibody positive, with wild zebra showing higher prevalences mainly attributed to higher exposure than captive zebra. Pooled antibody prevalence was high (58%; CI: 9–98%; Fig. 7B) in wild plains and Grevy's zebra populations sampled in Kenya and Tanzania, but we are not confident that this estimate represents the true prevalence (Tab. 4). It is worth noting that the peptide-based ELISA technique can detect and distinguish EHV-1 and EHV-9, allowing simultaneous comparison and correlation between the two pathogens (Abdelgawad et al., 2015; Guevara et al., 2018). Interspecies transmission between captive equids (Schrenzel et al., 2008) and several non-equid captive animals, including polar bear (Schrenzel et al., 2008), gazelle and antelopes (Borchers et al., 2008), and white and black rhinoceros (Abdelgawad et al., 2015) have been reported. Additionally, EHV-9 and the associated symptoms caused by the disease has also been detected in experimentally inoculated non-human primates (Yanai et al., 2011), raising concerns as to whether humans are also susceptible to EHV-9.

Role of zebra for the equine piroplasms

Theileria equi is one of the five most reported pathogens detected in all zebra species, both in captivity and in the wild (Fig. 6A, B, C) and in the absence of any clinical signs. Various molecular methods were used for pathogen detection, and the newly developed multiplex EP real-time qPCR allows for simultaneous detection of both *T. equi* and *B. caballi* infections (Bhoora et al., 2020). Across all agent detection tests, the pooled prevalence of *T. equi* was extremely high and precise i.e. 100% (95% CI: 94–100%; Fig. 7C) with no differences observed between zebra species. This result highlights the endemicity of the Apicomplexan pathogen in wild zebra populations living in South Africa and Kenya. In addition, considerably higher levels of genetic diversity were reported to occur in zebra than in horses suggesting that zebra is an ancestral host for this piroplasmid species (Bhoora et al., 2020). To summarise, zebra is to be considered a natural, super-infected and carrier host that may also act as a source of infection of *T. equi* for the tick vectors (i.e. several species of the genera *Hyalomma*, *Dermacentor* and *Rhipicephalus*) (World Organisation for Animal Health, 2019b). Further evidence (Scoles and Ueti, 2015; Spickler, 2018; Smith et al., 2019; Bhoora et al., 2020) suggests that wild zebra and competent tick vectors constitute a maintenance host community for *T.equi* in endemic areas, thus meeting all the requirements to act as a reservoir for other susceptible equid populations. Indeed, translocation of wild zebra has been suggested a potential corridor for the transmission of equine piroplasms to horses (Smith et al., 2019). Such an event has the potential to cause a dangerous outbreak with catastrophic consequences like the 1987 AHSV epidemic in Spain. The horse is indeed a very susceptible host to clinical EP, eventually dying if not treated. An outbreak of this disease in an area previously free of EP may cause the death of numer-

ous valuable horses (either natural or for stamping out) and may require huge eradication efforts and costs.

In contrast to *T. equi*, *B. caballi* infection has been reported in zebra from only 3 studies (Fig. 6A) and with a low pooled agent tests prevalence i.e. 8% (95% CI: 0–28%) with low QoE (Tab. 4). Despite the use of highly sensitive quantitative PCR tests, this outcome is attributable to the considerably low parasitaemia of *B. caballi* infections, estimated to range between 0.1% and 1% in horses. As such, there is no evidence to say that *B. caballi* is endemic in wild zebra populations, nor to assess the role of zebra for the spread of the pathogen to other susceptible zebra or equid hosts.

Role of zebra for Bacillus anthracis

A relatively high number of studies reported *B. anthracis* as the cause of mortality in all wild zebra species. Indeed, zebra mortality was reported in almost every major anthrax outbreak occurring in sub-Saharan Africa, with raw proportions (zebra mortality/total animal mortality) ranging from 1.7% in Malilangwe Wildlife Reserve, Zimbabwe (Clegg et al., 2007), to nearly 45% in Etosha National Park, Namibia (Lindeque et al., 1994). Such differences can be explained both by the absolute numbers of zebra populations (511 individuals in Malilangwe WR as of 2003 vs 13000 individuals in Etosha NP) (Clegg et al., 2007; Zidon et al., 2017), by temporal occurrence (Malilangwe outbreak occurred in 2004 while the mortalities reported from ENP cover 28 years of outbreaks), and, above all, by correlation with environmental factors, especially drought, rainfall and grassland structure. Although all zebra species are highly susceptible to anthrax infections, the disease is not contagious and therefore not transmissible to other hosts. Therefore they can be considered dead-end hosts or rather source of infection under certain conditions i.e. if zebra carcass is opened allowing the spread of spores and if control measures are not applied (Hassim et al., 2017). As such, zebra is in all respects part of the maintenance host community of *B. anthracis*, made up by the interplay of a wide range of vertebrate hosts (both carnivores and herbivores), invertebrate mechanical vectors (horse flies, blowflies, common houseflies, bottle flies, ticks etc.) (Basson et al., 2018; Hugh-Jones and De Vos, 2002; Fasanella et al., 2010) and the environment, especially soil (but also water and vegetation). An exception to this rule may be represented by zebra populations living in endemic systems, that can experience sublethal anthrax infections thus surviving and developing a short-term immunity (Zidon et al., 2017).

Role of zebra for the other infectious agents

One study reported similar seroprevalences (25%; Tab. 3) for Akabane virus (AKAV) and Wesselbron virus (WSLV) in wild plains zebra populations living in South Africa (Barnard, 1997), but no information on clinical disease was provided. Seroprevalence was found to be positively correlated in the vegetation zones with higher average rainfall (i.e. forest vegetation zones) while negatively correlated in the vegetation zones with lower average rainfall (i.e. semi-desert). Therefore, AKAV and WSLV are not widespread throughout South Africa. Relocation of zebra to habitats where they do not naturally occur and to which they may become exposed to viruses that are rare or absent in their original location, should be either be considered carefully or avoided. However, the role of zebra in either transmitting or maintaining these pathogens, is still largely unknown.

Group-specific antibodies to *Anaplasma* were detected with high seroprevalence (73%; CI: 39–94%; Tab. 3) in wild plains zebra from the Machakos area of Kenya, where wildlife populations share grazing land with cattle, sheep and goats (Ngeranwa et al., 2008). The *Anaplasma* species infecting zebra was not investigated, but literature suggests that *A. equi* or *A. phagocytophilum* could be the putative organism causing infection in zebra.

Bovine papillomavirus 1 and 2 (BPV-1 and -2) have been detected in sarcoid tumours of captive plains zebra (Löhr et al., 2005) and of free-ranging Cape mountain zebra, mostly as a mixed infection (van Dyk et al., 2009). Neoplastic lesions were observed in different parts of the body, including upper eyelid, medial aspect of the thigh and abdomen. While examination of sarcoids for BPV has identified viral DNA, to date there has been no visualization of viral particles in sarcoids and infection is thus generally considered non-productive Gaynor et al. (2016). However, the presence of BPV-1 and BPV-2 DNA has been evindenced also in healthy skin of sarcoid affected Cape mountain zebra, suggesting that a productive infection may occur in the epithelium of equids like in bovids. Even more intriguing is the detection of BPV-1 and BPV-2 DNA in the blood of non sarcoid affected Plains zebra, Cape mountain zebra and Hartmann's mountain zebra living in sarcoid-unaffected parks (van Dyk et al., 2009). These pathogens may therefore circulate to a high extent in zebra populations, and genetic factors associated with MHC class II gene and/or immune suppression conditions or immunodeficiency may be the predisposal factors for sarcoid development (Broström et al., 1988; Sundberg et al., 2000; Muñoz et al., 2003). Indeed, concurrent infection with *T. equi*, widespread in zebra populations (see above), or other infectious/parasitic pathogens may decrease the immune status of zebra. Cape mountain zebra populations are also known to be highly inbred thus having low genetic variation and inbreeding depression, which is also conditioned by harsh environmental conditions (Sasidharan, 2006). Finally, the transmission role of invertebrate vectors from affected to unaffected zebra is not to be excluded and should be carefully investigated (Finlay et al., 2009).

Positive antibody titres against *Brucella* spp. were detected using SAT (Tab. 2) in wild plains zebra living in Zimbabwe (Condy and Vickers, 1972), but typing and confirmatory methods were not performed. Other studies failed to detect *Brucella* spp. in zebra in Tanzania, Botswana, Zimbabwe and South Africa (de Vos and van Niekerk, 1969; Masden and Anderson, 1995; Alexander et al., 2012; Assenga et al., 2015a), although very small sample sizes (<20 units) and non-probabilistic sampling strategy were employed in such studies. Therefore, the role of zebra, if any, in the epidemiology of *Brucella* spp. remains largely unknown because of the lack of epidemiological information.

A zoo plains zebra foal was demonstrated to shed toxigenic *Clostridium difficile* ribotype 078 in faecal matter. The isolate was also resistant to metronidazole, an antimicrobial compound commonly used for protozoal infections in many animal species. In the same study, other three zebras resulted ab-positive to *C. difficile* without showing clinical signs (Álvarez-Pérez et al., 2014). Zebra is therefore a susceptible host, but the pathogen was evident only in a zoological context and with low prevalences. Investigating the role of zebra in the transmission of *Clostridium difficile*, especially to humans, can be useful to prevent zoonotic infections.

The Crimean-Congo haemorragic fever virus (CCHFV) is known to circulate in plains zebra living in South Africa and Zimbabwe, for which antibodies were detected from a study using reversed passive hemagglutination inhibition (RPHI) and indirect immunofluorescence (IF) tests (Tab. 2) (Shepherd et al., 1987). The authors observed that seroprevalence (17%; 95% CI: 10–26%; Tab. 3) was positively correlated with the feeding preference of ticks of the genus *Hyalomma*, suggesting that *Hyalomma* spp. are the principal CCHF vectors in the wild. Thus, zebra is a natural host for CCHFV but the role of zebra in the epidemiology of CCHFV is more likely to be minor since *Hyalomma* ticks do not usually feed on equids (Walker, 1991; Walker et al., 2003).

Cryptosporidium spp. oocysts were identified in faecal samples of free-ranging plains zebra living in Mikumi National Park, Tanzania, with a prevalence of 28% (95% CI: 12–49%; Tab. 3) (Mtambo et al., 1997). The isolation of the oocysts from faecal samples does not provide direct evidence of zebra susceptibility to infection but suggests that under natural conditions zebra may be able to shed *Cryptosporidium* oocysts into the environment, where they are able to survive for extended periods (World Organisation for Animal Health, 2018). Further studies are suggested to understand the nature of *Cryptosporidium* infection in zebra and the role of zebra in transmission to other animal species, as well as the role of the underlying environment. Molecular investigations may assist in identifying the species/genotype of *Cryptosporidium* associated with zebra and in understanding if they are

among the those that are potentially zoonotic (mainly *C. pestis* and *C. hominis*) (Šlapeta, 2013). However, the role of wild zebra in zoonotic cryptosporidiosis is most probably negligible, while it may have some interest for the captive ones.

Escherichia coli serotype O157:H7 was isolated from faecal samples of captive zebras in Al Zawraa zoological society of Baghdad, Iraq. Zebra species as well as clinical signs were not specified/reported. However, the study highlighted the importance of the environmental replication of *E. coli* that may cause infection to humans if adequate and preventive sanitary measures are not applied in zoological contexts (Hamzah et al., 2013). Like *Cryptosporidium* spp., zebra might contribute to the spread of *E. coli* in the environment, where replication is able to persist. In addition to accurate information warning the public of risk factors, disease surveillance and rigorous sanitary measures are suggested in zoological gardens.

Infection with the equine Gammaherpesvirus species EHV-2, EHV-5, AHV-5, EHV-7 and WAH has been proven in captive and wild zebra from a large variety of sample types (whole blood samples, serum samples, faecal samples, environmental samples, rectal swabs) and using several diagnostic techniques (pan-herpes PCR, nested PCR, conventional PCR, SNT, IFAT) (Tab. 2). All the infected zebras were clinically healthy (Tab. S4). Zebra might be considered a natural host and carrier for -EHV, but its significance is still unkown.

Neutralizing antibodies against equine arteritis virus (EAV) (seroprevalence=24%, 95% CI: 13–37%; Tab. 3) were detected in plains zebra living in Serengeti National Park, Tanzania (Borchers et al., 2005), demonstrating that zebra is a natural host for this pathogen. However, other studies failed to find any evidence of EAV antibodies in a large number of zebra samples from several countries (South Africa, Kenya, Botswana, Zimbabwe, Germany, Netherlands, Australia, USA and Canada) (Barnard and Paweska, 1993; Barnard, 1997; Paweska et al., 1997). While EAV is widespread in Serengeti NP, the virus is virtually absent in the other zebra populations. EAV may be endemic in the Serengeti National Park because it hosts one of the largest migratory zebra population (Borchers et al., 2005), estimated around 200000 individuals (https://www.serengeti.com/serengeti-animals.php), which may act as a super-abundant host thus providing an environment for persistent transmission and maintenance. This hypothesis may be supported by the limited host range of EAV, which is restricted to equids and a few camelids (alpaca and llama) (World Organisation for Animal Health, 2018) in which the transmission is mainly direct (Timoney and McCollum, 1993), not allowing the persistence of the pathogen in the environment and the spread in multiple hosts.

A distinct hepatitis B virus (HBV) species, namely eqHBV, has just been discovered in zebra using both molecular (PCR) and ab-based tests. Infection with eqHBV was evidenced in plains zebra from Tanzania using a nested PCR, and anti-HBV antibodies were detected in mountain and plains zebra sera originating from almost all sampled countries. Molecular, histopathological, and biochemical analyses revealed that infection patterns of EqHBV resembled those of HBV in humans, including hepatotropism, moderate liver damage, evolutionary stasis, and potential horizontal virus transmission (Rasche et al., 2021). Based on what is known, zebra might be considered a susceptible and natural host, which may possibly develop the disease and transmit the pathogen to other susceptible animal species. Further studies are needed to understand the prevalence and zoonotic potential of the pathogen in wild zebra populations. Parasitism with *Klossiella equi* was considered an incidental finding in zebra. To understand the role, if any, of zebra, further understanding of *K. equi* life cycle is needed. The presence of *Klossiella equi* may be investigated when evaluating renal disease in equids, including zebras (Suedmeyer et al., 2006).

A high seroprevalence (70%; Tab. 3) against *Neospora caninum* was evident in wild plains zebra living in a game ranch in Kenya, suggesting a steady presence of the pathogen in this area. No pathological effects could be found in infected animals, but the existence of a sylvatic cycle of *N. caninum* was highly probable (Ferroglio et al., 2003).

Infection with SFG *Rickettsia* spp. was identified in a wild plains zebra living in Maasai Mara National Reserve, Kenya, using a PCR assay to amplify the intergenic spacer rpmE-tRNAfMet (Ndeereh et al., 2017). However, the disease occured at low prevalence (3%; 95% CI: 0–13%) and no clinical signs were reported. Zebra is therefore a natural host, but it is not known how entrenched the pathogen is in wild zebra populations, and what their role, if any, is in trasmitting *Rickettsia* to other animal species.

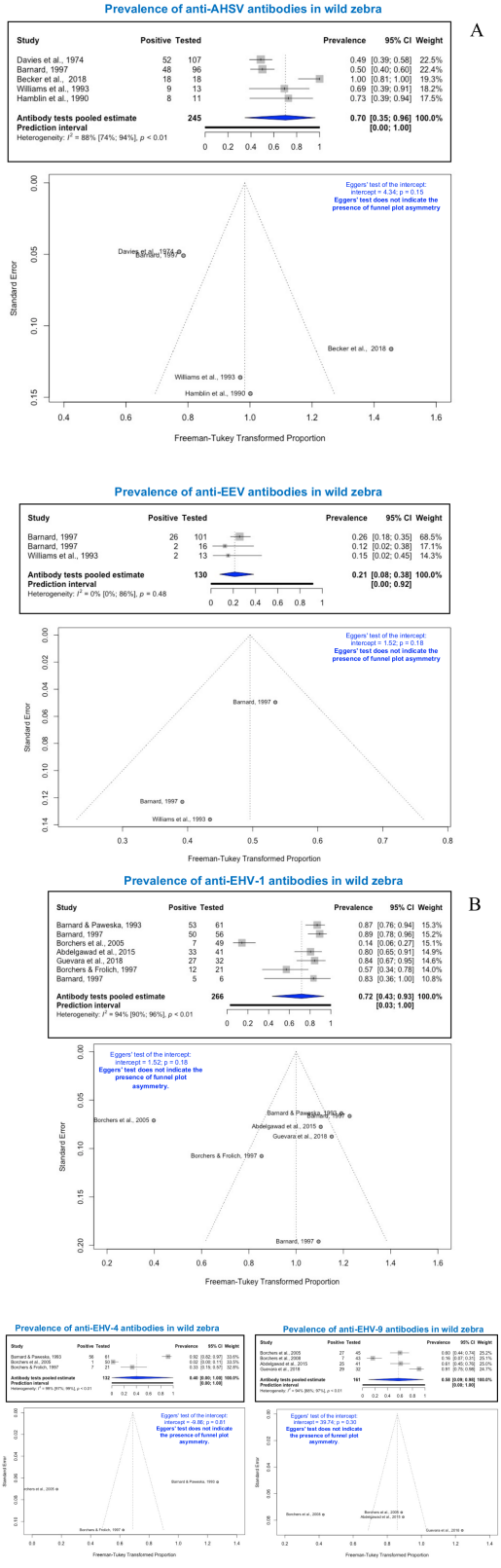
A cutaneous staphylococcal granuloma was confirmed in a wild plains zebra, believed to be initiated from a skin injury caused by a foreign body contaminated with *Staphylococcus aureus* (Pandey et al., 1998). Even if susceptibility of zebra has been discovered, the role of zebra as source of infection is to be considered insignificant because the pathogen is ubiquitous.

Infection with *Trypanosoma brucei rhodesiense* was evidenced from the cerebrospinal fluid of a plains zebra from Luangwa Valley in Zambia, proving that zebra are natural hosts for this pathogen (Mulla and Rickman, 1988) and may be a risk factor for the transmission to humans (Wyatt et al., 1985). However, tsetse flies do not normally feed on zebra (Weitz, 1963) and different studies failed to find any *Trypanosoma* species in zebra (Anderson et al., 2011). Another study identified ITS sequences from a plains zebra that closely matched *T. brucei* brucei (97% sequence similarity) (Auty et al., 2012). The identification in wildlife of *Trypanosoma* species, that are important pathogens of livestock, emphasizes the importance of understanding the transmission dynamics of these parasites. Further epidemiological surveys and extensive phylogenetic analysis of the *Trypanosoma* species identified, would assist in determining the precise role of wildlife in the spread of trypanosomosis.

Conclusions

A wide range of pathogens have been detected in zebra (30 in total). Many of these are listed by the World Organization for Animal Health (OIE: Office International des Epizooties) as notifiable animal diseases. In particular, zebra is most probably a component of the reservoir from which the OIE-listed AHSV, EHV-1 and *T. equi* can be directly or indirectly transmitted to horse populations, causing huge and onerous damages. Moreover, zebra can be naturally infected with zoonotic pathogens like *B. anthracis*, *Brucella* spp., *A. phagocytophylum*, CCHFV and *T. brucei*, providing direct or indirect infection to humans. Finally, zebra is host to pathogens like EHV-9, BPV-1 and BPV-2 which have the potential to affect threatened wild animal species. These observations support our hypothesis that zebra could be an important source of multiple and dangerous diseases for captive and wild animals, and for humans. Therefore, comprehensive studies focused on the prevalence of infectious agents present in zebra populations and the associated risk factors are required.

However, our analyses were limited by low number of studies, small sample sizes, high risk of bias and overall low evidence for our pooled estimates (Tab. 4). Influencing factors that facilitate disease outbreaks and/or circulation were poorly investigated in wild zebra populations. Age and gender factors are likely to be risk factors for different health related problems and should therefore always be reported in observational studies. Environmental variables, which represent the multifaceted ecological niche of a species accordingly to the geographic and biotic contexts, were considered only for the infections with *Bacillus antrhacis* (Muoria et al., 2007; Turner et al., 2013; Cizauskas et al., 2014, 2015; Gachohi et al., 2019). When environmental data are missing, remotely sensed data can be accessed and used in applied epidemiology (Ceccato et al., 2018). We propose the combination of the geographic information systems (GIS) and remote sensing to analyse climatic, environmental and biodiversity factors that influence disease transmission directly and indirectly, as already applied by several authors (Gomes et al., 2010; Atkinson et al., 2012; Bhunia et al., 2012; Machault et al., 2014; Moreno-Madriñán et al., 2014; Skouloudis and Rickerby, 2015; Bermúdez et al., 2016; Steenkamp et al., 2018). The knowledge gained from these analyses is then crucial for evidence-based decision making in animal and public health (Escobar and Craft, 2016; Ceccato et al., 2018). For example, environmental risk factors are useful for understanding disease outbreaks and the formulation of



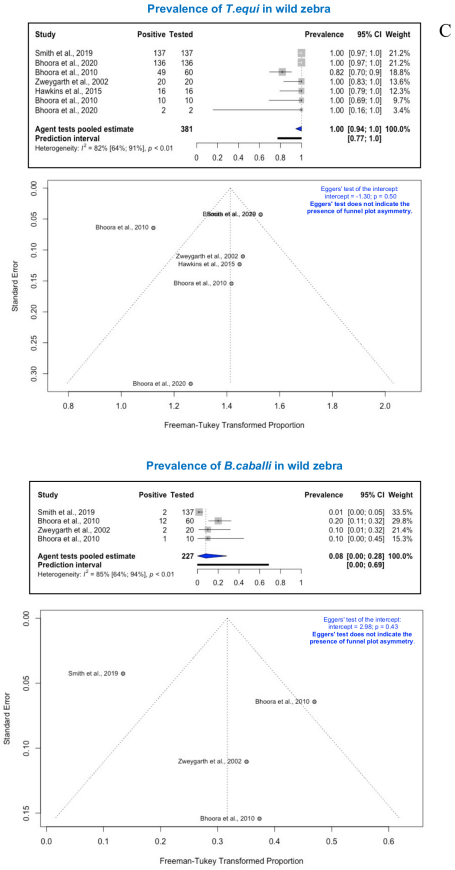


Figure 7 – Meta-analysis results displayed in forest and funnel plots. (A) Seroprevalence of equine Orbiviruses(B) Seroprevalence of equine herpesviruses;(C) Prevalence of equine piroplasms.

control measures prior to moving equids from endemic zones to pathogen free areas (Kirby et al., 2017). Clinical signs and lesions are more difficult to investigate in wild animals than domestic animals. A well-structured and defined sampling frame — as in van Dyk et al. (2009) and Bhoora et al. (2020) — can help to systematically take into account for the risk factors, and to significantly lower the risk of bias. Another limitation may be related to our background question (that is "What pathogens are able to infect zebra?"), since we excluded studies that didn't report the outcome of interest (i.e. the presence of viruses, bacteria or protozoan in zebra samples), either because they were not investigated or because they were investigated but not detected. References excluded for the last reason were a total of 7 (Fig. 2), and they were focused on: *Trypanosoma* spp. (Black et al., 1999; Anderson et al., 2011), Rift Valley Fever virus (Evans et al., 2008; Lwande et al., 2015), *B. anthracis* (Kirby et al., 2011), EAV (Paweska et al., 1997) and *Leptospira* spp. (Assenga et al., 2015b). Negative results as well could have provided interesting information, as we would have known that those pathogens have been searched in zebra species but not found for some reason.

OIE-listed infectious diseases of importance in the equine industry, but for which susceptibility in zebra has not been demonstrated, include: dourine, surra, glanders (infection with *Burkholderia mallei*), strangles (infection with *Streptococcus equi*), leptospirosis (*Leptospira* spp.), equine infectious anaemia (EIA), and Eastern, Western and Venezuelan encephalomyelitis (EEE, WEE, VEE). Should cases of these diseases be reported in zebra, epidemiologic studies are encouraged to clarify the role of zebra in disease epidemiology.

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Supplemental information

Additional Supplemental Information may be found in the online version of this article:

Table S1 PRISMA Checklists

Table S2 Checklists based on Migliavaca et al. (2020)

Table S3 JBI critical appraisal checklists

Table S4 Clinical signs, influencing factors and genotype/serotypes associated with microbial infections in zebra