

Coxiella burnetii in wild birds from Europe

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ABSTRACT

Coxiella burnetii is a highly infectious bacterium responsible for causing Q fever, an emerging public health problem of global concern and the cause of severe economic losses in livestock farming. Many species can be asymptomatic carriers and shed this bacterium in various secretions and excreta. Humans can acquire the infection mainly through environmental contamination due to bacterial shedding of infected animals, and through tick-borne or foodborne transmissions. If this agent is aerosolized, it is considered a potential biological weapon. Advanced molecular and serological diagnostic techniques for Q fever over the last decade have made it possible to detect clinical cases and carry out population screening effectively. There needs to be more research on *C. burnetii* control in wildlife despite the increasing evidence that wildlife is a source of *C. burnetii* for both domestic animals and humans. Birds can transmit this pathogen directly or indirectly to other animals or humans, but data about the spreading of *C. burnetii* in avian populations still need to be available. The present work aimed to revise the literature about the involvement of wild birds in the epidemiology of Q fever in Europe. Reports of this pathogenic agent in wild birds in European countries since 2007 were considered.

Introduction

Q fever is a common ubiquitous zoonosis caused by *Coxiella burnetii* and a notifiable disease in Europe (ECDC, 2012). Natural reservoirs are extensive and include several domestic and wild animals, most showing no clinical signs. Due to the high resilience of *C. burnetii* in the environment, humans are most often infected by inhalation of aerosols produced in contaminated areas. Still other routes of infection have been documented. The status of *C. burnetii* as an emerging disease impacting livestock and human health has been highlighted from different outbreaks over the last two decades in Europe (van der Hoek *et al.*, 2012; Schimmer *et al.*, 2014).

The most critical risk factor for human Q fever is living close to an infected ruminant farm (van der Hoek *et al.*, 2012; Schimmer *et al.*, 2014). Birth products of *C. burnetii*-infected ruminants are an important source of human infections (Maurin and Raoult, 1999). Zoonotic transmission of *C. burnetii* is considered a high risk to public health, and controlling endemic coxiellosis may play an essential role in reducing disease. Most member-states of the European Union (EU), reporting was based on clinical investigation and passive monitoring. Domestic ruminants (sheep, goats and cattle) are the main animal species tested. Samples are most frequently blood samples, samples from fetuses and stillborn animals, placentas, vaginal swabs from animals suspected of being infected with *C. burnetii*, and milk samples for screening. Samples are tested by serological methods (proving past or recent exposure to the agent) or direct detection (EFSA and ECDC, 2021).

Birds are involved in the epidemiology of Q fever disease in different ways. Wild birds can be affected by *C. burnetii*, a threat to their own health, and consequently be able to transmit the pathogenic agent to

other animals and humans. Outbreaks of Q fever have been reported in humans following exposure to infected birds (Stein and Raoult, 1999). Birds are also a means of transport to ixodid ticks, regarded as vectors of *C. burnetii*. Especially when we talk about migratory birds, the stopover areas are particularly densely populated (even temporarily), enhancing the probability of disease transmission, including for local settlements and farms. However, avian feces can contaminate any area, becoming a threat to other animals (Ebani and Mancianti, 2022).

This study aimed to gather the information published on the prevalence and detection of *C. burnetii* in wild birds and the ticks that feed on them and to raise awareness of the potential for the spread of this pathogen in Europe.

Coxiella burnetii

Etiology

Coxiella burnetii is a Gram-negative obligate intracellular bacterium and the causative agent of coxiellosis or Query (Q) fever, a zoonosis spread almost worldwide, except Antarctica and New Zealand (Maurin and Raoult, 1999; OIE, 2018; Devaux *et al.*, 2020). It was first described among abattoir workers in Australia when the term Q fever was suggested by Edward Holbrook Derrick in 1937 (Derrick, 1937), and it is still a cause of significant disease in human health around the world. The disease is now considered an occupational hazard among laboratory, livestock, and veterinary workers (Long *et al.*, 2021). Although *Coxiella* was historically regarded as a Rickettsia, 16S rRNA gene sequence analysis and genome analysis classify the *Coxiella* genus as a γ -proteobacteria in the order Legionellales, family Coxiellaceae (Seshadri *et al.*, 2003).

C. burnetii has two antigenic forms. Phase variation is related mainly to mutational variation in the lipopolysaccharide (LPS) (Hackstadt *et al.*, 1985). Phase I is pathogenic and the natural phase found in infected individuals. It is highly infectious and corresponds to smooth LPS. Phase II is attenuated and corresponds to rough LPS, is not very contagious, and is obtained in laboratory by repeated passages in cell cultures in vitro or embryonated egg cultures. Compared to phase I, phase II displays a truncated LPS and lacks some protein cell surface determinants (Amano and Williams, 1984; Maurin and Raoult, 1999). The transition between the two phases may be a strategy of *Coxiella* to bypass the host's immune response (Arricau-Bouvery and Rodolakis, 2005).

C. burnetii is remarkably resistant, and presents distinct morphological forms during the developmental cycle, capable of being present in the environment without a reservoir (Arricau-Bouvery and Rodolakis, 2005). It presents a biphasic developmental cycle, where two forms of this microorganism can be observed: the bacterium's large-cell variant (LCV) and the small-cell variant (SCV). LCV is an exponentially replicating form, whereas SCV is a stationary nonreplicating form, highly infectious and capable of resisting at ambient temperature for months on wool, straw or hay, for example (Angelakis and Raoult, 2010; Eldin *et al.*, 2017). Spore-like particles (SLP), which are infectious and very resistant to environmental conditions are also described (Gürtler *et al.*, 2014).

Transmission routes

The main transmission route (Figure 1) is through the inhalation of contaminated aerosols (originating from faeces and birth products) and contact with secretions or animal products from infected individuals (Angelakis and Raoult, 2010). Per os infection is suggested through consumption of raw dairy products or meat, but the transmission to humans by food is not yet confirmed. The bacterium is also found in the urine, feces, and milk of infected animals, mostly often cattle, sheep, and goats. Ticks and other arthropods may be responsible for Q fever transmission (Eldin *et al.*, 2017; Bolaños-Rivero *et al.*, 2017; OIE, 2018). Person-to-person, congenital infections (transplacental pathway), intradermal inoculation, blood transfusion, and sexual transmission are other forms of transmission already described (Maurin and Raoult, 1999).

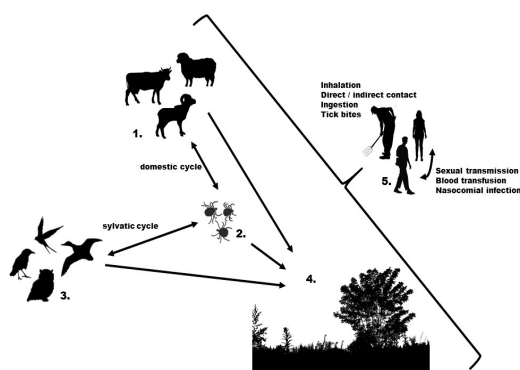


Fig. 1. Main transmission routes of *Coxiella burnetii*. Livestock (1) are considered the main reservoirs of the bacteria. Other domestic animals can also be involved in the transmission to humans. Ticks (2) play an important role in the transmission between wild birds (3) and the others. All of these can contaminate the environment (4). Humans can be infected from any of the represented sources (5): inhalation of contaminated aerosols, tick bite, ingestion of contaminated/unpasteurized products and direct or indirect contact with contaminated materials. Among humans (5), the transmission can occur through different pathways (blood transfusion, sexual, nosocomial and vertical transmission).

Reservoirs

There is a wide variety of reservoirs, including mammals, birds, and arthropods (mainly ticks). Domestic ruminants are the main reservoir, but other domestic animals and wildlife can transmit the infection to humans (Angelakis and Raoult, 2010; OIE, 2018). *C. burnetii* needs to be addressed

in wildlife in wildlife, despite the evidence that particular wild species behave as true reservoirs. Roughly, transmission in the wildlife has been proven in Eurasian wild boar (*Sus scrofa*), European wild rabbit (*Oryctolagus cuniculus*) and European hare (*Lepus europaeus*), red deer (*Cervus elaphus*) and other cervids, red fox (*Vulpes vulpes*) and different small mammals, among others (González-Barrio and Ruiz-Fons, 2019; González *et al.*, 2020; Celina and Cerný, 2022). Although domestic animals are less frequent than livestock, *C. burnetii* infection has been reported in camels, horses, pigs, rabbits, and water buffalo (*Bubalus bubalis*) (Celina and Cerný, 2022).

Infection in humans

In humans, Q fever can have an acute, chronic, or subclinical presentation (Anderson *et al.*, 2013). The acute form of the disease generally responds well to antimicrobial therapy and has a quick resolution, but the chronic form may persist over a long period of time under therapy. Acute Q fever has an estimated incubation period of approximately 20 days but ranges between two to six weeks (OIE, 2018; Rathish *et al.*, 2023). There is no typical clinical presentation, and the clinical signs may vary among patients. A self-limited febrile illness is present in the majority of the cases, with significant pulmonary alterations and elevated serum hepatic enzymes at the same time. Chronic Q fever may develop many months to years after the initial infection, manifesting as bacterial culture negative endocarditis in up to 75% of cases (Gami *et al.*, 2004; Angelakis and Raoult, 2010). Beyond endocarditis, valvular, vascular, or aneurismal infections, hepatitis, pneumonia, or chronic fatigue syndrome are the main clinical signs. *C. burnetii* infection in pregnant women can cause placentalitis and lead to premature delivery, growth restriction, spontaneous abortion, or foetal death (ECDC, 2012; OIE, 2018).

In 2021, the number of human Q fever cases in the EU was the lowest recorded in the last five years (EFSA and ECDC, 2021).

Infection in domestic animals

Infections with *C. burnetii* in domestic animals are usually asymptomatic. During the acute phase, the pathogen can be detected in blood and organs such as the lungs, liver, and spleen. In the chronic phase, most animals are asymptomatic carriers, shedding *C. burnetii* in urine, faeces and urine, and other secretions, like milk. In ruminants, the most common clinical signs are infertility, sudden abortions, or the production of a weak offspring that may die. In small ruminants, abortions are more common, and cattle usually produce offspring with low birth weight. Products of parturition, like the placenta and the newborn itself, have a high microbial load (Arricau-Bouvery and Rodolakis, 2005; Gürtler *et al.*, 2014).

Diagnosis

The prevalence of *Coxiella burnetii* in animals is thought to be highly underestimated, because approximately half of the infections are subclinical, and the clinical signs, whenever present, are very unspecific (ECDC, 2012; Anderson *et al.*, 2013). The OIE terrestrial manual listed several techniques used to diagnose Coxiellosis in animals at population and individual levels (OIE, 2018).

For a definitive diagnosis in the early stages of acute Q fever illness, combining serologic testing with polymerase chain reaction (PCR) is recommended. PCR is a valuable and reliable test for screening large numbers and various types of samples, and kits for ruminants are already available. It's considered the gold standard for Q fever diagnosis. PCR of whole blood or serum can be positive early after symptom onset but becomes negative as the antibody titer increases and after antibiotics administration (Anderson *et al.*, 2013). In ruminants, PCR testing of vaginal swabs collected at parturition can be useful to determine the immune status of the group (OIE, 2018).

Serological testing may be carried out using enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence assay (IFA), or complement fixation test (CFT). CFT has low sensitivity despite presenting high specificity for high-level antibodies. IFA has the disadvantage of being less reproducible between operators and, therefore, between laboratories and seems to have slightly lower sensitivity than ELISA. Therefore, ELISA is recommended for routine serological analysis, as it is considered a robust method (Rousset *et al.*, 2007; OIE, 2018).

A serological survey is useful to evaluate the prevalence of the agent, but serology presents some disadvantages. The presence of specific IgG anti-*C. burnetii* antibodies don't allow to distinguish a recent infection from a previous exposure to the agent (recovered individuals) and don't allow to differentiate an acute or chronic infection (OIE, 2018).

Serological diagnosis of acute Q fever performed by analysis of paired sera samples, targeting both IgM and IgG antibodies, is considered more specific than a single serum sample. IgM results provide complementary information to the IgG titer, but the absence of IgM antibodies in acute Q fever and prolonged persistence of IgG antibodies makes the IgM test limited as an independent test and with no reliable diagnostic value. It could lead to incorrect classification between active or past infections (Wegdam-Blans *et al.*, 2012; Sahu *et al.*, 2020). Besides, IgM antibodies have a much lower specificity than IgG (Anderson *et al.*, 2013). Serological assays are suitable for screening herds or flocks but are not valid for individual interpretation for different reasons. Firstly, many animals shedding *C. burnetii* bacteria, and even some Q fever aborted animals, are found to be seronegative (Rousset *et al.*, 2007; Rousset *et al.*, 2009). Additionally, serological diagnosis of Q fever in the early stage of infection can be unsuccessful due to the time frame of seroconversion, which covers 3–4 weeks post-infection (Howe *et al.*, 2009; Niemczuk *et al.*, 2014). Serological antigens are based on the two major antigenic forms of *C. burnetii*: phase I, obtained from spleens after inoculation of laboratory animals, and phase II, obtained by repeated passages in embryonated eggs or cell cultures. Currently available commercial tests allow the detection of phase II, which appear to be present whatever the infection stage or form, or of both phases II and I anti-*C. burnetii* antibodies (OIE, 2018). Phase II antibodies have been found to persist for a long period of time (even after one year) following infection. Therefore, its presence does not confirm active infection (Wegdam-Blans *et al.*, 2012). In contrast with acute Q fever infection, chronic infection is associated with continued increasing phase I IgG titers (typically $\geq 1:1024$) that might be higher than phase II IgG (Sahu *et al.*, 2020).

An avidity test to improve the accuracy of the serological diagnosis in dating the onset of the infection and to distinguish past from recent Q fever infections is already available. However, it is only recommended for sera with an IgG titer of $\geq 1:200$. A denaturation of phase I and II IgG with urea is observed in cases of recent infection. On the opposite, a strong avidity is found in patients who suffered from a past infection. To date the infection more accurately, a low avidity refers to a Q fever infection occurring in the three months preceding the onset of symptoms. In contrast a high avidity excludes one within the last six months (Luciani *et al.*, 2019).

For specific laboratory investigations, it may be necessary to isolate the agent. Direct isolation by inoculating specimens into embryonated chicken eggs or conventional cell cultures is possible (OIE, 2018; Sahu *et al.*, 2020).

The bacteria can be visualised in stained tissue or vaginal mucus smears using a microscope with an oil-immersion objective lens. It is acid resistant, so a better visualization can be achieved by staining with specific colorations, such as Stamp, modified Ziehl–Neelsen, Gimenez, Macchiavello, Giemsa, and modified Koster. However, these findings are not specific, so a finding is only presumptive. A definitive diagnosis of Q fever requires further confirmatory tests, with PCR the preferred option (OIE, 2018; Sahu *et al.*, 2020).

Immunohistochemical staining is more specific and sensitive than classical staining methods. No specific antibodies for immunochemistry

(IHC) are commercially available. The technique is done by in situ hybridisation (OIE, 2018).

Vaccination

It has been suggested that vaccination is useful in reducing the risk of outbreaks and in the event of an outbreak. Several inactivated vaccines against Q fever have been developed, but only vaccines containing or prepared from phase I *C. burnetii* should be considered effective. An inactivated phase I vaccine is commercially available. Repeated annual vaccination, particularly for young animals, is recommended in at-risk areas (OIE, 2018; Long, 2021). Vaccination is currently one of the most effective management strategies to reduce abortion rates and the spread of the pathogen and authorized vaccines for sheep, goats, and cattle are available in Europe (Celina and Cerný, 2022).

The immunogenicity of commercially available vaccines may be more effective in non-pregnant females than in pregnant ones (at least in does and ewes), for long-term control of the spread of *C. burnetii* in the herd (Porter *et al.*, 2011). Vaccination was found to be effective in goats and has been shown to reduce the risk of excretion in the milk of sensitized goats and in the milk, vaginal secretions, and faeces of virgin goats. Nevertheless, some studies reported no evidence of the effect of vaccination on sheep (O'Neill *et al.*, 2014), while others concluded that vaccination significantly reduced the average level of overall excretion, including in sheep (Hogerwerf *et al.*, 2011).

Coxiella burnetii in wild birds

Wild birds can be affected by pathogens that may be prejudicial to their health and can also be transmissible to other animals and humans. The role of migratory birds in the spread of tick-borne diseases has long been highlighted. Birds are a means of transport for ticks that can carry viral, bacterial or parasitic agents into new areas. Most of these tick-borne pathogens have zoonotic potential (Hasle, 2013; Toma *et al.*, 2014; Berthová *et al.*, 2016; Buczek *et al.*, 2020). *C. burnetii* has also been detected in mites, i.e. *Dermanissus gallinae*. Most avian species remove mites through feather picking and consequently ingest infected mites, which represent another route of transmission (Raele *et al.*, 2018).

The role of birds as a natural reservoir of *C. burnetii* has already been confirmed and detected in several countries (Figure 2). The involvement of avian populations in the epidemiology of *C. burnetii* was first suggested in the 1950s (Babudieri and Moscovici, 1952; Raska and Syrucek, 1956), with the first report of wild bird infection by *C. burnetii* (Babudieri and Moscovici, 1952), continued into the 1970s (Enright *et al.*, 1971), and having gained more attention in recent decades.



Fig. 2. Map with the European countries that have reported cases in wild birds since 2007 marked in dark grey. Created using <https://mapchart.net/europe.html>.

Wild birds are not frequently relevant faecal spreaders of *Coxiella burnetii* and other common bacteria and parasitic pathogens responsible for livestock infections. However, although not very often, birds may

Table 1. *Coxiella burnetii* detection in birds or ticks collected from birds, in Europe, since 2007 onwards.

Country	Year	Sample	Bird species	Diagnostic Tests	Results	Study
Italy	Jan-Dec 2016	Intestine	<i>Anas crecca</i> , <i>Anas platyrhynchos</i> , <i>Anas penelope</i> , <i>Anas clypeata</i> , <i>Anas acuta</i> , <i>Ardea cinerea</i> , <i>L. micha-hellis</i> , <i>Tadorna tadorna</i> , <i>Fulica atra</i> , <i>Falco tinnunculus</i> , <i>Falco peregrinus</i> , <i>Athene noctua</i> , <i>Accipiter nisus</i> , <i>Columba livia</i> , <i>Columba palumbus</i>	PCR	1/11 (9.09%) (<i>Anas penelope</i> samples); 1/121 (0.83%) (all samples)	Ebani et al. (2021)
			---		0/78 (0%)	
			<i>Coturnix coturnix</i> , <i>Larus ridibundus</i> , <i>Motacilla alba</i> , <i>Passer domesticus</i> , <i>Sturnus vulgaris</i> ; Total	PCR	1/10 (10.00%), 1/252 (4.00%), 2/52 (3.85%), 1/6 (16.67%), 1/16 (6.25%); 6/416 (1.44%)	
Russia and Bulgaria	2011-2012	Blood	<i>Luscinia luscinia</i> , <i>Phylloscopus trochilus</i> ; Total		1/4 (25.00%); 3/3 (100%); 4/874 (0.46%)	Tokarevich et al. (2019)
			<i>Pelecanus onocrotalus</i> ; Total		1/30 (3.33%); 1/175 (0.57%)	
		Faeces	<i>Acrocephalus scirpaceus</i> , <i>Motacilla flava</i> , <i>Pelecanus onocrotalus</i> , <i>Turdus merula</i> , <i>Tringa graleola</i> ; Total	PCR	3/16 (18.75%), 1/7 (14.29%), 1/18 (5.56%), 1/3 (33.33%), 1/7 (14.29%); 7/51 (13.73%)	
			<i>Erithacus rubecula</i>	PCR	3/143 (2.10%) (nr positive ticks for <i>Coxiella</i> /nr ticks tested)	
Italy	2016-2017 (Sep-Jan)	Serum	<i>Anas platyrhynchos</i> , <i>Corvus cornix</i> , <i>Turdus merula</i> , <i>Turdus pilaris</i> ; Total	Complement fixation test (CFT)	1/7 (14.29%), 1/7 (14.29%), 1/6 (16.67%), 3/21 (14.29%); 6/74 (8.11%)	Ebani et al. (2019)
			<i>Anas crecca</i> , <i>Anas penelope</i>	PCR	4/133 (3.01%)	
			<i>Columba livia domestica</i>	PCR	5/84 (5.95%)	
Slovakia	2012 (Apr-Aug)-2013 (Apr-Oct)	Ticks collected from captured birds	<i>Erithacus rubecula</i> , <i>Parus major</i> , <i>Passer montanus</i> , <i>Sitta europaea</i>	PCR	16/594 (2.69%) (nr positive ticks for <i>Coxiella</i> /nr ticks tested)	Berthová et al. (2016)
			<i>Erithacus rubecula</i> , <i>Sylvia atricapilla</i>	PCR	3/336 (0.90%)	
Italy	2010-2011 (Apr to Oct)	Ticks collected from captured birds	<i>Luscinia megarhynchos</i> , <i>Sylvia atricapilla</i> , <i>Sylvia communis</i> , <i>Saxicola rubetra</i> , <i>Pernis apivorus</i> , <i>Buteo buteo</i> ; Total	PCR	8/25 (32.00%), 1/2 (50.00%), 3/15 (20.00%), 2/17 (11.80%), 26/40 (65.00%), 2/7 (28.60%) (nr positive ticks for <i>Coxiella</i> /nr ticks tested)	Toma et al. (2014)
				PCR	42/127 (33.07%)	
France	Apr-10	Ticks collected from captured birds	<i>Sylvia atricapilla</i> , <i>Erithacus rubecula</i> , <i>Turdus merula</i> , <i>Passer domesticus</i> , <i>Acrocephalus scirpaceus</i> , <i>Luscinia megarhynchos</i> ; Total	PCR	0/17 (0%)	Socolovschi et al. (2012)
Spain	Jan 2001-Apr 2006	Spleen and/or lung from dead birds	<i>Milvus migrans</i> , <i>Gyps fulvus</i>	PCR	1/7 (14.30%), 1/9 (11.00%)	Astobiza et al. (2011)
Cyprus	Oct 2004-Oct 2006	Ticks collected from captured birds	<i>Accipiter gentilis</i> , <i>Anas platyrhynchos</i> , <i>Ardea cinerea</i> , <i>Asio otus</i> , <i>Buteo buteo</i> , <i>Buteo rufinus</i> , <i>Caprimulgus europaeus</i> , <i>Ciconia ciconia</i> , <i>Circus cyaneus</i> , <i>Columba palumbus</i> , <i>Falco peregrinus</i> , <i>Falco tinnunculus</i> , <i>Fulica atra</i> , <i>Gallinula chloropus</i> , <i>Grus grus</i> , <i>Hieraaetus fasciatus</i> , <i>Isobrychus minutus</i> , <i>Larus cachinnas</i> , <i>Larus fuscus</i> , <i>Nycticorax nycticorax</i> , <i>Pernis apivorus</i> , <i>Phoenicopiterus ruber</i> , <i>Sreptopelia turtur</i> , <i>Tyto alba</i> ; Total	PCR	3/3 (100%), 1/1 (100%), 1/4 (25.0%), 1/4 (25.0%), 1/4 (25.0%), 1/3 (33.3%), 1/3 (33.3%), 1/1 (100%), 2/3 (66.7%), 3/10 (30.0%), 2/4 (50.0%), 4/11 (36.4%), 1/5 (20.0%), 1/5 (20.0%), 1/1 (100%), 1/2 (50.0%), 1/1 (100%), 1/4 (25.0%), 1/2 (50.0%), 1/1 (100%), 1/3 (33.3%), 1/3 (33.3%), 1/7 (14.3%), 2/5 (40.0%); 34/135 (25.2%)	Ioannou et al. (2009)
Bulgaria	1950-2006	Serum	<i>Corvus corax</i> , <i>Pica pica</i> , <i>Phasianus colchicus</i> , <i>Columba palumbus</i>	CFT	27.27%, 26.22%, 33.33%, 11.84%	Martinov (2007)
			Crows, pigeons, <i>Corvus corax</i> , <i>Phasianus colchicus</i> , <i>Sreptopelia turtur</i>	Isolation	---	

excrete *C. burnetii* in their droppings, potentially contaminating the environment (Stein and Raoult, 1999; Ebani et al., 2016; Ebani et al., 2021).

A few studies have been conducted that detected the presence of *C. burnetii* in wild birds from Europe, either directly or in ectoparasites collected from captured birds (Table 1).

In Italy, faecal samples collected from 121 free-ranging wild birds belonging to 15 species of the genera Accipiter, Anas, Ardea, Athene, Columba, Falco, Fulica, Larus and Tadorna were submitted to bacteriological and molecular analyses that detected seven pathogenic agents, including *C. burnetii*. The portion of the intestine used to do the tests was collected from birds hunted during the hunting season or birds that died in a local wildlife rehabilitation centre. The prevalences found for all pathogens were relatively low, and only one Eurasian wigeon (*Anas penelope*) was positive for *C. burnetii* (Ebani et al., 2021).

Another study captured wild birds in migratory stopover sites in four different regions: Kaliningrad and St. Petersburg in Russia, and Atanasovsko Lake and Sofia in Bulgaria. Blood samples from the captured birds were collected in Kaliningrad, St. Petersburg, and Atanasovsko. Faecal samples were collected from birds captured in St. Petersburg and Atanasovsko. Ticks were collected from birds at Kaliningrad site. Ticks were also collected from vegetation, but these have not been considered for this review. Blood, faeces and ticks were tested for the presence of *C. burnetii* DNA by PCR, and blood sera for antibodies to *C. burnetii*. At Kaliningrad region, all blood samples (n=78) were negative for *C. burnetii* DNA, so the tested species have not been listed in Table 1. In St. Petersburg and Atanasovsko, prevalences of 1.44% (6/416) () and 0.46% (4/874) () were found, among five and two species out of 34 tested, respectively. Regarding faecal samples, in Atanasovsko, the only stool sample that tested positive out of 175 (0.57%) belonged to a great white pelican (*Pelecanus onocrotalus*); in St. Petersburg, seven individuals out of 51 from five species were positive (13.73%). Ticks were collected from birds of the following species in Kaliningrad: tree pipit (*Anthus trivialis*), hawfinch (*Coccothraustes coccothraustes*), European robin (*Erithacus rubecula*), chaffinch (*Fringilla coelebs*), great tit (*Parus major*), common redstart (*Phoenicurus phoenicurus*), common chiffchaff (*Phylloscopus collybita*), common starling (*Sturnus vulgaris*), Eurasian wren (*Troglodytes troglodytes*), common blackbird (*Turdus merula*) and song thrush (*Turdus philomelos*), but only three (2.10%) found in European robins tested positive for *C. burnetii*. Migratory birds are likely to act as effective vehicles in the dispersal of *C. burnetii*-infected ixodid ticks. Finally, in St. Petersburg, out of 74 sera, antibodies to *C. burnetii* were found in six samples (8.1%) from four species of birds. The places of wild bird stopover in these regions proved to be natural foci of *C. burnetii* infection (Tokarevich et al., 2019).

Among animals hunted in Pisa, Italy, in a migration corridor of waterfowl, a prevalence of 3.01% was found: three Eurasian teal (*Anas crecca*) and one Eurasian wigeon. Individuals of other species were tested, but the results were negative: northern pintail (*Anas acuta*), northern shoveler (*Anas clypeata*), mallard (*Anas platyrhynchos*), garganey (*Anas querquedula*), gadwall (*Anas strepera*), greylag goose (*Anser anser*), common pochard (*Aythya farina*), tufted duck (*Aythya fuligula*), Eurasian coot (*Fulica atra*), common snipe (*Gallinago gallinago*) and common shelduck (*Tadorna tadorna*) (Ebani et al., 2019).

In a research article that evaluated the presence of some zoonotic tick-borne bacteria in feral pigeons (*Columba livia domestica*) from Tuscany, Italy, a prevalence of 5.95% was detected by PCR assays for *C. burnetii* on animals that were found dead (Ebani et al., 2016).

Birds were captured in three distinct sites in Slovakia and samples of blood and ticks carried by the birds were taken. *C. burnetii* DNA was found in 16 ticks out of 594 (2.69%) and in blood samples from two European robins and one Eurasian blackcap (*Sylvia atricapilla*), 3/336 (0.90%) (Berthová et al., 2016).

As in previous studies, birds were captured from nets during regular ringing procedures and checked for the presence of ticks. All examined ticks were collected on 41 birds belonging to 17 species (Toma et al.,

2014).

Ticks from birds trapped in nets in the region of Camargue, France, were collected. Seventeen ticks were analyzed from six bird species; all the DNA samples extracted were negative for *C. burnetii*. Only one tick collected from the environment was positive for this agent (Socolovschi et al. 2012).

Wild animals that were hunted or found dead were sampled in the Basque Country, northern Spain, and organs were processed by PCR for *C. burnetii* detection. *C. burnetii* DNA was detected in 1.2% ($\pm 1.6\%$) of wild birds. The two positive specimens were diurnal birds of prey: griffon vulture (*Gyps fulvus*) and black kite (*Milvus migrans*). The remaining 121 individuals from other families tested negative (Astobiza et al. 2011).

Ticks and other ectoparasites were removed from trapped birds in Cyprus and processed for DNA extraction. From 557 bird samples representing 51 bird species, 135 pools were prepared, from which 34 (25.2%) were positive for *C. burnetii*. Throughout the main text, the authors refer to a higher prevalence, but we have chosen to follow the data in the table included in the article (Ioannou et al. 2009).

C. burnetii isolates have been obtained from wild birds in Bulgaria: two crows, three pigeons, one raven (*Corvus corax*), two pheasants (*Phasianus colchicus*), and two turtle-doves (*Streptopelia turtur*). Serological investigations in wild birds from different regions of the country revealed prevalences of 27.27% in ravens, 26.22% in magpies (*Pica pica*), 33.33% in pheasants, and 11.84% in woodpigeons (*Columba palumbus*) (Martinov, 2007).

Coxiella in the One Health approach

One Health is increasingly gaining recognition and has become more widespread in recent years. It is an approach arguing that the people's health is closely linked to the health of animals and also that of the environment in which they all live. Human populations are expanding into new geographic areas, formerly uninhabited. Nowadays, people live in close contact with wild and domestic animals, and disruptions in environmental conditions (changes in climate and land use) and habitats can provide new opportunities for disease transmission. Furthermore, with globalization, the movement of people, animals and products around the world has increased dramatically, consequently increasing the spread of pathogens across borders, namely existing (endemic) and new (emerging) zoonotic diseases (CDC, 2023). Today's health problems are frequently complex, transboundary, multifactorial, and across species, and a holistic and multidisciplinary approach is the best sustainable way to mitigate these zoonosis and other threats (Mackenzie and Jeggo, 2019). Birds and other animals are susceptible to several pathogens that are responsible for causing disease in humans, which is why they can act as sentinels for possible outbreaks of zoonotic diseases, such as Q fever. An approach based on One Health can achieve the best health outcomes for all those involved, by promoting collaboration across different sectors and working groups (CDC, 2023).

Bioterrorism is a different concept, which involves the deliberate release of bioweapons (bacteria, viruses, toxins, or fungi) to cause death or disease in humans, animals, or plants. According to the Centers for Disease Control and Prevention's (CDC) classification, *C. burnetii* is a category B biological weapon (second highest priority) due to its widespread availability, environmental stability, small size, low infectious dose, persistence in infected hosts, the potential for aerosol transmission, high morbidity, and substantial mortality (Azad, 2003; Rathish et al., 2023). The virulent strains of *C. burnetii* are highly infective, and aerosolized organisms are often the source of the reported laboratory-acquired infections. Because of that, biological safety level 3 conditions are required when working with this microorganism and its propagation, purification, and molecular or biochemical manipulation (Azad, 2003).

In most Q fever outbreaks, infected ruminants are typically the primary and main source of infection to humans (Cruz et al., 2018; Pexara et

al., 2018; Cruz et al., 2020; Pouquet et al., 2020). In some places, wildlife may not represent a direct source of human *C. burnetii* infection, but they contribute to the maintaining of the agent in nature (Kazar, 2005; Pires et al., 2023). Nevertheless, changes in land use and urbanisation have increased the proximity between wildlife and domestic animals and humans, thus increasing the potential for transmission of zoonotic diseases (Kazimírová et al., 2018; Tomassone et al., 2018).

C. burnetii has been isolated from over 40 species of hard ticks and 14 soft tick species collected from vegetation, domestic, and wild animals (Celina and Cerný, 2022). However, about public health, the epidemiological importance of *C. burnetii* tick-borne transmission is lower compared to airborne transmission (Duron et al., 2015). The transmission of the Q fever agent to humans through a direct tick bite is rare (Kazimírová et al., 2018), but should not be ruled out. On the other hand, transmission through domestic animals or wild birds that live near people who have been infected by ticks is a strong possibility. The growth of urban green areas and the geographical expansion of urbanized areas into agricultural habitats has also increase the dispersal and abundance of vectors (Tomassone et al., 2018).

The environment influences the spreading of *C. burnetii*, and humid environments favour the transmission of this pathogen. Most of the animals whose faeces were positive came from birds living in this kind of environment, such as waterfowls (Ebani et al. 2019; Ebani and Mancianti, 2022). This can be a factor to take into consideration in regards to hygiene and prophylaxis in farms.

Some carrion birds (e.g. black kite and griffon vulture) were identified as potential sources of *C. burnetii* infection in northern Spain (Astobiza et al. 2011). Scavenging behaviour has already been pointed out as a source of exposure to the microorganism, making scavengers more susceptible to infection than other species (To et al., 1998). Again, avoiding birth products, meat leftovers and other debris outdoors that could attract this type of wild birds is important when it comes to preventing transmission.

Conclusion

Q fever is a zoonosis of major importance, causing harm to human and animal health and significant economic losses on livestock farms. *C. burnetii* is highly infective and has the potential to spread rapidly, easily causing dramatic epidemic focuses. Although it has been classified as a notifiable animal disease by the World Organization for Animal Health, Q fever might still be considered a neglected zoonosis.

Birds are not the most likely source of outbreaks, but their potential involvement in the transmission of the infection and disease should be considered. In terms of conservation, considering that many wild birds are protected species, Q fever should also be considered a threat to populations, and other emerging infectious diseases. Further seroprevalence studies are recommended to assess the disease real status and be aware of its distribution. Additional studies are also required to understand better the pathogenicity and virulence of *C. burnetii* for its wild bird hosts.

Based on the interactions between wildlife, livestock and humans, the triad of which needs to be more widely assessed, it is important to develop effective prevention and control strategies in line with the One Health approach.

Conflict of interest

The authors declare that they have no conflict of interest.

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