microbiology research

MDPI

Article

Bartonella alsatica in Wild and Domestic Rabbits (*Oryctolagus cuniculus*) in The Netherlands

Marja J. L. Kik [1,2,*], Ryanne I. Jaarsma [3], Jooske IJzer [1,2], Hein Sprong [3], Andrea Gröne [1,2] and Jolianne M. Rijks [1]

1 Dutch Wildlife Health Centre, Utrecht University, 3584 CL Utrecht, The Netherlands; j.ijzer@uu.nl (J.I.); A.Grone@uu.nl (A.G.); J.M.Rijks@uu.nl (J.M.R.)
2 Veterinary Pathology Diagnostic Centre, Utrecht University, 3584 CL Utrecht, The Netherlands
3 Centre for Infectious Disease Control, National Institute for Public Health and the Environment, 3720 BA Bilthoven, The Netherlands; RI_Jaarsma@hotmail.com (R.I.J.); Hein.Sprong@rivm.nl (H.S.)
* Correspondence: m.kik@uu.nl; Tel.: +31-65-3524693

Abstract: Members of the genus *Bartonella* are Gram-negative facultative intracellular bacteria that are transmitted by arthropod vectors. *Bartonella alsatica* was detected in the spleens and livers of 7 out of 56 wild rabbits (*Oryctolagus cuniculus*) and in the liver of 1 out of 87 domestic rabbits in the Netherlands. The molecular evidence of *B. alsatica* infection in wild as well as domestic rabbits indicates the possibility of exposure to humans when these come in close contact with rabbits and possibly their fleas with subsequent risk of *Bartonella* infection and disease.

Keywords: *Bartonella alsatica*; domestic rabbit; endocarditis; lymphadenitis; *Oryctolagus cuniculus*; wild rabbit; zoonosis

Citation: Kik, M.J.L.; Jaarsma, R.I.; IJzer, J.; Sprong, H.; Gröne, A.; Rijks, J.M. *Bartonella alsatica* in Wild and Domestic Rabbits (*Oryctolagus cuniculus*) in The Netherlands. *Microbiol. Res.* **2021**, *12*, 524–527. https://doi.org/10.3390/microbiolres12020036

Academic Editor: Seyed Ali Ghorashi

Received: 18 May 2021
Accepted: 13 June 2021
Published: 16 June 2021

1. Introduction

Bartonella genus members are facultative intracellular Gram-negative bacteria. Many of them have been identified as zoonotic agents. Blood-sucking arthropods transmit the bacteria. The bacteria can infect erythrocytes of mammals potentially causing persistent bacteremia. *Bartonella henselae*, *B. bacilliformis*, and *B. quintana* are the most relevant species for human beings [1], but other species such as *B. alsatica* can also cause disease. *B. alsatica* has been described as the causative agent of two human cases of endocarditis, one aortobifemoral graft infection and one case of lymphadenitis [2–5]. All four patients had been in close contact with wild or domestic rabbits. In continental Europe, the pathogen has been detected both in France and in Spain in live caught, apparently healthy, wild European rabbits (*Oryctolagus cuniculus*) and in rabbit fleas (*Spilopsyllus cuniculi* and *Xenopsylla cunicularis*) [6,7]. Pathology on two euthanized infected rabbits provided no evidence of disease [7].

The incidence of human bartonellosis in the Netherlands is not well-known but estimated to be 2 per 100,000 inhabitants per year [8]. Several different zoonotic *Bartonella* species are known to circulate in the Netherlands, including *B. henselae*, *B. clarridgeiae*, *B. schoenbuchensis*, *B. grahamii* and *B. washoensis* [9,10]. Information on *B. alsatica* is lacking.

This study retrospectively investigated wild and domestic rabbits examined post-mortem in the Netherlands for evidence of *B. alsatica* infection.

2. Materials and Methods

Full necropsy was performed following a standard protocol on 51 wild and 87 domestic rabbits. From five wild rabbits only spleen and liver were obtained. The wild rabbits were offered for necropsy to the Dutch Wildlife Health Centre as part of disease surveillance in wildlife, and the domestic rabbits presented to the Veterinary Pathology Diagnostic Centre for diagnostic purposes. Necropsy was performed following a standard protocol on 51 wild rabbits and all 87 domestic rabbits. Necropsy included macroscopic examination, cytological analysis of liver, spleen, lungs, and intestinal contents stained

with Hemacolor® (Hemacolor quick stain, Merck, Darmstadt, Germany), and histological examination of samples of various organs fixed in 4% buffered formalin, embedded in paraffin, cut into 4-µm sections, and stained with hematoxylin and eosin. Organ tissues were frozen at −80 °C.

DNA was extracted from 56 wild rabbits (spleen and liver) and 87 domestic rabbits (liver) using the Qiagen DNeasy Blood & Tissue Kit according to the manufacturer's protocol (Qiagen, Venlo, the Netherlands). These samples were then tested for the presence of *Bartonella* spp. using qPCR [9]. From the qPCR-positive samples, a fragment of the gltA gene was amplified by conventional PCR and sequenced for species identification [11]. Distilled water was used as negative control and *B. schoenbuchensis* from deer keds (*Lipoptena cervi*) from a previous study as a positive control. Positive controls and negative water controls were used on every plate tested. To prevent contamination, and false-positive samples, the DNA extraction, PCR mix preparation, sample addition, and (q)PCR analyses were performed in separated air locked dedicated labs.

3. Results

The PCR-test results are summarized per necropsy result in Table 1. Twelve wild rabbits were positive by qPCR for *Bartonella* spp. From these, seven could be identified as *B. alsatica* by conventional PCR and sequencing: The 338 base pairs of the PCR product were 100% identical to GenBank accession number AF204273. Two domestic rabbits were positive by qPCR from which one could be identified as *B. alsatica* (identical to AF204273). Failure of successful sequencing of the other six of the PCR products might be due to low DNA concentration. The *B. alsatica* PCR-positive wild animals died of rabbit hemorrhagic disease virus (RHDV) infection (3), myxomatosis (2), or no pathologic examination was performed (2). The positive domestic rabbit died of RHDV infection. None of the six cases examined post-mortem and positive for *B. alsatica* showed evidence of endocarditis, myocarditis or vascular pathology.

Table 1. *Bartonella* PCR-test results of 56 wild and 87 domestic rabbit per necropsy result.

Type	Diagnosis	% of Animals	*Bartonella* # qPCR Positive %	*Bartonella alsatica* # %
Wild	Rabbit hemorrhagic disease virus infection	60.71	7.14	5.3
	Myxomatosis (3 with also trauma)	10.71	3.5	3.5
	Pneumonia (*Pasteurella multocida*)	1.79	0	0
	Hepatic parasites	1.79	0	0
	Enteric parasites	1.79	1.79	0
	Trauma (including shot)	7.14	1.79	0
	No clear lesions/etiology	8.93	3.5	3.5
	No pathology performed	7.14	3.5	3.5
	Total	100	21.22	15.8
Domestic	Rabbit hemorrhagic disease virus infection	50.57	2	1
	Tyzzer's disease	1.15	0	0
	Yersinia pseudotuberculosis * infection	1.15	0	0
	Bacterial septicemia (including pasteurellosis),	6.9	0	0
	Encephalitis (*Encephalitozoon cuniculi* *)	2.3	0	0
	Pneumonia (including *Pasteurella multocida*)	8.05	0	0
	Hepatic parasites	2.3	0	0
	Hepatitis	2.3	0	0
	Enteritis or colitis	2.3	0	0
	Mastitis (1 with also trauma)	3.45	0	0
	Multiple organ failure	1.15	0	0

Table 1. *Cont.*

Type	Diagnosis	% of Animals	*Bartonella* # qPCR Positive %	*Bartonella alsatica* # %
	Decompensatio cordis	6.9	0	0
	Metabolic bone disease	1.15	0	0
	Trauma	2.3	0	0
	No clear lesions/etiology	8.05	0	0
	Total	100	2.3	1.15

* Other zoonotic agents. # The presence of *Bartonella* spp. was first tested by qPCR. Only qPCR-positive samples were subjected to conventional PCR followed by Sanger sequencing. All the DNA sequences that were obtained were designated as *B. alsatica*. (Accession number GenBank MZ367378). Failure of successful sequencing the PCR product might be due to low DNA concentration.

4. Discussion

This study shows presence of *B. alsatica* in both domestic and wild rabbits in the Netherlands. The positive rabbits died of common viral diseases of rabbits and showed no evidence for typical *Bartonella*-associated lesions.

Human infections with *Bartonella* spp. have been described; however, in an unknown number of cases infection was suspected but proof could not be established by serology, culture or PCR. It is known that *B. alsatica* can cause blood culture-negative disease and, therefore, the incidence of infection may be under-estimated [3]. Marquez et al. [6] concluded, based on presence of *B. alsatica* in rabbits and their fleas, that more research should be performed to determine if *B. alsatica* could be responsible for unknown febrile disease in humans. This study supports the role of wild and domestic rabbits as a reservoir for human infections, and the addition of *B. alsatica* to the list of zoonotic agents [12]. Moreover, domestic rabbits are also kept in children's farms where people with variable immune status are in close contact with rabbits. The molecular evidence of *B. alsatica* infection in wild as well as domestic rabbits indicates the possibility of exposure to humans when these come in close contact with rabbits and possibly their fleas with subsequent risk of *Bartonella* infection and disease.

Author Contributions: Original Draft Preparation: M.J.L.K., J.M.R., H.S. Writing—Review and Editing: M.J.L.K., J.I., A.G., J.M.R. Methodology: M.J.L.K., J.I., R.I.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as the organs used were derived from wild rabbits offered for necropsy to the Dutch Wildlife Health Centre as part of disease surveillance in wildlife, and 87 domestic rabbits presented to the Veterinary Pathology Diagnostic Centre for diagnostic purposes.

Informed Consent Statement: Not applicable.

Data Availability Statement: GenBank accession number AF204273.

Acknowledgments: The authors thank Natashja Ennen-Buijs, Manon Lock, Ruby Wagensveld-van den Dikkenberg and Louis van den Boom for technical assistance.

Conflicts of Interest: None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence (bias) the content of the paper.

References

1. Cheslock, M.A.; Embers, M.E. Human Bartonellosis: An Underappreciated Public Health Problem? *Trop. Med. Infect. Dis.* **2019**, *4*, 69. [CrossRef] [PubMed]
2. Jeanclaude, D.; Godmer, P.; Leveiller, D.; Pouedras, P.; Fournier, P.-E.; Raoult, D.; Rolain, J.-M. *Bartonella alsatica* endocarditis in a French patient in close contact with rabbits. *Clin. Microbiol. Infect.* **2009**, *15*, 110–111. [CrossRef] [PubMed]
3. Raoult, D.; Roblot, F.; Rolain, J.-M.; Besnier, J.-M.; Loulergue, J.; Bastides, F.; Choutet, P. First Isolation of *Bartonella alsatica* from a Valve of a Patient with Endocarditis. *J. Clin. Microbiol.* **2006**, *44*, 278–279. [CrossRef] [PubMed]

4. Puges, M.; Ménard, A.; Berard, X.; Geneviève, M.; Pinaquy, J.-B.; Edouard, S.; Pereyre, S.; Cazanave, C. An unexpected case of *Bartonella alsatica* prosthetic vascular graft infection. *Infect. Drug Resist.* **2019**, *12*, 2453–2456. [CrossRef] [PubMed]
5. Angelakis, E.; Lepidi, H.; Canel, A.; Rispal, P.; Perraudeau, F.; Barre, I.; Rolain, J.-M.; Raoult, D. Human Case of *Bartonella alsatica* Lymphadenitis. *Emerg. Infect. Dis.* **2008**, *14*, 1951–1953. [CrossRef] [PubMed]
6. Márquez, F.J. Molecular Detection of *Bartonella alsatica* in European Wild Rabbits (*Oryctolagus cuniculus*) in Andalusia (Spain). *Parasites Vectors* **2015**, *8*, 731–734. [CrossRef] [PubMed]
7. Heller, R.; Kubina, M.; Mariet, P.; Riegel, P.; Delacour, G.; Dehio, C.; Lamarque, F.; Kasten, R.; Boulouis, H.-J.; Monteil, H.; et al. *Bartonella alsatica* sp. nov., a new *Bartonella* species isolated from the blood of wild rabbits. *Int. J. Syst. Evol. Microbiol.* **1999**, *49*, 283–288. [CrossRef] [PubMed]
8. Rijksinstituut voor Volksgezondheid en Milieu; Ministerie van Volksgezondheid, Welzijn en Sport. *Bartonella henselae*-infectie. Richtlijn (in Dutch). Available online: https://lci.rivm.nl/richtlijnen/bartonella-henselae-infectie (accessed on 3 June 2020).
9. Tijsse-Klasen, E.; Fonville, M.; Gassner, F.; Nijhof, A.M.; E Hovius, E.K.; Jongejan, F.; Ke, H.E.; Reimerink, J.R.; Overgaauw, P.A.M.; Sprong, H. Absence of zoonotic *Bartonella* species in questing ticks: First detection of *Bartonella clarridgeiae* and *Rickettsia felis* in cat fleas in the Netherlands. *Parasites Vectors* **2011**, *4*, 61. [CrossRef] [PubMed]
10. Von Loewenich, F.D.; Seckert, C.; Dauber, E.; Kik, M.J.L.; De Vries, A.; Sprong, H.; Buschmann, K.; Aardema, M.L.; Brandstetter, M. Prosthetic Valve Endocarditis with *Bartonella washoensis* in a Human European Patient and Its Detection in Red Squirrels (*Sciurus vulgaris*). *J. Clin. Microbiol.* **2019**, *58*, 1404–1419. [CrossRef] [PubMed]
11. Müller, A.; Reiter, M.; Schötta, A.M.; Stockinger, H.; Stanek, G. Detection of *Bartonella* spp. in *Ixodes ricinus* ticks and *Bartonella seroprevalence* in human populations. *Ticks Tick-Borne Dis.* **2016**, *7*, 763–767. [CrossRef] [PubMed]
12. Breitschwerdt, E.B. Bartonellosis, One Health and all creatures great and small. *Veter. Dermatol.* **2017**, *28*, 111–121. [CrossRef]