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To our knowledge, detection of Bartonella spp. DNA in sea turtle blood represents the first molecular evidence of Bartonella infection in nonmammalian vertebrates. B. henselae infection, now reported in porpoises and sea turtles, may represent an emerging infection of marine animals. According to previous studies, immune status appears to affect disease severity, variation in clinical manifestations, the pattern of histopathologic features, and the relative ease of diagnostic detection of the organism (4,7). Although healthy at the time of sample collection, the captive rehabilitated sea turtles were known to have been sick or injured before sampling, potentially reflecting immunocompromise. Whether detection of Bartonella spp. in blood of sea turtles is a function of prior immunosuppression induced by stressors is not known. Such stressors could include mechanical injury, malnutrition, environmental toxins, parasites, or concurrent bacterial or viral infections. Alternatively, sea turtles may be a natural marine reservoir for B. henselae or for a Bartonella sp. genetically related to B. vinsonii subsp. berkhoffii.

In summary, documentation of *B. henselae* and an organism genetically similar to *B. vinsonii* subsp. *berkhoffii* in the blood of loggerhead sea turtles provides evidence that this genus is not ecologically limited to terrestrial reservoirs. The geographic distribution, prevalence of infection, carrier potential, mode of transmission, and pathogenicity of bloodborne *Bartonella* spp. in sea turtles await additional studies.

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References

- Mandle T, Einsele H, Schaller M, Neumann D, Vogel W, Autenrieth IB, et al. Infection of human CD34+ progenitor cells with *Bartonella henselae* results in intraerythrocytic presence of *B. henselae*. Blood. 2005;106:1215–22.
- Dehio C. Bartonella interactions with endothelial cells and erythrocytes. Trends Microbiol. 2001;9:279–85.
- Vermi W, Facchetti F, Riboldi E, Heine H, Scutera S, Stornello S, et al. Role of dendritic cell-derived CXCL13 in the pathogenesis of *Bartonella henselae* B-rich granuloma. Blood. 2006;107:454–62.
- Breitschwerdt EB, Kordick DL. Bartonella infection in animals: carriership, reservoir potential, pathogenicity, and zoonotic potential for human infection. Clin Microbiol Rev. 2000;13:428–38.
- Chomel BB, Boulouis HJ, Breitschwerdt EB. Cat scratch disease and other zoonotic *Bartonella* infections. J Am Vet Med Assoc. 2004;224:1270–9.
- Boulouis HJ, Chang CC, Henn JB, Kasten RW, Chomel BB. Factors associated with the rapid emergence of zoonotic *Bartonella* infections. Vet Res. 2005;36:383–410.
- Chomel BB, Boulouis HJ, Maruyama S, Breitschwerdt EB. *Bartonella* spp. in pets and effect on human health. Emerg Infect Dis. 2006;12:389–94.
- Mexas AM, Hancock SI, Breitschwerdt EB. *Bartonella henselae* and *Bartonella elizabethae* as potential canine pathogens. J Clin Microbiol. 2002;40:4670–4.
- Maggi RG, Harms CA, Hohn AA, Pabst DA, McLellan WA, Walton WJ, et al. *Bartonella henselae* in porpoise blood. Emerg Infect Dis. 2005;11:1894–8.

 De Medici D, Croci L, Delibato E, Di Pasquale S, Filetici E, Toti L. Evaluation of DNA extraction methods for use in combination with SYBR green I real-time PCR to detect *Salmonella enterica* serotype enteritidis in poultry. Appl Environ Microbiol. 2003;69:3456–61.

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Human *Oestrus* sp. Infection, Canary Islands

To the Editor: Myiasis due to *Oestrus ovis* is a well known zoonosis that affects a variety of animals. Human myiasis has also been described and affects mainly persons in rural areas such as shepherds (1) and farmers (2). Although this disease has been reported in both humans and mammals in Spain (3,4), no human case has been described on the Canary Islands. We describe what we believe is the first confirmed case on the islands and discuss the potential utility of serologic diagnosis for this disease.

A 55-year-old farmer from the island of El Hierro, with a medical history of hypercholesterolemia, Q fever, and murine typhus, but currently not being treated, consulted a physician in August 2005 concerning a wormlike sensation in his nose and sinuses that had lasted 2 days. Three days before noticing this sensation, he had been working in his neighbor's barn, when he noticed that a passing fly "dropped" something in his nose. He also reported sneezing and watery rhinorrhea. These symptoms were self-treated with nasal anticongestants, which provided temporary relief. He finally sought medical attention when a severe cough developed and the wormlike sensation extended to his throat.

On physical examination, the patient's vital signs were normal, although a turbinate hypertrophy and mild redness of the throat were noted. No foreign objects or insects were seen on otorhinolaryngologic examination. The patient's blood count showed 8,480 leukocytes/µL with 6.1% (520/µL) eosinophils. Because of his stated symptoms, myiasis was suspected, and symptomatic treatment was started, consisting of antihistamines, nasal anticongestants, cough suppressants, and asphyxiant methods, i.e., swallowed olive oil. The patient was monitored closely and had complete remission of his symptoms after 6 days. No relapse has occurred.

In the meantime, we discovered that a serologic test for O. ovis was available (5). We requested and obtained a convalescent-phase serum sample from the patient on day 14 of his illness. Blood was also obtained from different "healthy" animals in the patient's neighborhood, including 2 dogs, 4 sheep, and 5 goats. This serologic assay had not previously been used in testing humans. Excretory and secretory antigens from O. ovis L2 (OL2ES) were obtained as previously described (6), and samples were analyzed by an immune enzymatic assay technique (7). Appropriate testing with different dilutions of the antigens, sera, and immunoconjugates was conducted. Immunoglobulin G (IgG) was detected in the patient, sheep, goats, and dogs following a similar protocol. OL2ES concentrations were 1, 1, 3, and 5 μ g/mL, respectively. Serum samples were diluted 1:100 for the patient and the dogs and 1:50 for the goats; immunoconjugates were diluted 1:1,500 for all species. *O. ovis* IgG was found in the patient's sera, as well as in sera of the 2 dogs, 2 of 4 sheep, and all 5 goats (Table).

Human infection by *O. ovis* is generally considered to be an accidental occurrence (8). This case confirms, however, that myiasis caused by *O. ovis* must be considered in the differential diagnosis of a patient with typical symptoms and eosinophilia. Most farmers in this area have reported similar symptoms. Most, however, do not seek medical attention because they prefer to use homemade remedies, such as topical oil.

The diagnosis of oestrosis is usually made by direct visualization of the larvae, since the most frequent symptoms are pharyngeal myiasis and ophthalmomyiasis. Immunodiagnostic methods, however, could be a viable alternative to the clinical examination when no larvae are directly seen but a high degree of suspicion exists. The ELISA was noted to have a sensitivity of 96.1% and a specificity of 55.8% (positive predictive value of 86.7% and negative predictive value of 82.8%) in various investigations made with sheep and goats (6).

Although allergic symptoms are frequent in animals, the pathophysiologic process seems to be different in humans (8). Nevertheless, other authors have also described coughing and sneezing (1), probably attributable

to irritation of the mucosa. In animals, a primary peak in eosinophil numbers has been noted 4 days after infection with a primary increase 48 hours after infection (9). In humans this pattern has not been described, but we did note a mild eosinophilia that disappeared after the patient recovered from his symptoms.

Outcome of the disease in humans is generally benign. Treatment includes removal of the larvae and, in some cases, prevention of local infections. Ivermectin has also been found useful in animal and human infections (10).

To our knowledge, this is the first case of human oestrosis on the Canary Islands, as well as the first human case described with eosinophilia. Physicians should be aware of the possibility of this disease in our region and of the fact that a serologic test is available for its diagnosis.

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Human		Dogs		Sheep		Goats	
OD	Interpretation	OD	Interpretation	OD	Interpretation	OD	Interpretation
0.658	Positive	0.677	Positive	0.639	Positive	0.838	Positive
		0.824	Positive	0.685	Positive	0.535	Positive
				0.226	Negative	0.594	Positive
				0.187	Negative	0.673	Positive
						0.622	Positive

*Results are expressed as optical density (OD), and interpretation (positive/negative) was made by using the following cut-offs: in sheep: 0.369 (0.1718 + 3x 0.066); goats: 0.406 (0.211 + 3 x 0.065); human 0.32 (0.17 + 3 x 0.049); dogs: 0.493 (0.37 + 3 x 0.041).

†One sample of positive and negative control samples was added to each plate. Sheep and goat sera from animals with a known history of *O. ovis* exposure were used. When positive sera were not available (human and dogs), we used only negative sera, and the cut-off was estimated as the mean OD of the negative sera plus 3 SDs (7).

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References

- Masoodi M, Hosseini K. The respiratory and allergic manifestations of human myiasis caused by larvae of the sheep bot fly (*Oestrus ovis*): a report of 33 pharyngeal cases from southern Iran. Ann Trop Med Parasitol. 2003;97:75–81.
- Fathy FM, El-Barghathi A, El-Ahwal A, El-Bagar S. Study on human ophthalmomyiasis externa caused by *Oestrus ovis* larva, in Sirte-Libya: parasite features, clinical presentation and management. J Egypt Soc Parasitol. 2006;36:265–82.
- Beristain X, Alkorta M, Egana L, Lacasta A, Cilla G. Nasopharyngeal myasis by third stage larvae of *Oestrus ovis*. Enferm Infecc Microbiol Clin. 2001;19:86–7.
- Lucientes J, Ferrer-Dufol M, Andres MJ, Peribanez MA, Gracia-Salinas MJ, Castillo JA. Canine myiasis by sheep bot fly (Diptera: Oestridae). J Med Entomol. 1997;34:242–3.
- Sanchez-Andrade R, Romero JL, Suarez JL, Pedreira J, Diaz P, Arias M, et al. Comparison of *Oestrus ovis* metabolic and somatic antigens for the immunodiagnosis of the zoonotic myasis oestrosis by immunoenzymatic probes. Immunol Invest. 2005;34:91–9.
- Suarez JL, Scala A, Romero JA, Paz-Silva A, Pedreira J, Arias M, et al. Analysis of the humoral immune response to *Oestrus ovis* in ovine. Vet Parasitol. 2005;134: 153–8.
- Scala A, Paz-Silva A, Suarez JL, Lopez C, Diaz P, Diez-Banos P, et al. Chronobiology of *Oestrus ovis* (Diptera: Oestridae) in Sardinia, Italy: guidelines to chemoprophylaxis. J Med Entomol. 2002;39:652–7.
- Dorchies P. Comparative physiopathology of *Oestrus ovis* (Linne 1761) myiasis in man and animals. Bull Acad Natl Med. 1997;181:673–84.
- Yacob HT, Jacquiet P, Prevot F, Bergeaud JP, Bleuart C, Dorchies P, et al. Examination of the migration of first instar larvae of the parasite *Oestrus ovis* (Linne 1761) [Diptera: Oestridae] in the upper respiratory tract of artificially infected lambs and daily measurements of the kinetics of blood eosinophilia and mucosal inflammatory response associated with repeated infection. Vet Parasitol. 2004;126:339–47.
- Macdonald PJ, Chan C, Dickson J, Jean-Louis F, Heath A. Ophthalmomyiasis and nasal myiasis in New Zealand: a case series. N Z Med J. 1999;112:445–7.

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European Hedgehogs as Hosts for *Borrelia* spp., Germany

To the Editor: The European hedgehog, Erinaceus europaeus, is known to host a variety of tickborne pathogens, including the virus that causes tickborne encephalitis (1) and at least 3 species of the Borrelia burgdorferi sensu lato group: B. burgdorferi sensu stricto, B. afzelii, and B. garinii (2). Members of the *B. burgdorferi* s. 1. group are the most common vectorborne pathogens of humans in central Europe (3). The role of hedgehogs as hosts for these pathogens is, therefore, of considerable epidemiologic interest. Hedgehogs are a common synanthropic species that live in urban, suburban, and rural environments (4)and are known to carry not only the hedgehog tick, Ixodes hexagonus, but also the most common European tick, I. ricinus (2,5). Both of these ticks are known vectors of B. burgdorferi s. l. and tickborne encephalitis virus; I. ricinus is the most important vector of both throughout Europe (1,5). To date, however, only limited information has been available on the role of the hedgehog as a host or reservoir for B. burgdorferi s. l. in Germany.

We report the presence of 3 species of the *B. burgdorferi* s. l. group in European hedgehogs from Germany. To our knowledge, this is the first report of these species in hedgehogs in this country and the first report of *B. spielmanii* (A14S) (6) from this host.

The investigated hedgehogs came from 2 sources: 9 from the \approx 40 in an experimental plot in the city of Karlsruhe, state of Baden-Wuerttemberg, and the remainder from wild hedgehogs that had been brought to hedgehog care centers from various areas of Germany. All hedgehogs had died naturally, and tissue samples were taken from 43 animals (kidneys from 43, heart from 22, bladder from 33). The bodies had been frozen at -17° C before the samples were taken.

DNA isolation was done by using the Maxwell 16 Instrument and System (Promega, Madison, WI, USA). Tissue samples were $3 \times 3 \times 3$ mm. To detect B. burgdorferi s. l., we used 2 PCR protocols. The first was a nested PCR done according to the method of Rijpkema et al. (7). The target for the PCR was the spacer region between 5S and 23S rRNA genes of B. burgdorferi s. l. The nested primers generated a product of 226 bp. The amplified products were analyzed by agarose gel electrophoresis. The second protocol, a LightCycler-PCR hybridization assay (Roche Diagnostics, Mannheim, Germany) (8), simultaneously detects and genotypes the 3 genomic groups of B. burgdorferi s. l. This assay was specific for B. burgdorferi senso stricto, B. garinii, and B. afzelii (8) but also amplified B. spielmanii and B. valaisiana. The target for the PCR was the OspA gene.

The PCR products of both systems were sequenced. For DNA sequencing reaction, the fluorescence-labeled didesoxynucleotide technology (Applied Biosystems, Darmstadt, Germany) was used. The sequenced fragments were separated, and the data were collected with an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The obtained sequences were then analyzed and compared by using BLAST (www. ncbi.nlm.nih.gov/BLAST).

For 6 hedgehogs, *Borrelia* spp. could be clearly defined by using both gene sequences. Two additional animals had positive results, but sequencing was not possible because of either too little DNA or a mixed infection. *B. spielmanii* DNA was detected in the kidneys of 2 hedgehogs: 1 from Karlsruhe and 1 from 30 km west of this city in the German federal state of Rhineland-Palatinate. When sequences were compared by using BLAST, 4 BLAST sequences (AM055823, AM055822, DQ133518, AY 995900)