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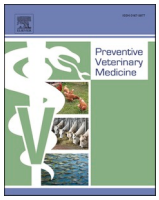

Occurrence and molecular prevalence of *Anaplasmataceae*, *Rickettsiaceae* and *Coxiellaceae* in African wildlife: A systematic review and meta-analysis

C.A. Cossu [a,b,*], R. Cassini [b], R.V. Bhoora [a], M.L. Menandro [b], M.C. Oosthuizen [a], N.E. Collins [a], J. Wentzel [c,d], M. Quan [a], D.M. Fagir [a], H. van Heerden [a]

[a] Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa
[b] Department of Animal Medicine, Production and Health, University of Padova, Legnaro 35020, Italy
[c] Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa
[d] Wildlife Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa

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ABSTRACT

Introduction: Tick-borne pathogens (TBPs) constitute an emerging threat to public and animal health especially in the African continent, where land-use change, and wildlife loss are creating new opportunities for disease transmission. A review of TBPs with a focus on ticks determined the epidemiology of *Rhipicephalus* ticks in heartwater and the affinity of each *Rickettsia* species for different tick genera. We conducted a systematic review and meta-analysis to collect, map and estimate the molecular prevalence of *Anaplasmataceae*, *Rickettsiaceae* and *Coxiellaceae* in African wildlife.

Materials and methods: Relevant scientific articles were retrieved from five databases: PubMed, ScienceDirect, Scopus, Ovid and OAIster. Publications were selected according to pre-determined exclusion criteria and evaluated for risk of bias using the appraisal tool for cross-sectional studies (AXIS). We conducted an initial descriptive analysis followed by a meta-analysis to estimate the molecular prevalence of each pathogen. Subgroup analysis and meta-regression models were employed to unravel associations with disease determinants. Finally, the quality of evidence of every estimate was finally assessed.

Results: Out of 577 retrieved papers, a total of 41 papers were included in the qualitative analysis and 27 in the meta-analysis. We retrieved 21 *Anaplasmataceae* species, six *Rickettsiaceae* species and *Coxiella burnetii*. Meta-analysis was performed for a total of 11 target pathogens. *Anaplasma marginale, Ehrlichia ruminantium* and *Anaplasma centrale* were the most prevalent in African bovids (13.9 %, CI: 0–52.4 %; 20.9 %, CI: 4.1–46.2 %; 13.9 %, CI: 0–68.7 %, respectively). Estimated TBPs prevalences were further stratified per animal order, family, species and sampling country.

Discussion: We discussed the presence of a sylvatic cycle for *A. marginale* and *E. ruminantium* in wild African bovids, the need to investigate *A. phagocytophilum* in African rodents and non-human primates as well as *E. canis* in the tissues of wild carnivores, and a lack of data and characterization of *Rickettsia* species and *C. burnetii*.

Conclusion: Given the lack of epidemiological data on wildlife diseases, the current work can serve as a starting point for future epidemiological and/or experimental studies.

1. Introduction

Emerging diseases relevant to human and animal health are often linked with a wildlife origin as wild animals are considered to be the source of 70 % of zoonoses worldwide (Jones et al., 2008; Rhyan and Spraker, 2010). Several systematic reviews have already highlighted the wide range of pathogens that wild animals may carry without necessarily showing overt clinical signs (Cossu et al., 2022; Bonilla-Aldana et al., 2021; González-Barrio and Ruiz-Fons, 2019; Rahman et al., 2020; Simpson et al., 2021; Thomas et al., 2021; Vieira et al., 2018). Diseases such as tuberculosis (Michel et al., 2006; Thomas et al., 2021; Tschopp et al., 2010), brucellosis (lMadzingira et al., 2021; Ntivuguruzwa et al., 2020; Shirima and Kunda, 2016), rabies (*i.a.* Stuchin et al., 2018), avian influenza virus (Krauss and Webster, 2010), as well as emerging pathogens like Ebola virus (Leroy et al., 2004), Hendra and Nipah viruses (Letko et al., 2020), *Leptospira* spp. (Vieira et al., 2018)

* Corresponding author at: Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa.
E-mail address: ca.cossu@tuks.co.za (C.A. Cossu).

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and *Trypanosoma* spp. (Kasozi et al., 2021), have indeed been associated with a wildlife source and their management imposes serious challenges at the wildlife/human interface (Mogotsi et al., 2016; Bengis et al., 2002; Kurpiers et al., 2016).

Ticks are internationally considered to be the most important pathogen vectors for wildlife and domestic animals (Titcomb et al., 2017; Young et al., 1988). Among the emergent threats, tick-borne pathogens (TBPs) have a great impact on animal and human health throughout the African continent (Jongejan and Uilenberg, 2004; Asante et al., 2019). The epidemiology of ticks and TBPs is complex and multimodal: environmental variables and contact among wildlife, livestock and humans may create favorable conditions eventually driving the spread of TBPs. Consequently, wildlife loss and climate change may increase disease risk (Olivieri et al., 2021).

Anaplasmataceae, *Rickettsiaceae* and *Coxiellaceae* are three major taxa of obligate intracellular bacteria conveyed by blood-sucking arthropods (especially ticks) that were historically grouped in the order Rickettsiales. Due to recent re-classification, the *Coxiellaceae* family has now been moved to the order Legionellales (Dumler et al., 2001). Members of the family *Anaplasmataceae* are frequently reported in African wildlife, especially African buffalo (*Syncerus caffer*) and several antelope species (Brothers et al., 2011; Eygelaar et al., 2015; Henrichs et al., 2016; Khumalo et al., 2016; Penzhorn et al., 2018). *Anaplasma marginale* and *Ehrlichia ruminantium* are known to have a moderate prevalence in African ticks (12.8 %, 95 %CI: 4.1–24.4 %; and 6.4 %, 95 %CI: 4.0–9.2 %, respectively), but the importance of tick vectors for the other *Anaplasmataceae* species is questionable (Cossu et al., 2023). On the other hand, *Rickettsia* species are believed to be mainly human pathogens (Parola et al., 2005) transmitted and maintained by tick and other invertebrate vectors. *Rickettsia* spp. prevalence has been estimated as high as 18.4 % (95 %CI: 14.2–22.9 %) in African tick populations (Cossu et al., 2023). However, *Rickettsia* spp. were detected in non-primate wildlife (Ndeereh et al., 2017; Krücken et al., 2021; Essbauer et al., 2018). *Coxiella burnetii*, which is the main representative of the family *Coxiellaceae* and the causative agent of the zoonotic Q-fever, is also severely under-reported and under-appreciated throughout Africa, where the role of ticks in maintaining the disease has been considered negligible (Cossu et al., 2023). Nevertheless, wildlife (red deer, rabbit, wild boar) has been demonstrated to play an important role in the epidemiology of Q fever in Europe and elsewhere (González-Barrio and Ruiz-Fons, 2019).

On these premises, we conducted a systematic review and meta-analysis aiming to collect good-quality evidence of important and closely related TBPs (*i.e. Anaplasmataceae*, *Rickettsiaceae* and *Coxiellaceae*) in African wildlife, recording their occurrence either as present or past infection and estimating molecular prevalence using methodology that is unbiased and current.

2. Material and methods

2.1. Search strategy

This systematic review and meta-analysis is registered in the international database of prospectively registered systematic reviews (PROSPERO) with the following ID: CRD42021270290. To ensure that this manuscript has all the elements and characteristics required for a systematic review, we populated the PRISMA checklist as well as an additional comprehensive checklist provided by Migliavaca et al. (2020). Both checklists are reported in supporting material Table S1, Table S2 and Table S3. We used the PICO (Population-Intervention-Comparison-Outcome) model to set our objectives, research questions, search strategy and inclusion/exclusion criteria. In particular, our population of interest is African wildlife living in Africa; intervention is laboratory detection tests *i.e.* nucleic acid (molecular) tests, antigen tests, antibody (serological) detection tests or direct identification (*e.g.* microscopy); comparison is the difference among tests of the same test type *e.g.* conventional PCR vs real-time PCR or ELISA vs IFAT; the outcome of interests is the presence or absence of *Anaplasmataceae*, *Rickettsiaceae* and/or *Coxiellaceae*. Consequently, our research questions are: Which species of the target pathogens have been detected in African Wildlife? What is the prevalence of the target pathogens in African wildlife? What role, if any, does the target population play in the epidemiology of pathogens and diseases?

To retrieve such information, we formulated the following search algorithm: "Africa AND wildlife AND (anaplasma OR ehrlichia OR rickettsia OR coxiella)". The algorithm was run in four different electronic databases: ScienceDirect, PubMed, Scopus and Ovid. In PubMed, MeSH terms were searched and entered in the search strategy to retrieve more relevant publications (PubMed algorithm: Africa[MeSH] AND animal,wild[MeSH] AND (anaplasma[MeSH] OR ehrlichia[MeSH] OR rickettsia[MeSH] OR coxiella[MeSH])). An additional database *i.e.* OAIster (https://oaister.on.worldcat.org/discovery), was searched for grey literature. Records were imported into Mendeley, where duplicates were removed and the selection process was applied.

2.2. Selection process

Studies retrieved with our search strategy underwent an initial title and abstract screening, and subsequently a full-text examination. Articles were selected according to the following exclusion criteria: i) Inapplicable study designs such as poster sessions, interviews, abstracts, symposia, oral presentations, reviews and experimental studies; ii) studies conducted outside Africa; iii) non-wildlife target population; iv) inapplicable interventions such as therapeutic rather than diagnostic; v) inapplicable outcomes where the investigated pathogens or microbes differ from the target pathogens. A detailed list of the reasons why studies were excluded during full-text examination is reported in supporting information (List of papers excluded during full-text examination and relevant exclusion criteria). While examining full-text manuscripts, two additional studies (Nakayima et al., 2014; Ramsauer et al., 2007) were included in our analyses. For meta-analysis, only the following inclusion criterion was selected: i) Studies using suitable quantitative molecular tests (no sequencing data; see "Quantitative analyses" paragraph).

2.3. Data extraction and management

Information was gathered for a set of 27 variables, grouped into five categories: details related to publications, specifics about the animals (*e.g.* species, sex, age, clinical signs), details about samples (*e.g.* sampling date, location, sample type), particulars about the laboratory procedures (pathogens investigated, detection technique) and epidemiological data (*e.g.* number tested, number positive, variables correlated). Raw data were then entered and stored into a Google spreadsheet shared among all the authors. Each row of the spreadsheet consisted of a unique set of information for all variables. Raw data were imported into R studio software (version 2022.12.0+353) where it underwent aggregation, manipulation, and transformation according to analysis and variables of interest.

2.4. Critical assessment of included studies

Concurrently with data extraction, a critical assessment of the risk of bias in individual studies was performed using a modified version of the Appraisal Tool for Cross-Sectional Studies (AXIS). This appraisal tool comprises a checklist of 20 questions to be responded to with either "yes", "no" or "don't know". Questions regarding non-responders were not considered because they are not applicable to animal studies. Papers having less than 50 % positive responses were assessed as "high" risk of bias, those with 50–70 % positive responses were assessed as "moderate" and papers with >70 % positive responses were considered to have a "low" risk of bias. Details of critical appraisal are reported in table S4.

2.5. Qualitative and quantitative analyses

A qualitative analysis was initially performed using descriptive statistics. The study/publication unit underwent calculations for frequency distributions of different variables, which were finally visualized in bar and pie charts. A summary table was used to present records of "pathogen species" and "animal species", while map charts illustrated the variable "sampling country" stratified based on the "pathogen species".

Meta-analysis was conducted to estimate the pooled molecular prevalence of each pathogen species in African wildlife. In this context, molecular prevalence is defined as the likelihood that an individual from the target population will yield a positive result in a nucleic acid detection test for a pre-determined pathogen (*e.g. Rickettsia* spp. or *Anaplasma marginale*) at a specific point in time. Molecular sequencing does not provide a ratio of positive cases to the total tested cases, does not focus on a predetermined pathogen and requires interpretation through BLAST (Basic Local Alignment Search Tool) searches and additional phylogenetic analyses. As a consequence, studies using sequencing to characterize/speciate a pathogen, were considered unsuitable for our quantitative analysis of proportions and were then only included in the qualitative analysis. Meta-analysis was not performed for pathogens documented in fewer than four appropriate studies, with this threshold being arbitrarily selected to ensure representativeness of our analysis.

The elements of our meta-analytical approach can be found in the supplementary checklist (Migliavaca et al., 2020), as detailed in supporting information Table S3. A justification for the choice of each component is provided elsewhere (Cossu et al., 2023). Finally, our meta-analysis results were visualized in summary tables and maps. Codes and functions utilized can be retrieved from the first author's GitHub website using the URL: https://github.com/CarlVet/Scientific_papers/blob/main/Meta_analysis_codes.

2.6. Quality assessment of the body of evidence

To ensure appropriate methodologic consistency, we evaluated the quality of evidence (QoE) for our pooled prevalence estimates using the GRADE (Grading of Recommendations Assessment, Development, and Evaluation) guidelines (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. We adopted a semi-quantitative GRADE rating as indicated elsewhere (Cossu et al., 2023). If the final score fell within the interval 0–1 we rated the QoE as "Very low +", 1–2 as "Low ++", 2–3 as "Moderate +++", and 3–4 as "High ++++".

2.7. Reliability

To ensure reliability, each author was randomly allocated an equivalent portion of papers for cross-verifying data extraction and conducting an independent critical evaluation of the included studies. Any discrepancies were discussed and resolved between the authors.

Literate programming and search update

Plots, tables, and results were combined with narrative text in a dynamic R markdown document (Xie et al., 2018), which was later rendered into Word format (using the "officedown" package) to allow the authors to revise the document. The concept referred to as "literate programming" (Knuth, 1984), is based on the idea that a computer program should be documented in a way that is comprehensible to humans. This involves creating a single document that integrates data analysis (executable code) with textual documentation, thereby establishing connections between data, code, and explanations. Any changes applied to the raw data (in the Google Sheet) were then automatically updated in the manuscript. This approach significantly reduces bias in data handling, processing, and writing. Upon completion of the reliability process, the application of the literate programming automatically updated the data in the manuscript when adjustments were made to the original search strategy for articles published between 2021 and 2022.

3. Results

3.1. Qualitative analysis

According to our search strategy and selection process, a total of 41 papers were originally included in the qualitative analysis and 27 in the quantitative analysis (Fig. 1). After the search update, five additional studies (Krücken et al., 2021; Gakuya et al., 2022; Mwale et al., 2023; Makgabo et al., 2023; Broecke et al., 2023) were included in the qualitative analysis, three of which (Krücken et al., 2021; Mwale et al., 2023; Makgabo et al., 2023) were suitable for inclusion in the quantitative analysis.

As a general trend, studies focused on selected bacteria in wildlife increased in the last ten-year (2013–2023) period (26/41 studies; 63 %, Fig. 2), highlighting a substantial increase in interest and research in this topic.

The laboratory analyses were mainly conducted on whole blood samples (27/41 studies; 66 %), and rarely on tissue samples such as bone marrow and spleen. All pathogen occurrences are documented in Table S5 and categorized by animal species (together with their scientific nomenclature), country, detection method and corresponding references.

In African wildlife, a comprehensive assessment revealed the presence and identification of 21 species within the *Anaplasmataceae* family. The most frequently observed species were *Anaplasma marginale* in 10 studies, followed by *Anaplasma centrale* in six studies, *Ehrlichia ruminantium* in five studies, *Anaplasma* sp. (Omatjenne) and *Ehrlichia canis* in four studies (Table S5). The presence of *Anaplasma marginale* has been documented in four African countries: South Africa, Nigeria, Botswana and Zambia (Fig. 3). *Anaplasma marginale* has been identified in 12 animal species, belonging to four different families: *Bovidae* (eight species), *Felidae* (two species), *Hippopotamidae* (one species) and *Equidae* (one species) (Fig. 4; Table S5). *Anaplasma centrale* has been identified in nine animal species. These species belong to four different families: *Bovidae* (six species), *Equidae* (one species), *Suidae* (one species) and *Felidae* (one species) (Fig. 4; Table S5). *Ehrlichia ruminantium* has been identified in four animal species all belonging to the *Bovidae* family (Fig. 4). These findings span across four African countries: South Africa, Botswana, Zimbabwe and Namibia (Fig. 3). *Anaplasma* sp. (Omatjenne) has been identified in four animal species also belonging to the *Bovidae* family (Fig. 4) and its presence has been documented in South Africa and Botswana (Fig. 3). The presence of *Anaplasma centrale* has been documented in South Africa and Botswana (Fig. 3). *Ehrlichia canis* was identified in eight animal species that belong to either the *Felidae* family (four species) or the *Canidae* family (three species) (Fig. 4; Table S5). The presence of *Ehrlichia canis* was reported in Zimbabwe, Nigeria, Kenya (Fig. 3).

In African wildlife, a total of six species from the *Rickettsiaceae* family were detected and identified. The most frequently documented species include *R. prowazekii* and *R. conorii* (in two studies) reported in spiny mouse (*Acomys*) and vervet monkey (*Chlorocebus pygerythrus*as) (Table 1).

Unspecified *Rickettsia* spp. were also detected in 12 animal species *i.e.* chimpanzee (*Pan troglodytes*), western gorilla, bonobo (*Pan paniscus*), yellow baboon (*Papio cynocephalus*), vervet monkey, African buffalo, plains zebra, blue wildebeest, topi (*Damaliscus lunatus jimela*), brown hyena (*Parahyaena brunnea*), spotted hyena, and African wild dog (Table 1) (three *Hominidae* spp., three *Bovidae* spp., two *Cercopithecidae* spp. and two *Hyenidae* spp.; Fig. 4; Table S5) and in six African countries (Cameroon, Democratic Republic of the Congo, Zambia, Kenya, Namibia, South Africa; Fig. 5).

As represented in Table 1, only *C. burnetii* has been detected in

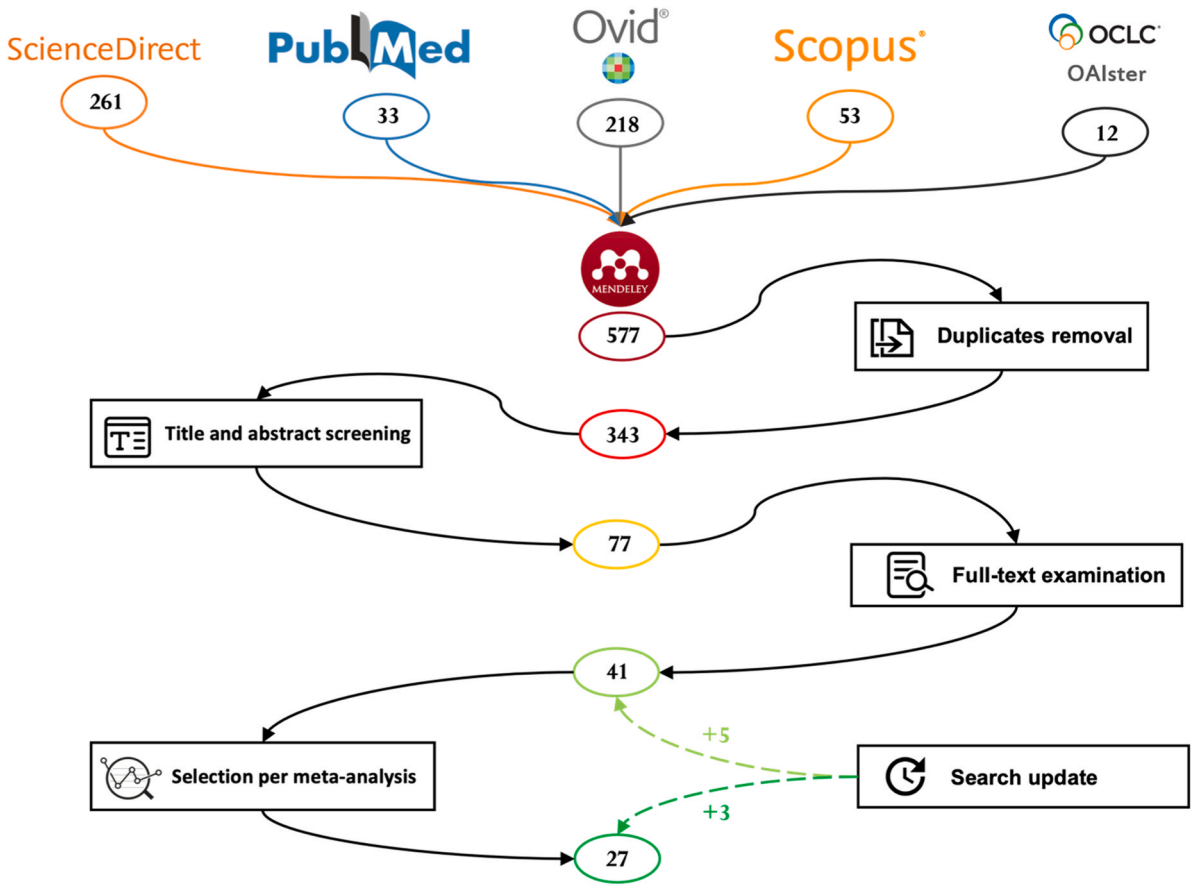

Fig. 1. Flow diagram depicting the steps performed in conducting the systematic search. A total of 577 papers were initially retrieved from ScienceDirect, PubMed, Ovid, Scopus and OAIster. Following the selection process, we included 41 papers in the qualitative analysis and 27 in quantitative analysis.

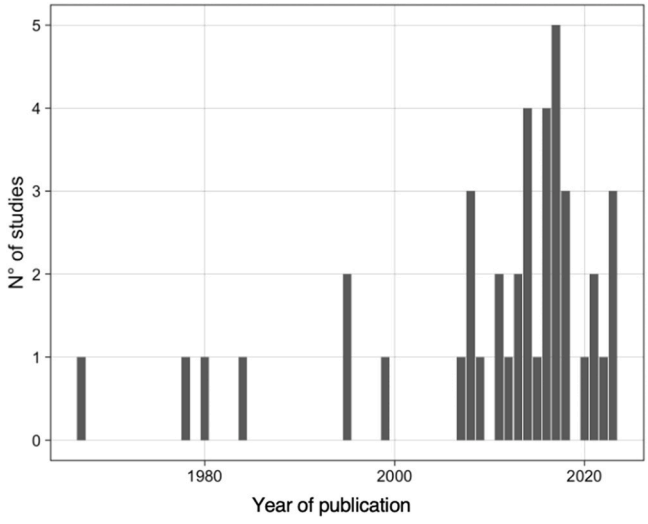

Fig. 2. Studies included in qualitative analysis stratified per publication year. Note that only a few papers were published before 2000.

African wildlife *i.e.* in spiny mouse (*Acomys*), brown rat (*Rattus norvegicus*), black rat (*Rattus rattus*), vervet monkey, African wild dog, African buffalo, eland, impala, waterbuck, giraffe, plains zebra, black rhinoceros (*Diceros bicornis*). These species belong to seven families, *Bovidae* (four species), *Muridae* (three species), *Cercopithecidae, Canidae, Giraffidae, Equidae* and *Rhinocerotidae* (one species each) (Fig. 5) documented in Egypt, Nigeria, South Africa, Kenya (Fig. 6).

3.2. Quantitative analysis

A meta-analysis was performed on 11 target pathogens identified using molecular tests in African wildlife, utilizing data from 27 articles. Pathogens not meeting the inclusion criteria, either due to being investigated in fewer than four studies or not being detected using molecular tests suitable for inclusion in the quantitative analysis, were excluded. Pooled prevalences, confidence and prediction intervals, and heterogeneity indices are displayed in Fig. 7.

Seven pathogens were subjected to subgroup analysis due to high heterogeneity indices ($I^2 > 70$ %). The subgroup analysis highlighted that factors mostly associated with pathogen prevalence were animal species (observed in 6/7 pathogens) and sampling country (observed in 3/7 pathogens) (Table 1).

The molecular prevalence estimates for the target pathogen species across various wildlife species are summarized in Table 2. The quantitative distribution across different African countries is depicted in Fig. 8.

According to the subgroup analysis, we combined the significant variables in multiple meta-regression models. The best-fitting models (*i.e.* the ones accounting for the biggest amount of heterogeneity) are represented in Table 3. The selected moderators had a significant impact on the prevalences of both *E. ruminantium* and *Rickettsia* spp. (*i.e.* test of moderators <0.05), and the respective residual heterogeneity was not significant (*i.e.* test for residual heterogeneity >0.05). Egger's test, revealed that none of the estimates showed significant funnel plot asymmetry, indicating the absence of publication bias.

The QoE for our prevalence estimates are summarized in Table 4. The prevalence of *Ehrlichia canis*, *A. phagocytophilum* and *C. burnetiid* in African wildlife were evaluated as high. This suggests a high level of confidence that the actual effects align closely with estimated effects.

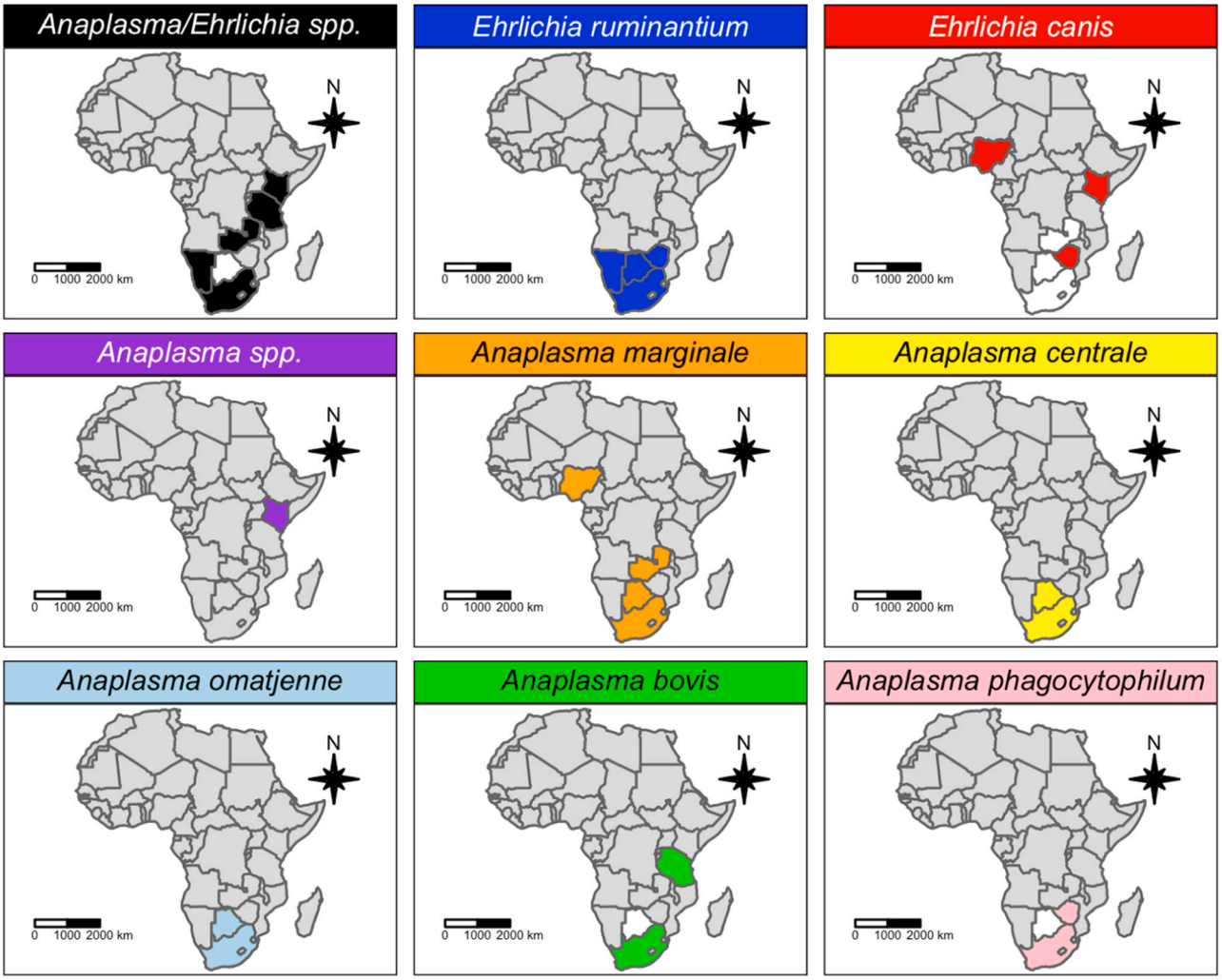

Fig. 3. Geographic distribution of main *Anaplasmataceae* species detected in African wildlife. Countries where the pathogen was investigated but not detected are represented in white, while countries where the pathogen has not been investigated are represented in grey.

However, the pooled effects for the prevalence of *Ehrlichia/Anaplasma* spp. and *Rickettsia* spp. in African wildlife have a low QoE, suggesting that the actual prevalence may be significantly different from the estimated prevalence. Lastly, the prevalence of other pathogens in African wildlife resulted in a moderate QoE, indicating that we can rely on the likelihood that the true effect is closely aligned with the estimated effect.

4. Discussion

Anaplasma and *Ehrlichia* species span eight orders (*Artiodactyla, Perissodactyla, Carnivora, Macroscelidea, Primates, Aves, Rodentia* and *Proboscidea*) and 14 families (*i.e. Bovidae, Giraffidae, Suidae, Macroscelididae, Felidae, Hyaenidae, Canidae, Hippopotamidae, Cercopithecidae, Equidae*, *Spheniscidae, Muridae, Elephantidae* and *Rhinocerotidae*) of wild African animals. The molecular prevalence of *Anaplasmataceae*, detected through genus-specific molecular assays (*i.e.* targeting genera *Anaplasma* and/or *Ehrlichia* spp), is considerable (21.2 %; 95 %CI: 7.6–39.1 %) across various African wildlife taxa (Table 2), thus indicating remarkable adaptability within these animal populations. Comparatively higher prevalences have been recorded in Namibia, Zambia, Malawi and Kenya in contrast to South Africa and Botswana.

Ehrlichia ruminantium, *A. marginale* and *A. centrale* are the primary *Anaplasmataceae* species documented in wild African bovids as they are reported almost exclusively in this animal family. Their prevalences are recorded as 13.9 % (95 %CI: 0–52.4 %) for *E. ruminantium*, 20.9 % (95 %CI: 4.1–46.2 %) for *A. marginale* and 13.9 % (95 %CI: 0–68.7 %) for *A. centrale* (Table 2). These trends are reflected in the behavior of these pathogens in domestic animals: *E. ruminantium* causes heartwater, while the intraerythrocytic *A. marginale* and *A. centrale* cause bovine anaplasmosis. These diseases rank among the most dangerous tick-borne diseases affecting the cattle industry in Africa (De Waal, 2000; Marais, 2010). *Ehrlichia ruminantium* and *A. marginale* are also prevalent in African ticks. Studies indicate that 9.4 % (95 %CI: 5.3–14.3 %) of *Amblyomma hebraeum* may carry *E. ruminantium,* while 59.7 % (95 %CI: 9.1–99.4 %) of *Rhipicephalus microplus* could be infected with *A. marginale.* Additionally, *Amblyomma variegatum* may be infected with both pathogens with prevalence of 7.5 % (95 %CI: 4.4–11.3 %) for *E. ruminantium*, and 19 % (95 %CI: 7.7–33.5 %) for *A. marginale* (Cossu et al., 2023). We can then hypothesize the presence of a sylvatic cycle of *E. ruminantium* and *A. marginale* in African bovids. This potential cycle might exist independently from domestic animal populations, suggesting that these pathogens could be maintained exclusively in certain locations within the wild (Allsopp, 2010). However, the wide confidence intervals suggest that focused studies on specific wildlife species are needed to determine the importance of *E. ruminatium* and *A. centrale* in African wildlife.

Although extensively investigated in even-toed ungulates (artiodactyls), *Anaplasma* sp. (Omatjenne) was detected only in giraffe (20 %; 95 %CI: 0.1–61.5 %), nyala (33 %; 95 %CI: 24–42.6 %), roan antelope (40 %; 95 %CI: 6–81.3 %) and African buffalo (1.3 %; 95 %CI: 0–72 %).

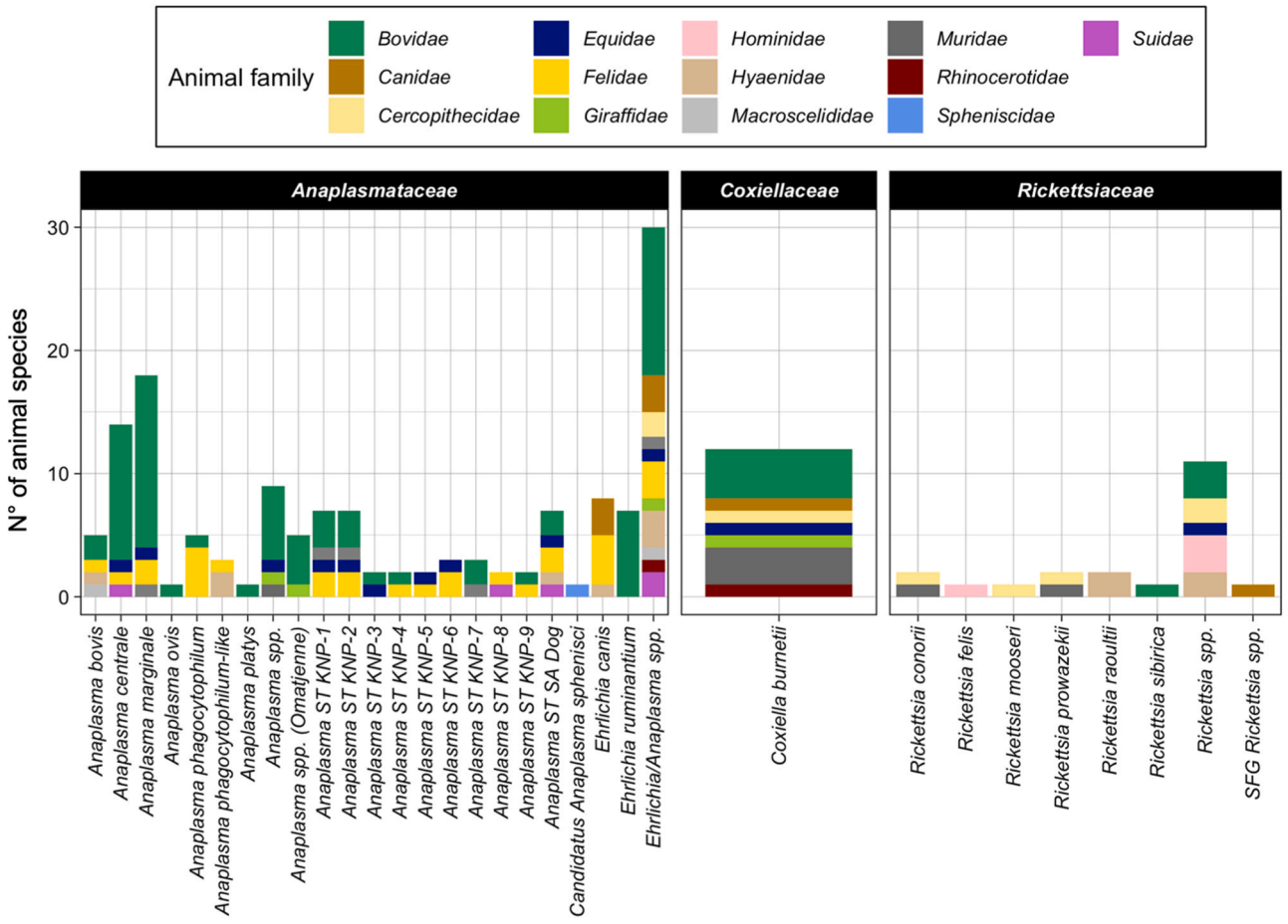

Fig. 4. Number of animal species infected with different pathogen species stratified per animal family.

Table 1
Significance (*p*-values) of different variables on the molecular prevalence of *Anaplasmataceae, Rickettsiaceae* and *Coxiellaceae* in African wildlife according to subgroup analysis. Only the estimates showing high heterogeneity (I^2> 75 %) are shown here. Significant values (p-value ≤ 0.05) are displayed in bold and indicate that variables are correlated with the estimate for a specific pathogen.

	Animal family	Animal species	Habitat status	Sampling country	Sampling period	Molecular test	Risk of bias
Ehrlichia/Anaplasma spp.	**<0.001**	**<0.001**	0.502	**<0.001**	**<0.001**	**0.032**	0.201
Ehrlichia ruminantium	1	**<0.001**	0.222	**<0.001**	0.425	**<0.001**	0.458
Anaplasma marginale	0.11	**<0.001**	1	0.848	0.138	0.848	**0.047**
Anaplasma centrale	0.737	0.391	0.822	0.585	0.364	0.585	0.242
Anaplasma sp. (Omatjenne)	0.666	**<0.001**	0.937	0.96	0.328	0.96	0.297
Anaplasma bovis	1	**<0.001**	0.589	0.587	0.508	0.587	0.587
Rickettsia spp.	**<0.001**	**<0.001**	N/A	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Habitat status: free-ranging vs captive; Sampling period: samples collected before 2002 vs 2002–2011 vs 2012–2022; Risk of bias: evaluated during critical assessment of included studies as "high", "moderate" or "low".

This suggests that nyala and roan antelope may serve as hosts, but further investigation is needed to understand the role of giraffe and African buffalo in the transmission of *Anaplasma* sp. (Omatjenne). This bacterium has been found in cattle across the African continent (Muhanguzi et al., 2010; Teshale et al., 2018). Further research is necessary to understand any potential effects of this infectious agent on the health of wildlife.

Anaplasma phagocytophilum is the most promiscuous *Anaplasma* species as it causes granulocytic anaplasmosis in humans, tick-borne fever in domestic ruminants, equine anaplasmosis in horses, and severe febrile illnesses in dogs and cats (Rar and Golovljova, 2011). The host plasticity of *A. phagocytophilum* has been highlighted also in tick vectors, since it has been detected in 14 African tick species belonging to five genera of Ixodid ticks (*i.e.*, *Amblyomma, Hyalomma, Rhipicephalus, Haemaphysalis* and *Ixodes*) and two of Argasid ticks (*i.e., Argas* and *Ornithodoros*) (Boucheikhchoukh et al., 2018; Hornok et al., 2016; Teshale et al., 2016; Mtshali et al., 2017; Hegab et al., 2022; Qiu et al., 2021). In African wildlife, an organism closely related to *A. phagocytophilum* was detected in four felid species (African wild cat, cheetah, lion and serval; Kelly et al., 2014) using a conventional PCR which detects a 478 bp fragment of the 16 S rRNA gene of *Ehrlichia* spp. and *Anaplasma* spp. and in African buffalo (Henrichs et al., 2016) using a reverse line hybridization assay which uses species-specific probes to distinguish between 16 S rRNA amplicons. It should be noted that sequence identities above 98.70 % are found between 16 S rRNA sequences of known *Anaplasma* species, and some *Anaplasma* 16 S rRNA gene sequences (*e.g.*, *A. platys*, *Anaplasma* sp. Omatjenne, *Anaplasma* sp. Mymensingh and "*Candidatus* Anaplasma camelii") share more than 99.5 % sequence identity (Caudill and Brayton, 2022). The 16 S rRNA sequence is therefore not an ideal target to resolve these organisms to

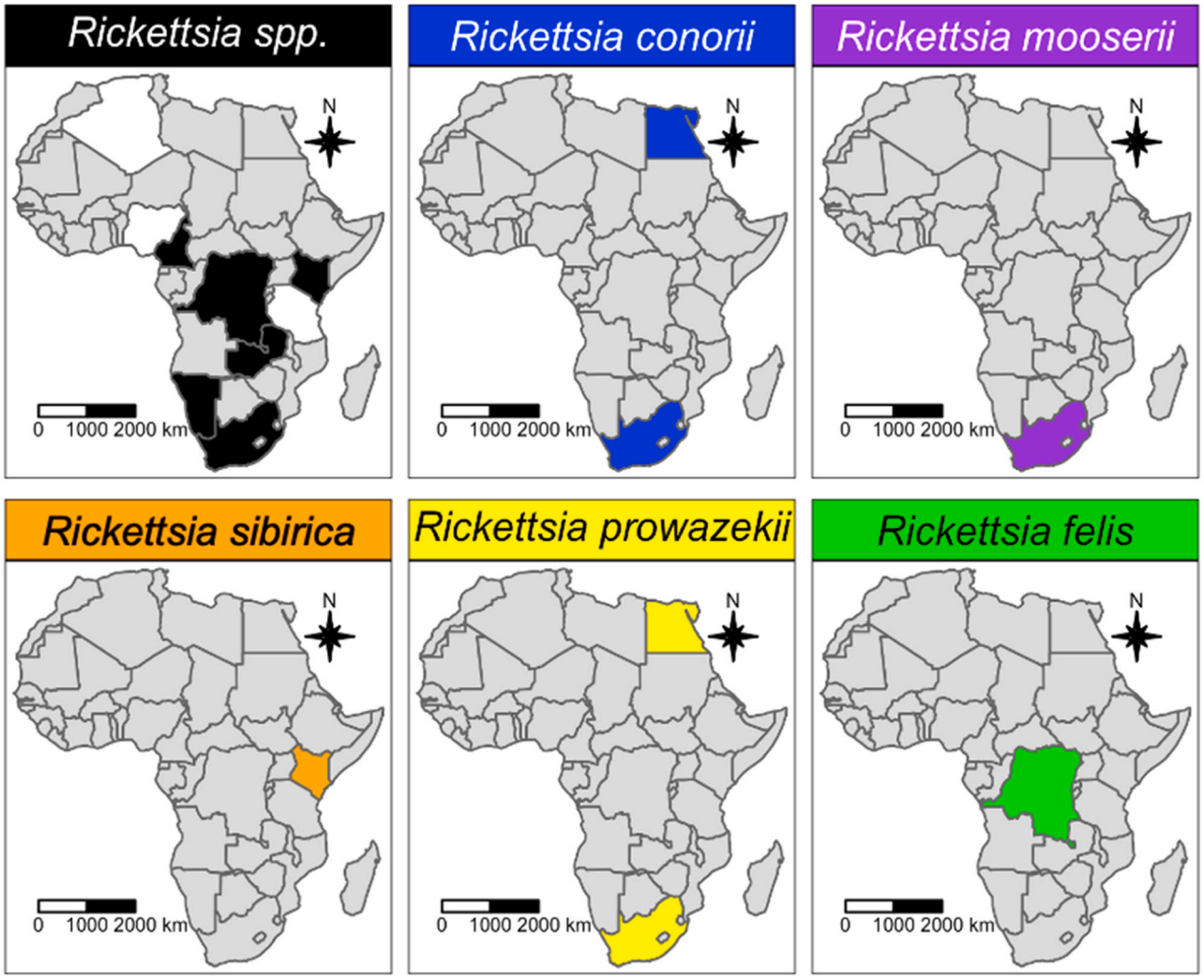

Fig. 5. Geographic distribution of *Rickettsiaceae* species detected in African wildlife. Countries where the pathogen was investigated but not detected are represented in white, while countries where the pathogen has not been investigated are represented in grey.

species level. When it comes to statistics, the prevalence of *A. phagocytophilum* in African ticks (Cossu et al., 2023) and wildlife (Fig. 7) is extremely low, suggesting that *A. phagocytophilum* may be circulating at a very low rate in the populations investigated. Alternatively, as already highlighted for *E. canis,* the species-specific technique used by the studies (*i.e.* the reverse line blot; McDermid et al., 2017; Penzhorn et al., 2018; Pfitzer et al., 2011; Viljoen et al., 2021; Viljoen et al., 2020; Matjila et al., 2008) may not be sensitive enough to detect *A. phagocytophilum* in wildlife blood. Finally, *A. phagocytophilum* has not been investigated in African rodents, which are suspected to act as the natural reservoirs for this bacterium in Europe and United States (*i.a.* Chastagner et al., 2016; Hulínská et al., 2004; Hartelt et al., 2008; Dugat et al., 2015; Stuen et al., 2013). Additionally, there is a lack of investigation in non-human primates, which may pose specific public health concerns and play an important role in understanding and modeling the effects of disease in humans.

Contrary to what the name may suggest, *A. bovis* was also sequenced from non-bovid wildlife (*i.e.* from Eastern rock sengis, leopard and spotted hyena) (Makgabo et al., 2023; Krücken et al., 2021) but attempts to detect it using species-specific techniques (*i.e.* reverse line blot) were unsuccessful in various canid and felid species (McDermid et al., 2017; Penzhorn et al., 2018; Viljoen et al., 2020; Viljoen et al., 2021). *Anaplasma bovis* is known to infect cattle but without causing clinical disease, while *A. marginale* is typically able to cause anaemia, jaundice and can also lead to death infected animals. Nevertheless, *A. bovis* has recently been found to cause disease (fever and thrombocytopenia) in humans in China (Lu et al., 2022). Based on these observations, *A. bovis* appears to have a broad host range, suggesting functional similarities to *A. phagocytophilum.* While the current data suggests limited veterinary importance, the emergent zoonotic potential of *A. bovis* is concerning (Lu et al., 2022). Again, we suggest conducting research in wild rodents and non-human primates to assess the risk of transmission to humans.

E. canis causes canine monocytic ehrlichiosis in domestic dogs (Rar and Golovljova, 2011) and has also been reported in domestic cats (Assis Braga et al., 2014), though its significance in this species is still unclear. As highlighted in the present review, *E. canis* was detected exclusively in wild carnivores (felids, canids and hyaenids) through various methods such as microscopy, serology, or genus-specific PCR, followed by sequencing (Fig. 4, Table S5). Attempts to identify *E.canis* in the same animal species using species-specific molecular assays (*i.e.* reverse line blot and nested PCR; Penzhorn et al., 2018; Pfitzer et al., 2011; Viljoen et al., 2021; Viljoen et al., 2020; Williams et al., 2014) proved unsuccessful. This could be indicative of either low sensitivity/limit of detection (LOD) or a genuinely low probability of detecting the *E. canis* DNA in the blood of wild African carnivores. Since the target cells of *E. canis* are monocytes and macrophages, there is a possibility that this bacterium may persist chronically in organs such as the spleen, bone marrow and liver (Rodríguez-Alarcón et al., 2020). This could

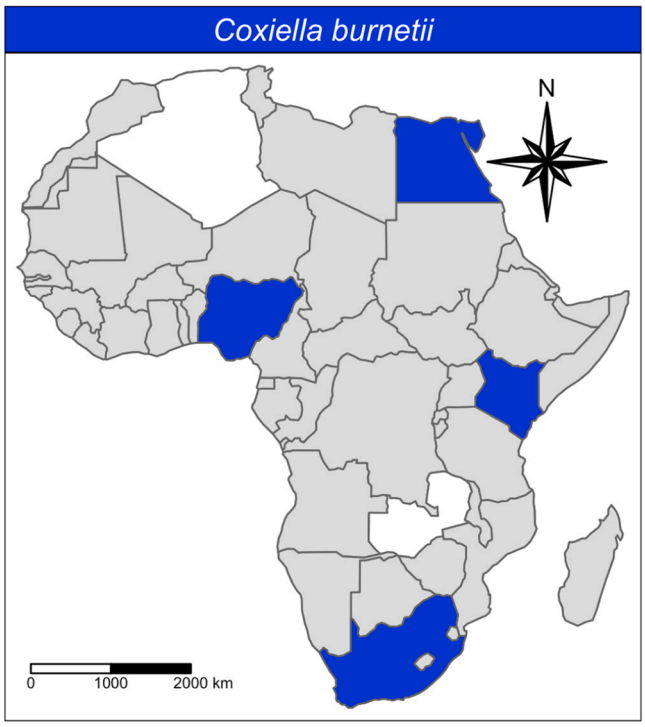

Fig. 6. Geographic distribution of *Coxiellaceae* species detected in African wildlife. Countries where the pathogen was investigated but not detected are represented in white, while countries where the pathogen has not been investigated are represented in grey.

potentially explain the challenges in detecting *E. canis* in the blood of wildlife. To confirm the absence of *E. canis* in wild animals, particularly carnivores, we recommend expanding investigations to include wildlife tissues when available. Additionally, we highlight that the moderate-high prevalence of *Anaplasmataceae* (as detected by genus-specific PCR) in non-bovid wildlife and concurrent low prevalence of Anaplasma/Ehrlichia species occurring in carnivores (*E. canis, A. platys, A. phagocytophilum*) may be attributed to the presence of as-yet-uncharacterized *Anaplasmataceae* species in wild carnivores.

Nine new putative *Anaplasma* species (with 16 S rRNA gene sequences designated *Anaplasma* ST KNP-1–9) and *Anaplasma* ST SA Dog (Inokuma et al., 2005; Vlahakis et al., 2018) have been described by Makgabo et al., (2023) using a third-generation sequencing approach with the Pacific Biosciences' (PacBio) single-molecule real-time (SMRT) sequel II platform (Pacific Biosciences, Menlo Park, CA). This novel technology allows for the real-time and massively parallel sequencing of long DNA fragments, providing a much lower LOD (since the nucleotide sequence of each molecule is analyzed individually) and a greater amount of information compared to the classic Sanger sequencing (*i.a.* Aigrain, 2021). The application of this high-throughput technique represents a rather innovative solution for the discrimination of highly homologous TBP species occurring in African wildlife and therefore should be considered when planning future studies with similar objectives.

Rickettsia species are transmitted by invertebrate vectors (ticks, mites, lice and fleas) (Gillespie et al., 2007), where the pathogen is known to be maintained through efficient multiplication, long-term maintenance, trans-stadial and trans-ovarial transmission, and extensive geographic and ecologic distribution (Azad and Beard, 1998). Animals are not essential in the life cycle of *Rickettsia* spp. and they are not considered to maintain the pathogen in the absence of invertebrate vectors. Usually, direct diagnosis in animals yields negative results. However, since all mammals are infested with ticks, they can be infected with a specific *Rickettsia* sp. hosted by the ticks feeding on them. In our study most of the rickettsias species were detected in the Old World monkeys (*Cercopithecidae*) and in the *Hominidae* family, both belonging to the Primates order. Nevertheless, *Rickettsia* species have also been documented in mammalian families such as *Bovidae* (Topi, wildebeest and buffalo), *Equidae* (plains zebra), *Hyenidae* (brown and spotted hyena) and *Canidae* (African wild dog) (Table S5). Several studies have reported negative results for *Rickettsia* in wild African mammals, with the selected sample sizes consistently being small (<20) (Kamani et al., 2018; Krücken et al., 2021; Leulmi et al., 2016; Ndeereh et al., 2017). We therefore conclude that the occurrence and characterization of species in wild mammals are severely under-investigated and that further research is necessary to clarify the clinical significance of *Rickettsia* in wild animals.

Coxiella burnetii is the only member of the *Coxiellaceae* family that has been investigated in African wildlife. While numerous wildlife species have been tested for *C. burnetii*, with some showing positive results through serology (Table S5; Gakuya et al., 2022; Hoogstraal et al., 1967; Kaschula et al., 1978; Van Heerden et al., 1995), the detection of *C. burnetii* DNA has been limited to brown rat and black rat through PCR and sequencing (Kamani et al., 2018) and at low prevalence. This is in contrast with reports from Europe, where different wild animal species (also African species kept in captivity such as waterbuck and roan antelope) tested positive for the pathogen. In these cases, the animals eventually exhibitied clinical signs (abortion and stillbirth) associated with Q fever (Clemente et al., 2008; Lloyd et al., 2010; Perugini et al., 2009; González-Barrio et al., 2019). With the available data, it is challenging to determine whether this discrepancy is due to different environmental conditions and vector distribution, the type of sample analyzed (blood or tissues), or if the occurrence of Q fever in African wildlife is indeed negligible. Given the extreme environmental resistance of *C. burnetii*, facilitated by its small-cell variant (functionally similar to *Bacillus* and *Clostridium* spores), dust, soil and water may act as the main reservoirs and sources of infection for hosts over considerable distances and time period from the deposition of the pathogen at a given source (Abeykoon et al., 2020).

Our analyses and interpretations come with some limitations. In some cases, potential cross-reactions with the laboratory tests used by the authors might have occurred. This is particularly relevant for serological techniques that targeted a specific pathogen species (*e.g.* the agglutinin test used by Hoogstraal et al., 1967 to identify *R. prowazekii*, *R. conorii* and *C. burnetii*). Serological cross-reactivity among the components of the *Anaplasmataceae*, *Rickettsiaceae* and *Coxiellaceae* families has previously been documented by various authors (*i.a.* Hechemy et al., 1989; Dreher et al., 2005; Waner et al., 1998). Data from pathogen species that were characterized only through sequencing were excluded from meta-analysis to prevent bias in the estimates. Many studies sequenced only a subset of samples that tested positive using a genus-specific technique (*e.g. Anaplasma/Ehrlichia* spp., *Rickettsia* spp. or *Coxiella* spp.-based assays), therefore including only a portion of the analysis could introduce considerable bias into the results. Our quantitative analysis was hampered by the limited number of studies (29), small sample sizes and lack of proper randomization in the sampling strategy. Indeed, random samples and justification of the sample sizes were very rarely considered by the studies included in our work. Only two out of 36 of the studies (6 %) met the criteria for Question n°3 regarding the justification of sample sizes according to the AXIS tool. Additionally, only 3/36 of the studies (8 %) affirmed the presence of randomization as indicated by Question n°6.

5. Conclusion

In the present study, we comprehensively pooled all the epidemiological literature on *Anaplasmataceae, Rickettsiaceae* and *Coxiellaceae* in African wildlife. We highlighted and discussed the main qualitative findings, and we provided reference values for the measure of prevalence. Five tick-borne pathogens circulate in wild herbivores,

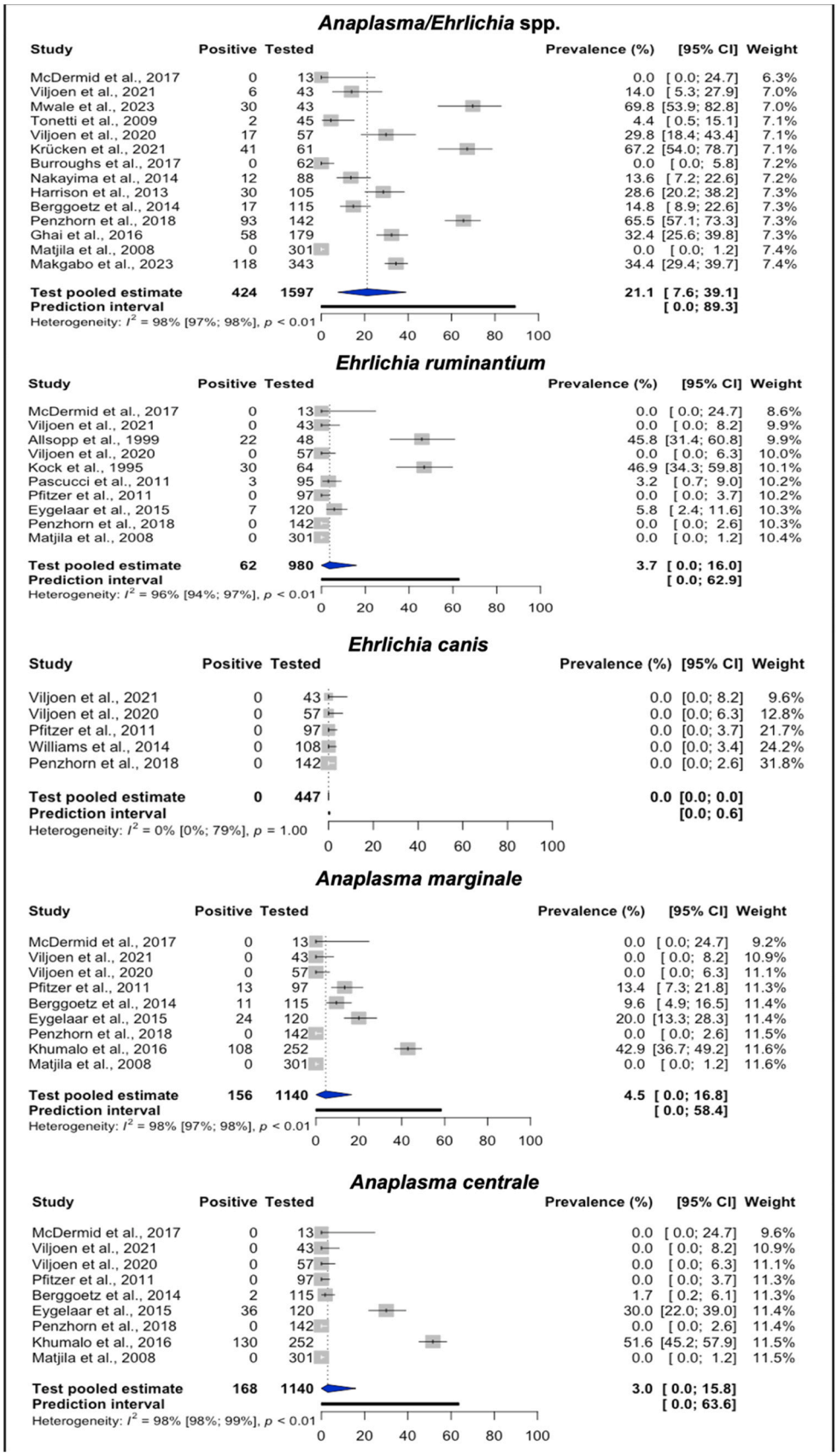

Fig. 7. Forest plots illustrating the results of meta-analysis on the molecular prevalence of *Anaplasmataceae, Rickettsiaceae* and *Coxiellaceae* in African wildlife. The pooled prevalence can be seen as a blue diamond at the bottom of each plot, where the vertices represent the confidence intervals. The weight of each study is calculated basing on the inverse-variance method.

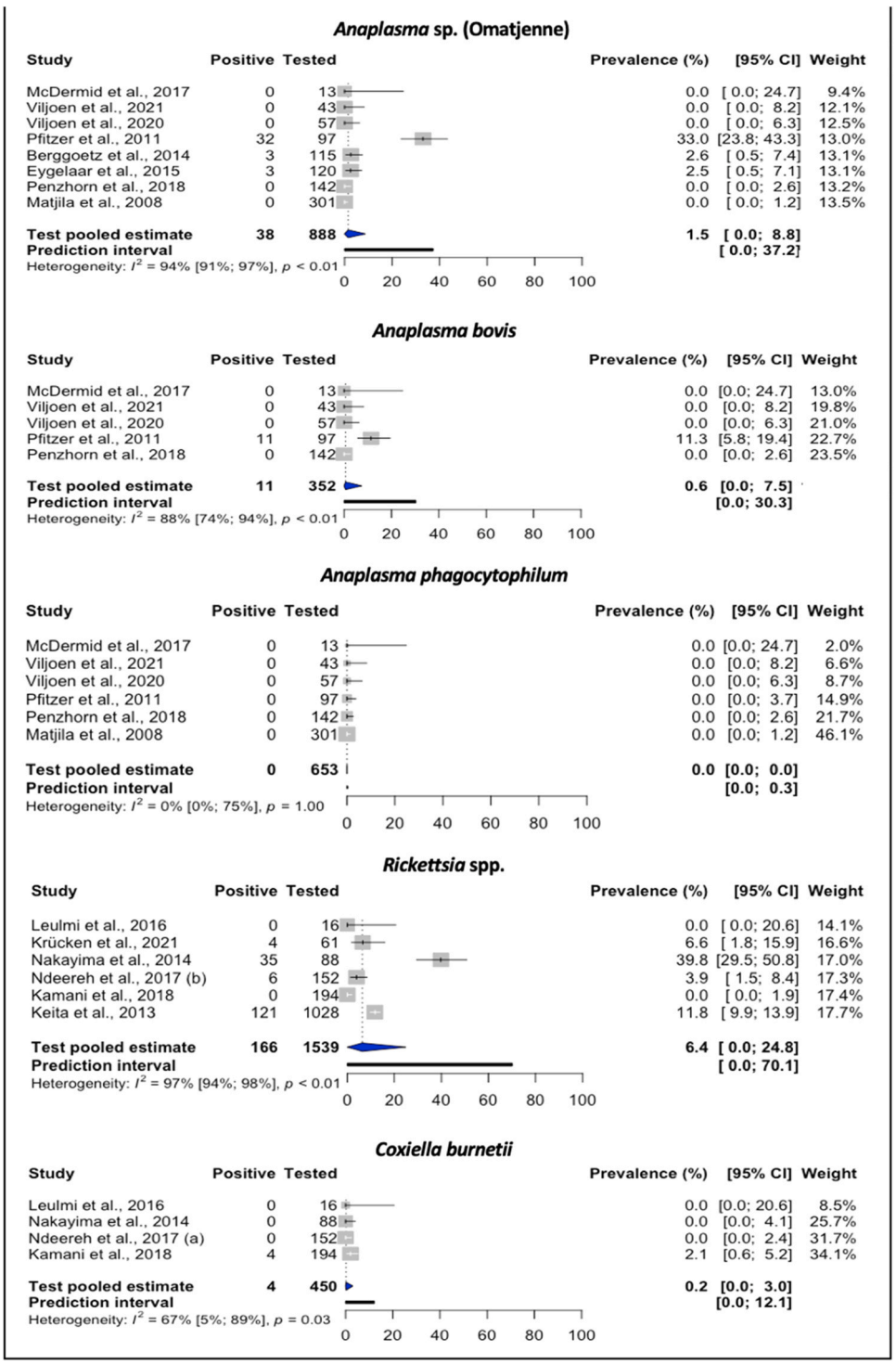

Fig. 7. (*continued*).

particularly bovids, namely *A. marginale, A. centrale, Anaplasma* sp. (Omatjenne), *A.bovis* and *E. ruminantium.* The next step in future research would be to untangle the epidemiological context of these pathogens in African wildlife, with a focus on clarifying their potential role as maintenance hosts. In other animal families, pathogens attributable to the genera *Ehrlichia, Anaplasma*, and *Rickettsia* have been identified, but the species-specific identification remains quite vague. Therefore, the next steps should focus primarily on clarifying which species are circulating in these other wild populations. The epidemiological role can be considered only at a later stage.

Given the lack of standardization and comprehensive data for the topic of interest across the African continent, this systematic review and meta-analysis can serve as a foundational reference for future epidemiological and/or experimental studies.

CRediT authorship contribution statement

C.A. Cossu: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **R. Cassini:** Writing – review

Table 2
Estimated prevalences in different wildlife species.

Animal species	*A. bovis*	*A.centrale*	*A.marginale*	*Anaplasma* sp. (Omatjenne)	*E. ruminantium*	*Ehrlichia/Anaplasma* spp.	*Rickettsia* spp.
African buffalo (*Syncerus caffer*)		**29.8 %** [0–94.7 %]	**40.8 %** [0–98.5 %]	**1.3 %** [0–72 %]	**14.1 %** [0–83.2 %]	**47 %** [0–100 %]	**3.2 %** [0–12.2 %]
African wild dog (*Lycaon pictus*)		**0 %** [0–0.3 %]	**0 %** [0–0.3 %]	**0 %** [0–0.3 %]	**0 %** [0–0.3 %]	**0.5 %** [0–85.6 %]	
Bat							**0 %** [0–9.3 %]
Black rat (*Rattus rattus*)							**0 %** [0–2 %]
Black wildebeest (*Connachaetes gnou*)		**16.4 %** [0–100 %]	**0 %** [0–0 %]	**0 %** [0–5.2 %]		**0 %** [0–0 %]	
Black-backed jackal (*Canis mesomelas*)	**0 %** [0–0 %]	**0 %** [0–0 %]	**0 %** [0–0 %]	**0 %** [0–0 %]	**0 %** [0–0 %]	**38.2 %** [0–100 %]	
Blesbok (*Damaliscus pygargus*)		**0 %** [0–28.7 %]	**0 %** [0–28.7 %]	**0 %** [0–28.7 %]		**0 %** [0–0 %]	
Blue wildebeest (*Connachaetes taurinus*)		**9.2 %** [0–100 %]	**0 %** [0–0 %]	**0 %** [0–18 %]		**0 %** [0–18 %]	**2.9 %** [0–10.8 %]
Bonobo (*Pan paniscus*)							**2.8 %** [1.2–5 %]
Brown hyena (*Parahyaena brunnea*)						**29.1 %** [0–100 %]	**5.9 %** [0–21.5 %]
Brown rat (*Rattus norvegicus*)							**0 %** [0–0.8 %]
Caracal (*Caracal caracal*)	**0 %** [0–1.7 %]	**0 %** [0–1.7 %]	**0 %** [0–1.7 %]	**0 %** [0–1.7 %]	**0 %** [0–1.7 %]	**29.8 %** [18.8–42.2 %]	
Chimpanzee (*Pan troglodytes*)							**12.5 %** [9–16.3 %]
Common eland (*Taurotragus oryx*)		**19.1 %** [0–100 %]	**25 %** [9.3–45.2 %]	**0 %** [0–7.2 %]		**20.9 %** [0–100 %]	
Crested porcupine (*Hystrix cristata*)							**0 %** [0–69 %]
Eastern rock sengis (*Elephantulus myurus*)						**28.6 %** [20.4–37.6 %]	
Egyptian mongoose (*Herpestes ichneumon*)							**0 %** [0–69 %]
Elephant (*Loxodonta africana*)						**16.7 %** [5.7–31.8 %]	
Gambian pouched rat (*Cricetomys gambianus*)							**0 %** [0–40.8 %]
Gemsbok (*Oryx gazella*)		**0 %** [0–69 %]	**0 %** [0–69 %]	**0 %** [0–69 %]		**57.1 %** [0–100 %]	
Giraffe (*Giraffa camelopardalis*)		**0 %** [0–18 %]	**0 %** [0–18 %]	**20 %** [0.1–61.5 %]		**40 %** [6–81.3 %]	
Golden jackal (*Canis aureus*)							**0 %** [0–40.8 %]
Grant's gazelle (*Nanger granti*)						**32.4 %** [25.8–39.4 %]	**0 %** [0–13.1 %]
Greater kudu (*Tragelaphus strepsiceros*)		**0 %** [0–10.3 %]	**33.3 %** [8.1–65.4 %]	**0 %** [0–10.3 %]		**17.2 %** [0–83.6 %]	
Hartebeest (*Alcelaphus buselaphus*)							**0 %** [0–18 %]
House mouse (*Mus musculus*)							**0 %** [0–4.3 %]
Impala (*Aepyceros melampus*)		**14.3 %** [0–47.3 %]	**0 %** [0–13.1 %]	**0 %** [0–13.1 %]	**33.3 %** [0.2–85.6 %]	**32.8 %** [0–100 %]	**0 %** [0–40.8 %]
Leopard (*Panthera pardus*)						**40 %** [23.5–57.8 %]	
Lion (*Panthera leo*)	**0 %** [0–7.2 %]	**0 %** [0–7.2 %]	**0 %** [0–7.2 %]	**0 %** [0–7.2 %]	**0 %** [0–7.2 %]	**5.3 %** [0–100 %]	
Nyala (*Tragelaphus angasii*)	**11.3 %** [5.8–18.4 %]	**0 %** [0–1 %]	**13.4 %** [7.4–20.9 %]	**33 %** [24–42.6 %]	**0 %** [0–1 %]		
Pack rat (*Neotoma* spp.)							**0 %** [0–69 %]
Plains zebra (*Equus quagga*)		**0 %** [0–15.2 %]	**0 %** [0–15.2 %]	**0 %** [0–15.2 %]		**13.2 %** [0–100 %]	**1.7 %** [0–6.4 %]
Red hartebeest (*Alcelaphus buselaphus caama*)		**0 %** [0–6.3 %]	**0 %** [0–6.3 %]	**0 %** [0–6.3 %]		**0 %** [0–0 %]	
Reedbuck (*Redunca* spp.)					**0 %** [0–28.7 %]		
Roan antelope (*Hippotragus equinus*)		**0 %** [0–18 %]	**0 %** [0–18 %]	**40 %** [6–81.3 %]		**28.4 %** [0–100 %]	
Sable antelope (*Hippotragus niger*)		**0 %** [0–40.8 %]	**0 %** [0–40.8 %]	**0 %** [0–40.8 %]		**28.2 %** [0–100 %]	

(*continued on next page*)

Table 2 (*continued*)

Animal species	*A. bovis*	*A.centrale*	*A.marginale*	*Anaplasma* sp. (Omatjenne)	*E. ruminantium*	*Ehrlichia/Anaplasma* spp.	*Rickettsia* spp.
Spotted hyena (*Crocuta crocuta*)						**20.1 %** [0–100 %]	**6.8 %** [1.3–16 %]
Springbok (*Antidorcas marsupialis*)		**0 %** [0–15.2 %]	**0 %** [0–15.2 %]	**0 %** [0–15.2 %]		**0 %** [0–0 %]	
Topi (*Damaliscus lunatus jimela*)							**30 %** [7.1–60.3 %]
Tsessebe (*Damaliscus lunatus lunatus*)					**50 %** [36.6–63.4 %]		
Vervet monkey (*Chlorocebus pygerythrus*)						**17.5 %** [7.5–30.6 %]	**47.5 %** [32.4–62.8 %]
Warthog (*Phacocoerus africanus*)		**0 %** [0–28.7 %]	**0 %** [0–28.7 %]	**0 %** [0–28.7 %]		**42.4 %** [10.4–78.6 %]	
Waterbuck (*Kobus ellipsiprymnus*)		**7.1 %** [0–25.8 %]	**7.1 %** [0–25.8 %]		**50 %** [14.1–85.9 %]		**0 %** [0–40.8 %]
Western gorilla (*Gorilla gorilla*)							**17.2 %** [13.8–20.9 %]
White rhinoceros (*Ceratotherium simum*)						**8.6 %** [1.7–19.9 %]	
Wild boar (*Sus scrofa*)							**0 %** [0–40.8 %]
Yellow baboon (*Papio cynocephalus*)						**10.4 %** [3.5–20.5 %]	**33.3 %** [20.8–47.2 %]

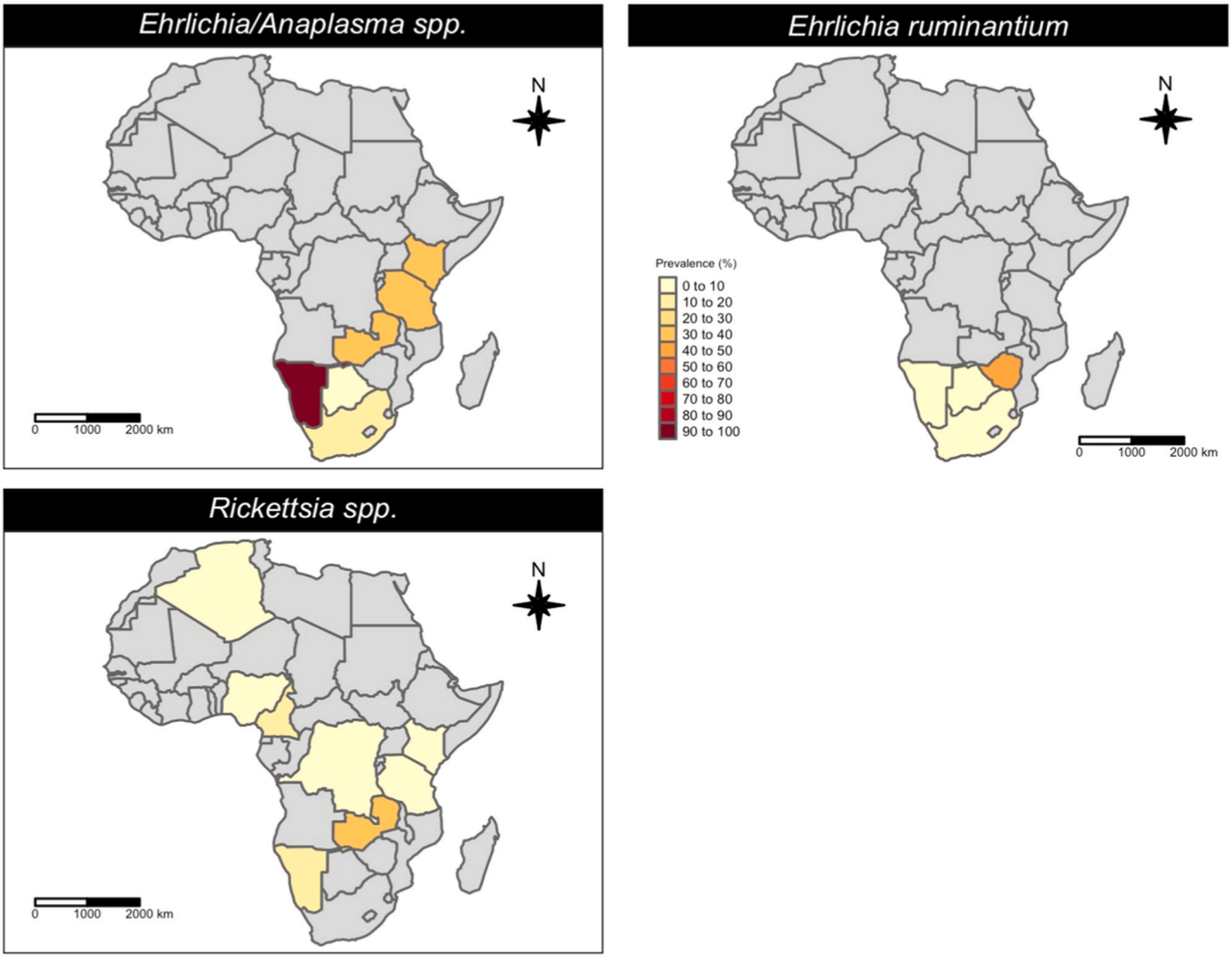

Fig. 8. Choropleth maps showing the molecular prevalence of selected TBPs in African wildlife. Only the estimates showing a significant association with the variable "sampling country" are displayed. Countries where the pathogen has not been investigated with molecular tests suitable for inclusion in the quantitative analysis or not investigated at all, are represented in grey.

Table 3
Meta-regression on the molecular prevalence of *Anaplasmataceae, Rickettsiaceae* and *Coxiellaceae* in African wildlife. Significant p-values (≤ 0.05) are displayed in bold.

Pathogen species	Formula	Residual heterogeneity (I^2), %	Amount of heterogeneity accounted for (R^2), %	Test for residual heterogeneity (p-value)	Test of moderators (p-value)
Ehrlichia/Anaplasma spp.	Animal species + Sampling country	83.46	38.18	**<0.001**	0.104
Ehrlichia ruminantium	Animal species + Sampling country	0	100	1	**0**
Anaplasma marginale	Animal species + Risk of bias	83.8	56.48	**<0.001**	0.142
Rickettsia spp.	Sampling country + Sampling period	61.18	71.24	0.595	**<0.001**

Table 4
Quality of Evidence (QoE) for our prevalence estimates.

Pathogen species	Pooled Estimate (%) [95 % CI]	Reasons to downgrade	Reasons to upgrade	Score	Resulting QoE
Ehrlichia/Anaplasma spp.	21.2 [7.6–39.1]	Risk of bias ~ Low Imprecision Inconsistency Indirectness		1.32/4	**Low** ++
Ehrlichia ruminantium	3.7 [0–16]	Risk of bias ~ Moderate Indirectness		2.33/4	**Moderate** +++
Ehrlichia canis	0 [0]	Risk of bias ~ Low	Large effect	4/4	**High** ++++
Anaplasma marginale	4.5 [0–16.8]	Risk of bias ~ Low Inconsistency		2.66/4	**Moderate** +++
Anaplasma centrale	3 [0–15.8]	Risk of bias ~ Low Inconsistency		2.66/4	**Moderate** +++
Anaplasma bovis	0.6 [0–7.5]	Risk of bias ~ Low Inconsistency		2.66/4	**Moderate** +++
Anaplasma phagocytophilum	0 [0]	Risk of bias ~ Low Indirectness	Large effect	3.33/4	**High** ++++
Anaplasma sp. (Omatjenne)	1.5 [0–8.8]	Risk of bias ~ Low Inconsistency		2.66/4	**Moderate** +++
Rickettsia spp.	6.4 [0–24.8]	Risk of bias ~ Low Imprecision Inconsistency		1.99/4	**Low** ++
Coxiella burnetii	0.2 [0–3]	Risk of bias ~ Low		3.33/4	**High** ++++

& editing, Validation. **R.V. Bhoora:** Writing – review & editing, Validation. **M.L. Menandro:** Writing – review & editing, Validation. **M.C. Oosthuizen:** Writing – review & editing, Validation, Conceptualization. **N.E. Collins:** Writing – review & editing, Validation. **J. Wentzel:** Writing – review & editing Validation. **M. Quan:** Writing – review & editing Validation. **D.M. Fagir:** Writing – review & editing. **H. van Heerden:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prevetmed.2024.106257.

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