

***Brucella suis* biovar 2 infection in humans in France: emerging infection or better recognition?**

A. MAILLES^{1*}, M. OGIELSKA², F. KEMICHE³, B. GARIN-BASTUJI⁴,
N. BRIEU⁵, Z. BURNUSUS⁶, A. CREUWELS⁶, M. P. DANJEAN⁷, P. GUIET⁶,
V. NASSER⁵, B. TOURRAND⁸, F. VALOUR⁹, M. MAURIN¹⁰,
D. O'CALLAGHAN¹¹, V. MICK⁴, V. VAILLANT¹, M. JAY⁴, J. P. LAVIGNE^{11,12}
AND H. DE VALK¹

¹ Santé Publique France, Saint-Maurice, France

² General Hospital, Blois, France

³ General Hospital, Pontoise, France

⁴ Bacterial Zoonosis Unit, French Agency for Food, Environmental and Occupational Health and Safety, Maisons-Alfort, France

⁵ General Hospital, Aix-en-Provence, France

⁶ General Hospital, Sens, France

⁷ General Hospital, Agen, France

⁸ General Hospital, Alès, France

⁹ Lyon University hospitals, Hospices civils de Lyon, Lyon, France

¹⁰ Grenoble Alpes University Hospital, and Grenoble Alpes University, Grenoble, France

¹¹ Institute National de la Santé et de la Recherche Médicale, Nîmes, France

¹² University Hospital Caremeau, Nîmes, France

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SUMMARY

Brucellosis is usually acquired by humans through contact with infected animals or the consumption of raw milk from infected ruminants. *Brucella suis* biovar 2 (BSB2) is mainly encountered in hares and wild boars (*Sus scrofa*), and is known to have very low pathogenicity to humans with only two case reports published in the literature. Human cases of brucellosis caused by BSB2 were identified through the national mandatory notification of brucellosis. The identification of the bacterium species and biovar were confirmed by the national reference laboratory. Epidemiological data were obtained during medical follow-up visits. Seven human cases were identified between 2004 and 2016, all confirmed by the isolation of BSB2 in clinical specimens. All patients had direct contact with wild boars while hunting or preparing wild boar meat for consumption. Five patients had chronic medical conditions possibly responsible for an increased risk of infection. Our findings suggest that BSB2 might be an emerging pathogen in hunters with massive exposure through the dressing of wild boar carcasses. Hunters, especially those with chronic medical conditions, should be informed about the risk of BSB2 infection and should receive information on protective measures.

Key words: *Brucella suis* biovar 2, brucellosis, emerging diseases, wild boar, zoonosis.

* Author for correspondence: A. Mailles, Santé publique France, 12 rue du Val d'Osne, 94415 Saint Maurice cedex, France. (Email: Alexandra.mailles@santepubliquefrance.fr)

INTRODUCTION

Brucellosis is a zoonotic disease caused by Gram-negative bacilli of the genus *Brucella*. The main sources and routes of infection in humans are (1) contact with animal abortive materials responsible for exposure through small skin wounds, (2) the inhalation of bacteria in aerosols that may be generated following abortion or when dressing a carcass and (3) oral exposure through the consumption of animal food products from infected animals (raw milk, offal, etc.) [1]. Ruminants are the usual reservoir of *Brucella melitensis* and *Brucella abortus*, and pigs the main reservoir of *Brucella suis*. *Brucella suis* biovar 2 (BSB2) is encountered in hares, wild boars (*Sus scrofa*) and pigs in open-air farms and has been reported in continental Europe only; it is considered to be barely pathogenic in humans, as only two case reports have been reported in the literature [2, 3].

France has been officially brucellosis-free in cattle since 2005 and the last outbreak in sheep or goats was identified in 2003. Most human cases diagnosed in France are 'imported cases' infected while visiting endemic countries [4]. A limited number of domestic cases occur in laboratory workers or elderly patients with latent infection reactivation [4]. In France, swine brucellosis disappeared in the 1970s with the industrialization of pig breeding farms. BSB2 re-emerged in France in pig herds in the 1990s following the development of open-air farms. From 1993 to 2001, 26 pig herds infected with BSB2 were identified in 22 different districts of mainland France [5].

A study was implemented in 2004 among farmers and their families exposed to pigs infected naturally by BSB2 to assess the risk of transmission to humans in close contact with infected animals. People working or living on 14 farms with pigs infected by BSB2 were examined for *Brucella*. None of the study subjects reported symptoms suggestive of *Brucella* infection and no case of brucellosis was identified [6]. These findings suggested low pathogenicity of BSB2 to humans.

Unexpectedly given these results, seven human cases of brucellosis caused by BSB2 were identified in France between 2004 and 2016. We report the clinical and biological details of these patients, as well as their risk factors, and propose recommendations to avoid the occurrence of further cases.

METHODS

Cases identification and definition

Human brucellosis is a mandatory notifiable disease in France. Physicians and microbiologists must notify

cases to the regional health agency and notifications are centralized at the French public health agency. For mandatory notification, a case of brucellosis is defined as a patient presenting with symptoms or clinical signs consistent with brucellosis and,

- (1) for a confirmed case, the isolation of a *Brucella* sp. strain from any clinical sample,
- (2) for a probable case: a fourfold or greater increase in *Brucella* antibody titers between acute and convalescent phase serum specimens obtained at least 3–4 weeks apart, or the detection of *Brucella* DNA in a clinical specimen by PCR,
- (3) for a possible case: a single elevated serum *Brucella* total antibody titer.

Confirmation of brucellosis diagnosis

For patients with confirmed brucellosis, isolated strains are systematically sent to the National Reference Center (NRC) where the species and biovar are determined by a combination of phenotypical tests (colonial morphology, Gram staining, growth characteristics, CO₂ requirement, H₂S production, urease and oxidase activities, slide agglutination with mono-specific sera (anti-*A*, anti-*M* and anti-*R*), dye sensitivity (basic fuchsin and thionin) and phage lysis (Tb, Wb, Iz, R/C) [7].

Due to the low specificity of in-house serology and commercial serological kits, and the low prevalence of brucellosis in France, the positive predictive value of a serological test is very low. Therefore, all positive serological results have to be confirmed by the NRC. The suspected sera are sent for analysis at +4 °C or –20 °C. They are kept frozen at –20 °C. Different techniques are used to corroborate the diagnosis: the two classical brucellosis serological diagnoses (Rose Bengal test and Wright test) and other more sophisticated tests (competitive ELISA, Brucellacapt, lateral flow immunochromatography and indirect immunofluorescence for IgM and IgG detection) [7–11].

Cases investigation and data collection

The standard notification form includes brief clinical information, biological diagnostic details and at-risk exposures in the 6 months before onset of symptoms.

When no at-risk exposure is mentioned on the form, or in the case of unusual findings such as the identification of BSB2, a detailed standardized questionnaire is completed with both the attending physician and

the patient to identify the possible origin of the contamination.

Data management

All variables included in the notification forms were input using Voozanoo© and analyzed with Stata12©. Due to the limited number of cases, only a descriptive analysis was performed.

Ethical requirements

The national surveillance of human brucellosis is carried out with the approval of the French Commission for Data Protection ('Commission Nationale Informatique et Liberté'). Nominative data are deleted 1 year after data have been collected.

RESULTS

From 1 January 2004 to 1 June 2016, seven patients with a diagnosis of brucellosis due to BSB2 confirmed by the NCR were notified to Santé publique France, the French National Public Health Agency (formerly known as Institut de Veille Sanitaire).

The seven patients represented 3% of all brucellosis patients ($N = 240$) identified during the period, and 25% of domestic cases ($N = 28$). None of the patients were related and cases were diagnosed in seven different regions of mainland France.

The patients were six men and a woman, with a median age of 68 years (range 43–76); only one patient was under 60 (Table 1). The six male patients were wild boar hunters. The only female patient had not hunted but handled, prepared and cooked a piece of a wild boar carcass. Three patients had chronic medical conditions that were potentially immunocompromising or known to be associated with an increased risk of infectious diseases, and two patients were diagnosed with type 2 diabetes during the hospitalization for brucellosis.

Clinical and diagnostic details are presented in Table 1. Three patients had acute brucellosis without focalized infection: in these three patients, blood cultures were positive for BSB2. Three others presented with arthritis (spondylodiscitis in two and hip arthritis in one): BSB2 was isolated from intervertebral disc and vertebral biopsies of the two patients with spondylodiscitis, and from the synovial fluid of the patient with hip arthritis. The seventh patient presented with

an abscess of soft tissues: BSB2 was isolated from pus collected from the abscess.

One patient had died at the time of notification, but death was considered to be a direct consequence of the lymphoma he was treated for and to be unrelated to brucellosis.

DISCUSSION

We report here on the largest number of human brucellosis cases due to BSB2 ever published. Only two cases were previously published in the literature. In 1989, BSB2 was isolated in France from a culture of blood obtained from a pig breeder with no relevant medical history [2]. The authors concluded that the origin of the infection was unclear as the pigs were apparently healthy. No information was available for this patient concerning a possible history of hunting. In 1998, Paton *et al.* reported BSB2 infection in a Chinese woman with diabetes mellitus and hypertension, who died from brucellosis 20 years after her last contact with farm animals [3]. BSB2 was also isolated from blood culture in this case. This case was remarkable as BSB2 has never been reported in pigs or wildlife in Asia.

In contrast, the seven patients reported here were exposed to wildlife, namely wild boars, but not to domestic pigs. Six patients were hunters with probable repeated and massive exposure to BSB2 as they dressed (namely skinned and gutted) wild boars after hunting without any individual protection. The seventh patient had been exposed only once to wild boar carcass and never to domestic pigs.

The identification of seven cases of infection by BSB2 was also unexpected with regards to the absence of symptomatic cases among farmers with daily exposures to pigs infected with BSB2 in a 2005 study, and the very low prevalence of anti-*Brucella* antibodies among them [6]. In this study, 58 people with close contacts with pigs infected by BSB2 were screened for anti-*Brucella* antibodies. They were the farmers, household members and employees of 14 pig farms with animals diagnosed positive for BSB2 infection. Among the 58 exposed subjects, none had experienced symptoms evocative of brucellosis after the diagnosis was obtained in the pigs. Only three individuals (5%) had antibodies against *Brucella* (IgG), although all had been massively exposed to infected pigs and their excreta [6]. Among these three, two displayed titers compatible with the timeline of infection of their herds. Two of the three persons with antibodies

Table 1. *Clinical details, diagnosis and at-risk exposures of patients infected by BSB2, France 2004–2016*

ID	Sex, age	Comorbidities/medical history	Clinical presentation	At-risk exposures	Delay-onset bacterial isolation	Site of isolation
1	Male, 43 y.o.	Systemic lupus erythematosus treated by high-dose steroids, resulting in bilateral femoral head necrosis and unilateral humeral head necrosis. Bilateral hip replacement occurred a couple of weeks before onset	Hip arthritis requiring prosthesis removal and spacer placement	Wild boar and hare hunter	3 months	Hip arthritis fluid
2	Male, 69 y.o.	Silicosis (20%), type 2 diabetes (under treatment), hypertension	Intermittent fever 39.5 °C, excessive sweating, asthenia, weight loss (3 kg in a 2-week period), muscle pain (legs)	Wild boar hunter, dressing an average of 100 wild boars a year, did not use individual protections, last exposure 11 days before onset [6]	17 days	Blood culture
3	Male, 73 y.o.	Recent history of T-cell lymphoma, with toxic chemotherapy-related cardiomyopathy requiring the placing of a pace maker	Bilateral abscesses of psoas and peri-aortic inflammation detected by PET scan while investigating sepsis. The patient reported back pain present for a few weeks. No other cause could be found for the sepsis. Endocarditis was excluded following TOE and TTE	Used to be a wild boar hunter. No hunting or exposure to wild boars after the diagnosis of lymphoma 1 year before brucellosis diagnosis	Several weeks (back pain)	Psoas abscess pus
4	Male, 76 y.o.	Hypertension, stroke	Spondylodiscitis T11/T12, no fever, no neurological signs, weight loss (6 kg). History of lower back pain for 8 months	Wild boar and deer hunter, regularly dressed kills	Estimated 8 months	Intervertebral disc and vertebral biopsies
5	Female, 67 y.o.		Fever 39 °C, asthenia, bacteremia	Not a hunter, but prepared only once a freshly skinned piece of wild boar given by a hunter just after the hunt	12 days	Blood culture
6	Male, 63 y.o.	Hypertension, bilateral hip replacement Type 2 diabetes and congestive cardiac insufficiency diagnosed during hospitalization	Spondylodiscitis L3–L4 with major back pain but no fever, no neurological symptoms. History of back pain for months	Wild boar and red deer hunter	6 months and 14 days	Intervertebral disc and vertebral biopsies
7	Male, 63 y.o.	Hypertension, heavy smoking, history of alcohol abuse	Fever 40 °C, recent lower back pain but normal spine MRI, night sweating, weight loss (8 kg)	Wild boar hunter, last at-risk exposure 2 months before onset	2 days	Blood culture

TOE, transesophageal echocardiography; TTE, transthoracic echocardiography; y.o., years old.

against *Brucella* were wild boar hunters and had skinned wild boars carcasses. None of the 58 ever presented with symptoms evocative of brucellosis or had a BSB2 strain isolated from a clinical sample. However, serology cannot distinguish between different species of *Brucella*, and in the absence of any isolated strains, we cannot with certainty attribute the antibody response to an infection with BSB2.

It is remarkable that five of the seven patients reported in this article had comorbidities that could increase susceptibility to infection, including severe immunocompromising conditions (treated systemic lupus, lymphoma). In these five cases, the medical conditions at the time of exposure may be responsible for an increased susceptibility to the infection, and be one of the determining factors for their disease caused a bacterium usually considered as non-pathogenic for human beings. However, the two other patients had no known medical conditions increasing their risk of infection, suggesting that BSB2 infection can also occur in previously healthy patients.

Moreover, wild boar hunters usually skin and gut the animals without any protections, such as gloves and masks, and they usually do not clean the tools (knives) used for skinning and gutting. At least two patients in our report had a very frequent risk of exposure and possibly high inoculum, as they were preparing the carcasses of the animals hunted by themselves and by the other hunters of their hunting group. The four other male patients had been hunting for years, and therefore also had at-risk exposures although possibly less frequent. Finally, the only female patient reported a unique exposure to a piece of a wild boar carcass. She was not a hunter and did not regularly cook wild boar meat, but she might have been exposed to a high inoculum on this unique occasion and she might have handled the meat for a long time if she was not used to preparing it. All of these patients had epidemiological data supporting a possible contact with BSB2.

Therefore, both the possible exposure to a high inoculum of bacteria and/or their deteriorated immune system or health might have contributed to the infection and the occurrence of the disease in the seven patients reported here.

The clinical symptoms of the patients reported here were very classical and did not suggest that human brucellosis due to BSB2 might present differently from brucellosis due to *B. melitensis* or *B. abortus* or even *B. suis* biovar 1. Three patients had focalized brucellosis and had experienced symptoms for months before seeking

medical attention, which was consistent with chronic brucellosis. In contrast, the three patients with positive blood cultures had no focalized infection and presented with fever at the time of the diagnosis. The delay between symptom onset and diagnosis of the seventh brucellosis case is more difficult to assess as he was suffering end-stage cancer, making it difficult to precisely give a date for the onset of symptoms of brucellosis. In the French case report in 1989, the clinical presentation was consistent with brucellosis although not specific: the patient presented with asthenia, fever and excessive sweating [2]. By contrast, the patient reported from China in 1998 experienced fever and chills associated with severe liver failure and disseminated intravascular coagulation (DIVC), and died [3]. Liver involvement is classically described in brucellosis but is usually not severe and not associated with DIVC.

Finally, only the specific exposure to wild boar carcasses and the existence of immunocompromising comorbidities seem to distinguish brucellosis due to BSB2 in humans from other types of Brucellosis. This is an important finding as it can result in specific recommendations to prevent further cases in this population. It also highlights that BSB2 in wildlife has a limited but true impact on human health.

Apart from the Chinese patient diagnosed in 1998, all reported cases were diagnosed in France although BSB2 is known to be enzootic in wild boars and hares in a number of other European countries [12–18]. No other European country ever reported human cases, which is surprising considering the occurrence in wildlife. One can hypothesize that other national surveillance systems do not systematically identify the species and biovar of *Brucella* strains isolated in humans, since this information is not of importance for the clinical management of the patient. Under this assumption, it is possible that the incidence of brucellosis due to BSB2 in Europe may be underestimated.

CONCLUSION

We report in this article the largest case series of human brucellosis cases due to BSB2. Our findings support the role of BSB2 as a pathogenic bacterium in humans with specific risk factors, namely chronic or immunocompromising conditions, and the exposure to wild boar carcasses and organs. Because the infection is enzootic in wild boars and hares, and the number of people with chronic medical conditions is increasing, it is likely that more cases will be identified. It is also possible that some cases might occur

in other European countries but might go unnoticed because of an incomplete identification of *Brucella* strains, but studies are needed to confirm our hypothesis. The zoonotic aspect of BSB2, although limited, should be taken into account for the management of brucellosis in wildlife. Finally, recommendations will be given to the hunting societies in France: first, hunters should wear gloves and masks when skinning and gutting wild boar and hare carcasses, and second, hunters with chronic conditions should avoid these high-risk exposures. Moreover, general hygiene practices apply for game meat cooking: any skin wound of the food handler should be covered before manipulating the meat, and hands should be washed after handling raw food products.

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DECLARATION OF INTEREST

None.

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