The role of wildlife in the epidemiology of *Toxoplasma gondii:* Surveillance and Public Health Implications

El papel de la fauna silvestre en la epidemiología del *Toxoplasma gondii:* vigilancia y repercusiones para la salud pública

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Abstract

Introduction: *Toxoplasma gondii* is a globally distributed zoonotic parasite capable of infecting numerous species of warm-blooded animals. Wildlife plays an important role in its ecology and epidemiology by participating in transmission cycles and reflecting environmental contamination. In Spain, evidence of infection in wildlife has increased, although it remains heterogeneous and poorly integrated into coordinated surveillance systems. This work analyses the role of wildlife in the epidemiology of *T. gondii* in Spain and opportunities to strengthen its surveillance within a One Health approach.

Method: Published evidence on *T. gondii* infection and exposure in Spanish wildlife was considered, together with relevant ecological, epidemiological, environmental, and public health information. The National Wildlife Health Surveillance Framework (PNVSFS*)* was also examined to identify opportunities for integrating parasite surveillance.

Results: *Toxoplasma gondii* is widely distributed among diverse wildlife species and ecosystems in Spain. Different wildlife groups provide complementary information on environmental and trophic exposure. Wild ungulates, carnivores, birds, and selected aquatic species may therefore serve as complementary sentinel groups. The National Wildlife Health Surveillance Plan provides a potential framework for incorporating *T. gondii* surveillance

Conclusions: Wildlife surveillance currently represents an underused component of *T. gondii* epidemiological surveillance in Spain. An integrated, risk-based One Health approach, supported by harmonized protocols, appropriate sentinel species, and coordinated data sharing, could improve understanding of parasite circulation and contribute to the identification and prevention of risks at the wildlife–domestic animal–human interface.

Keywords: Wildlife; zoonoses; *Toxoplasma gondii*; One Health; Surveillance.

Resumen

Introducción: *Toxoplasma gondii* es un parásito zoonósico de distribución mundial capaz de infectar a numerosas especies de animales de sangre caliente. La fauna silvestre desempeña un papel relevante en su ecología y epidemiología, participando en los ciclos de transmisión y reflejando la contaminación ambiental. En España, la evidencia de infección en fauna silvestre ha aumentado, aunque sigue siendo heterogénea y poco integrada en sistemas de vigilancia coordinados. Este trabajo analiza el papel de la fauna silvestre en la epidemiología de *T. gondii* en España y las oportunidades para reforzar su vigilancia desde un enfoque One Health.

Método: Se consideró la evidencia publicada sobre infección y exposición a *T. gondii* en fauna silvestre española, junto con información ecológica, epidemiológica, ambiental y de salud pública relevante. Asimismo, se examinó el marco nacional de vigilancia sanitaria de la fauna silvestre para identificar oportunidades de integración de la vigilancia del parásito.

Resultados: *Toxoplasma gondii* circula ampliamente entre diversas especies y ecosistemas en España. Los distintos grupos de fauna silvestre proporcionan información complementaria sobre la exposición ambiental y trófica. Ungulados silvestres, carnívoros, aves y determinadas especies acuáticas pueden actuar como grupos centinela complementarios. El Plan Nacional de Vigilancia Sanitaria de la Fauna Silvestre (PNVSFS) ofrece un marco potencial para incorporar la vigilancia de *T. gondii.*

Conclusiones: La vigilancia de la fauna silvestre constituye un componente actualmente infrautilizado de la vigilancia epidemiológica de *T. gondii* en España. Un enfoque One Health integrado y basado en el riesgo, con protocolos armonizados, especies centinela adecuadas e intercambio coordinado de datos, podría mejorar el conocimiento sobre la circulación del parásito y contribuir a la identificación y prevención de riesgos en la interfaz fauna silvestre–animales domésticos–ser humano.

Palabras clave: Fauna silvestre; zoonosis; *Toxoplasma gondii*; One Health; Vigilancia.

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INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan with a cosmopolitan distribution and one of the most widespread zoonotic parasites worldwide. It is estimated that approximately one-third of the human population has been exposed to the parasite, although prevalence varies considerably geographically depending on climatic, environmental, cultural, and dietary factors[1-3]. From a public health perspective, *T. gondii* represents an important zoonotic pathogen because, although infection is asymptomatic or mild in most immunocompetent individuals, it can cause severe and potentially life-threatening disease in immunocompromised patients and serious consequences following congenital infection. Infection acquired during pregnancy can result in miscarriage, fetal damage, or congenital toxoplasmosis, with neurological and ocular manifestations that may persist throughout life[4-6]. These different clinical outcomes highlight the relevance of *T. gondii* beyond its high prevalence and support the need for surveillance and prevention strategies addressing the different components involved in its transmission.

The complex life cycle of *T. gondii* contributes to its widespread distribution and involves a large number of animal species[7,8]. Both domestic and wild felids are the only known definitive hosts of *T. gondii*, in which sexual reproduction occurs in the intestinal epithelium and viable oocysts are subsequently shed into the environment through feces[9]. Once sporulated, these oocysts can remain infectious for months in soil and water, facilitating environmental exposure of a wide range of intermediate hosts, including wild mammals and birds, livestock, and humans[10]. Transmission can also occur through the consumption of tissues containing tissue cysts, particularly through the consumption of raw or undercooked meat from infected intermediate hosts, or, less frequently, through transplacental transmission following maternal infection[11,12].

Over the past two decades, increasing attention has been paid to the role of wildlife in the ecology and epidemiology of *T. gondii*, complementing the traditional focus on domestic cats, livestock, and human infection. Numerous studies have demonstrated the circulation of *T. gondii* across a wide diversity of terrestrial and aquatic species, highlighting that its transmission is shaped by a complex interplay among environmental conditions, biodiversity, host communities, human activities, and climate. In this context, wildlife plays a dual role: it contributes to the maintenance of transmission cycles while also serving as a valuable indicator of environmental contamination by oocysts[7,8,10].

Studying *T. gondii* infection in wildlife provides valuable information that may be difficult to obtain through surveillance of domestic animals or human populations alone. The detection of antibodies or the parasite itself in species with different feeding habits, geographic distributions, and trophic levels can help identify spatial patterns of parasite circulation, assess infection pressure across different ecosystems, and evaluate the influence of environmental and anthropogenic factors on transmission. Furthermore, selected wildlife species may serve as sentinels of environmental contamination and pathogen circulation, providing valuable information for assessing changes in transmission dynamics and potential risks to public health and biodiversity conservation[7,8,10,13]. From a One Health perspective, surveillance of *T. gondii* in wildlife is particularly relevant because wildlife can link environmental contamination with domestic animal and human exposure, while also providing information on the health of wildlife populations themselves[7,14]. The expanding interface between wildlife, domestic animals, and human populations, together with changes in land use, climate change, and human activities such as hunting and recreation, may influence transmission patterns and contribute to the emergence of new risk scenarios[7,15,16]. In this context, incorporating wildlife into surveillance systems represents an opportunity to improve our understanding of the epidemiology and ecology of this zoonosis and to strengthen prevention and control strategies[17].

The aim of this work is to review and critically analyse the role of wildlife in the epidemiology of *Toxoplasma gondii*, synthesise the available evidence on parasite circulation, and discuss its implications for epidemiological surveillance within a One Health framework, with particular emphasis on the challenges and opportunities for public health.

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WILDLIFE AND THE EPIDEMIOLOGICAL CYCLE OF *TOXOPLASMA GONDII*

The extraordinary ability of *Toxoplasma gondii* to infect virtually all warm-blooded animals has favoured the establishment of complex transmission cycles involving both domestic and wild species, as well as multiple environmental compartments[7,10,18]. Although felids are the only definitive hosts capable of completing the sexual stage of the life cycle and shedding oocysts into the environment, the persistence and dispersal of the parasite are sustained by a complex network of ecological interactions involving wildlife, domestic animals, and environmental compartments[7,10].

Following their shedding by domestic or wild felids, oocysts sporulate in the environment and may remain viable for extended periods depending on temperature, humidity, and solar radiation. Contamination of soil, surface water, and vegetation represents an important route of environmental exposure for numerous wildlife species[7,10]. Herbivores acquire infection primarily through the ingestion of oocysts present in contaminated pasture, vegetation, soil, or water, whereas carnivores and omnivores may become infected both through environmental exposure to oocysts and through the consumption of prey or carrion containing tissue cysts[7,19]. The diversity of feeding strategies and trophic positions among wildlife facilitates the circulation of *T. gondii* through different levels of the trophic chain, linking environmental and trophic transmission pathways[18,20]. Small mammals, such as rodents and insectivores, represent an important epidemiological link because they are common prey for carnivores and raptors[21]. Similarly, wild ungulates may reflect the intensity of environmental contamination with oocysts, as their herbivorous diet limits other potential sources of infection[18,22]. In turn, large predators and scavengers accumulate exposure along the food chain, making them useful indicators of parasite circulation in the ecosystems they inhabit[7,18,23]. Birds also contribute to the epidemiology of *T. gondii*, although their role varies according to their ecological characteristics, including habitat, feeding habits, trophic position, and migratory behaviour[13,24]. Terrestrial species that feed directly on the ground are at greater risk of exposure to oocysts, whereas raptors may become infected by consuming parasitized prey. Migratory birds have attracted particular attention because of their potential to transport *T. gondii* over long distances along their migratory routes, potentially contributing to the spatial dissemination of parasite strains between geographically distant regions, although the epidemiological significance of this mechanism remains incompletely understood[25,26]. In aquatic ecosystems, the detection of *T. gondii* in marine mammals has provided important insights into the environmental epidemiology of the parasite. Because felids do not inhabit marine environments, the presence of infection in cetaceans, pinnipeds, and sea otters highlights the importance of the transport of oocysts from terrestrial environments to rivers, estuaries, and coastal areas through surface runoff and other hydrological processes. This terrestrial-to-marine transfer makes certain marine species valuable sentinels of environmental contamination and illustrates the close connectivity between terrestrial and aquatic ecosystems[27-29]. The circulation of *T. gondii* in wildlife is influenced by numerous ecological and anthropogenic factors.

Ecological factors include climatic conditions, landscape structure, the density of definitive and intermediate hosts, and predator–prey relationships[7,20]. Humidity and moderate temperatures favour oocyst survival, whereas extreme drought conditions and prolonged exposure to solar radiation can reduce their viability[10]. Likewise, the composition of wildlife communities may alter transmission dynamics by modifying interactions among susceptible species[7,20]. Human activities also have a major influence on the epidemiology of toxoplasmosis. Urban expansion, habitat fragmentation, changes in land use, and increasing populations of domestic and free-ranging cats may promote environmental contamination with oocysts and increase opportunities for contact among wildlife, domestic animals, and humans[7,20]. Similarly, hunting activities and the consumption of inadequately cooked game meat may represent potential routes of *T. gondii* transmission to humans, particularly in regions where hunting and game meat consumption are widespread[30].

From an epidemiological perspective, wildlife should not be regarded solely as a reservoir of infection, but rather as a dynamic component of a complex ecological system in which environmental, biological, and anthropogenic factors converge. Studying wildlife can help identify areas of high parasite circulation, detect changes in transmission patterns associated with environmental transformations, and improve our understanding of the mechanisms determining exposure in domestic animals and human populations. Consequently, integrating information from wildlife surveillance with data obtained from

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domestic animals and humans represents a key component in the development of epidemiological surveillance systems based on the One Health approach. In Spain, the available evidence on *T. gondii* infection in wildlife has increased in recent years, although it remains fragmented and derives from studies conducted in different species, regions, and ecological contexts. This evidence provides an opportunity to comprehensively assess the role of wildlife in parasite circulation and to evaluate how wildlife surveillance could be incorporated into national wildlife health surveillance systems.

THE EPIDEMIOLOGICAL SITUATION OF *TOXOPLASMA GONDII* IN SPANISH WILDLIFE: FROM SCIENTIFIC EVIDENCE TO SURVEILLANCE

Knowledge of *Toxoplasma gondii* circulation in Spanish wildlife has increased considerably over the past two decades[7]. Although early studies focused mainly on game species of public health and economic importance, the development of wildlife health surveillance programmes and the incorporation of molecular diagnostic tools have progressively expanded the number of species investigated and the geographical coverage. Spain has accumulated substantial evidence on the epidemiology of *T. gondii* in wildlife, encompassing a wide range of host speciregions (geographical regions)[7].

This study is a narrative and critical review based primarily on the systematic review conducted by González-Barrio et al. (2024), which was selected as the main evidence base because it provides a recent and comprehensive synthesis of available information on *Toxoplasma gondii* in Spanish wildlife. Rather than conducting a new systematic review, the present work critically evaluates and contextualizes the evidence compiled by González-Barrio et al. (2024), with particular emphasis on its implications for wildlife surveillance and the identification of potential sentinel species within a One Health framework.

The evidence was considered according to the wildlife species and groups investigated, geographical distribution, type of evidence of *T. gondii* exposure or infection, and its potential relevance for surveillance. Particular attention was given to distinguishing evidence of exposure or infection from evidence supporting the use of a species as a surveillance sentinel. The proposed classification of sentinel species therefore represents a risk-based framework derived from the available epidemiological evidence and from ecological and practical considerations, rather than a formal validation of sentinel performance.

This review identified exposure in 96 species of wild vertebrates across most of Spain's biogeographical regions. The findings confirm that *T. gondii* is widely distributed in Spanish ecosystems and circulates among species with markedly different ecological characteristics and feeding strategies. Importantly, exposure varies according to trophic position, with the highest prevalence observed in wild carnivores, followed by birds, ungulates, and lagomorphs, supporting the importance of both environmental and trophic transmission.

Wild ungulates, particularly wild boar (*Sus scrofa*), represent promising sentinel species for environmental exposure to *T. gondii*. Their wide distribution, increasing populations, and frequent interactions with agricultural and livestock systems make them suitable candidates for long-term surveillance. In addition, the large number of animals obtained through hunting provides an opportunity to develop passive surveillance programmes with extensive geographical coverage. Conversely, wild carnivores such as red foxes (*Vulpes vulpes*), Iberian wolves (*Canis lupus signatus*), stone martens (*Martes foina*), and European badgers (*Meles meles*) provide complementary information by integrating parasite exposure across different trophic levels and potentially reflecting changes in prey availability, landscape structure, or the abundance of domestic and free-ranging felids.

The available evidence shows substantial spatial heterogeneity in both the distribution of studies and reported *T. gondii* exposure across Spain. While some regions have been relatively well investigated, important geographical gaps remain, particularly in areas where wildlife, livestock, and human populations overlap. Consequently, the absence of reported infection should not necessarily be interpreted as evidence of parasite absence but may instead reflect gaps in surveillance and research effort[7].

An important contribution of recent research has been the use of predictive models to investigate environmental determinants of *T. gondii* exposure. Climatic conditions, wetland availability, and

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host-community characteristics have been associated with spatial variation in wildlife exposure in Spain[7]. Building on these findings, the integration of wildlife surveillance data with climatic, land-use, livestock, domestic and free-ranging cat population, and hydrological variables could facilitate the development of spatial risk models to identify areas with a higher likelihood of *T. gondii* circulation and prioritize surveillance and public health interventions. Previous studies have demonstrated that climatic and landscape variables can influence *T. gondii* infection in wildlife, while spatial models incorporating cat distribution, land use and environmental characteristics have been used to predict areas of increased environmental contamination[31]. Furthermore, hydrological and land-use models have shown the potential to identify pathways of oocyst transport from terrestrial environments to aquatic ecosystems and to link predicted environmental contamination with infection in wildlife sentinels[32].

Nevertheless, several methodological limitations currently hinder the establishment of standardized surveillance programmes. A substantial proportion of the available studies are cross-sectional and are based on samples taken on an *ad hoc* basis from hunted animals, carcasses, or wildlife admitted to rehabilitation centres. Considerable heterogeneity also exists in diagnostic methods, serological cut-offs, and the tissues examined using molecular techniques, making direct comparisons between studies and the assessment of temporal trends difficult[7,33].

Future surveillance should therefore focus on harmonized protocols, including standardized diagnostic methods, the selection of appropriate sentinel species, representative sampling strategies, and the development of coordinated national databases. Harmonization and standardization are recognized as key challenges in wildlife health surveillance, particularly to improve comparability of data across regions and surveillance programs[34]. Similarly, recent evaluations of *T. gondii* diagnostic methods have highlighted substantial methodological heterogeneity and the need for improved validation and inter-laboratory comparability[35].

POTENTIAL SENTINEL SPECIES AND RISK-BASED SURVEILLANCE

One of the main challenges in developing wildlife surveillance for *Toxoplasma gondii* is identifying species that can provide informative epidemiological data. No single wildlife species is likely to provide a comprehensive picture of parasite circulation; rather, different wildlife groups may provide complementary information on distinct components of the transmission cycle. Sentinel species should therefore be selected according to their ecological characteristics, trophic position, geographical distribution, population abundance, sampling feasibility, and relevance to potential transmission pathways[36]. Based on the evidence synthesized by González-Barrio et al. (2024) and the existing wildlife health surveillance framework in Spain, we propose a tiered approach for the surveillance of *T. gondii* in wildlife (Table 1). Importantly, evidence of exposure or infection in a given species should not be interpreted as evidence that the species has been validated as a surveillance sentinel. Instead, the proposed classification considers the epidemiological evidence together with ecological characteristics, geographical distribution, sampling feasibility, and the potential of each species to provide complementary information on environmental or trophic exposure to *T. gondii*. Thus, the species included in Table 1 should be regarded as potential sentinel species within a proposed risk-based surveillance framework, rather than as formally validated indicators.

Several of these groups are already included in the National Wildlife Health Surveillance Plan (PNVSFS)[37] coordinated by the Spanish Ministry of Agriculture, Fisheries and Food (MAPA). The current PNVSFS includes, among others, wild boar, cervids, wild bovids, carnivores such as foxes, wolves and badgers, lagomorphs, and several groups of wild birds.

This existing framework provides an important opportunity to incorporate *T. gondii* surveillance into established wildlife health and sampling activities rather than creating an entirely separate surveillance system. In particular, wild boar, cervids, and red foxes could constitute a core group of sentinel species with the moderate to high seroprevalence rates recorded in these species: 59.6% in red foxes, 23.8% in wild boar and 14.7% in red deer, combining broad distribution, ecological relevance, and relatively good opportunities for sample collection. Other species could be incorporated according to regional ecological characteristics, specific transmission pathways, or particular conservation and public health priorities.

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Table 1. Proposed classification of wildlife species according to their potential value for *Toxoplasma gondii* surveillance in Spain.

Surveillance level	Species/groups	Potential epidemiological value for Toxoplasma gondii	Potential sampling framework
Core sentinel species	Wild boar (*Sus scrofa*)	Indicator of environmental exposure to oocysts; wide distribution and high availability of samples; direct relevance to game-meat safety.	Hunting activities and existing wildlife health surveillance programmes.
Core sentinel species	Red deer (*Cervus elaphus*) and other cervids	Complementary indicator of environmental exposure across different habitats and landscapes.	Hunting-derived samples and wildlife health surveillance.
Core sentinel species	Red fox (*Vulpes vulpes*)	Integrates exposure across the trophic network and may reflect transmission through both environmental and trophic pathways.	Passive surveillance and opportunistic sampling.
Complementary sentinel species	Other carnivores, including wolf (*Canis lupus*) and badger (*Meles meles*)	Provide information on exposure at higher trophic levels and across different ecological niches.	Passive and targeted surveillance.
Complementary sentinel species	Lagomorphs, particularly Iberian hare (*Lepus granatensis*) and rabbit (*Oryctolagus cuniculus*)	Provide information on environmental exposure in herbivorous species and represent an important prey resource for carnivores.	Hunting-derived and passive surveillance.
Complementary sentinel species	Selected terrestrial and raptor species	Reflect environmental and trophic exposure, depending on feeding ecology and habitat use.	Targeted sampling according to ecological and regional risk.
Complementary/targeted species	Aquatic and migratory birds	Potential indicators of terrestrial-to-aquatic transport and contamination of wetlands and river basins.	Targeted surveillance in wetlands, estuaries, and areas receiving terrestrial runoff.
Targeted/opportunistic surveillance	Protected carnivores, e.g. Iberian lynx (*Lynx pardinus*)	May provide information on exposure in high trophic-level predators while linking parasite surveillance with conservation health.	Opportunistic sampling within conservation and wildlife health programmes.
Targeted/opportunistic surveillance	Marine mammals	Potential sentinels of terrestrial-to-marine transport and contamination of coastal ecosystems.	Stranding networks and existing marine mammal health programmes.

Note: Classification reflects a proposed risk-based surveillance framework and does not imply formal validation of sentinel performance. Evidence of *T. gondii* exposure was considered together with ecological relevance, geographical distribution, sampling feasibility, and potential relevance to transmission pathways.

Importantly, *T. gondii* is not currently a core target of the PNVSFS. Its incorporation should therefore be considered as a potential extension of the existing surveillance framework rather than as an existing component of the national programme. The current plan nevertheless demonstrates that a structured national framework for wildlife health surveillance and passive and targeted sampling is already in place, providing a potentially valuable infrastructure for future *T. gondii* monitoring.

From an operational perspective, a risk-based and tiered surveillance strategy would allow resources to be concentrated on species and geographical areas where surveillance is most likely to provide epidemiologically relevant information. Such an approach would also facilitate the integration of *T. gondii* monitoring with existing wildlife health, veterinary, food-safety, and public-health surveillance systems, consistent with a One Health framework. In this context, wildlife surveillance could

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help identify spatial and ecological patterns of *T. gondii* exposure and highlight areas or transmission interfaces that may warrant further investigation. This information could support the prioritization of surveillance in areas where wildlife, livestock, companion animals, and humans frequently interact, including hunting areas, game-meat production and consumption systems, agricultural landscapes, and aquatic or wetland environments.

However, detection of *T. gondii* in wildlife should be interpreted as evidence of environmental or trophic exposure rather than as direct evidence of human infection risk. When integrated with animal health, environmental, food-safety, and human surveillance data, wildlife monitoring could contribute to identifying priority areas for further investigation and to informing targeted prevention and risk-communication strategies.

CONCLUSIONS AND FUTURE PERSPECTIVES: TOWARDS AN INTEGRATED ONE HEALTH SURVEILLANCE SYSTEM

Surveillance of *Toxoplasma gondii* is still largely conducted through separate systems covering wildlife, domestic animals, food safety, environmental contamination, and human health. Greater integration of these sources would improve the identification of transmission pathways and help assess how environmental and anthropogenic factors shape infection risk. In Spain, existing wildlife health surveillance programmes, veterinary services, reference laboratories, and public health networks provide a valuable basis for developing such an integrated approach, although stable mechanisms for data sharing and harmonized surveillance protocols are still needed.

Environmental surveillance should also be incorporated into this framework. Combining information from wildlife with data on surface waters, sediments, wetlands, land use, climate, and feline populations could improve the identification of areas at increased risk and support spatially targeted surveillance. Advances in molecular detection methods and predictive modelling may further strengthen this approach by allowing environmental contamination and changes in parasite circulation to be monitored more effectively.

Game species, particularly wild boar, represent an additional component of relevance for both wildlife surveillance and food safety. Monitoring hunted animals can provide information on parasite circulation while contributing to the assessment of potential foodborne risks. Targeted prevention measures, including appropriate handling, freezing, and cooking of game meat, should therefore complement surveillance activities, particularly for vulnerable populations.

Future surveillance in Spain should prioritise harmonized diagnostic methods, a defined set of sentinel species, representative and longitudinal sampling, interoperable databases, and closer collaboration among public health, veterinary, environmental, wildlife-management, and research sectors. The integration of spatial epidemiology, genomic surveillance, and predictive modelling could ultimately move surveillance beyond a predominantly descriptive approach towards earlier identification of changes in parasite circulation and more targeted prevention.

The substantial evidence accumulated in Spain over the past two decades, including the broad distribution of *T. gondii* exposure across wildlife documented by González-Barrio et al. (2024), provides a strong epidemiological basis for moving towards an integrated surveillance approach. Our analysis further highlights marked differences among wildlife groups and the potential value of combining epidemiological evidence with ecological and operational criteria to prioritize species and areas for surveillance. Rather than indicating a single universal sentinel species, the available evidence supports a complementary, risk-based approach in which wildlife data are linked with domestic animal, environmental, food-safety, and human health information. Such integration could improve understanding of transmission dynamics and strengthen the capacity to identify priority areas and interfaces for targeted investigation and prevention.

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