Re-emergence of brucellosis in cattle in France and risk for human health

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A case of human brucellosis was diagnosed in France in January 2012. The investigation demonstrated that the case had been contaminated by raw milk cheese from a neighbouring dairy farm. As France has been officially free of bovine brucellosis since 2005, veterinary investigations are being conducted to determine the origin of the infection and avoid its spread among other herds. Hypotheses about the source of this infection are discussed.

In January 2012, a human case of brucellosis was diagnosed by blood culture in a district of the French Alps. The isolated strain was identified as *Brucella melitensis* biovar 3. The patient had presented in late November 2011 with non-specific symptoms that had been ongoing since that date. Usual at-risk exposures were investigated: recent or ancient travel in an endemic/enzootic country, consumption of raw milk or raw milk products imported from an enzootic country, professional or accidental exposure to *Brucella* strains in a laboratory, direct contact with animals, etc. As the patient had not had such an exposure at any point before, the case was considered to be an autochthonous case of acute brucellosis of undetermined origin.

In April 2012, brucellosis was confirmed in a dairy cow in a herd of the same district of the French Alps. The seropositive cow had aborted in late January, and a strain of *Brucella melitensis* biovar 3 was isolated from the milk sampled from the animal. The animal belonged to a herd 21 dairy cows, and no other animal in the herd presented with symptoms suggesting brucellosis or showed any serological reaction. Approximately 20 kg of Reblochon cheese (soft raw milk cheese) are usually produced daily on the affected farm.

Brucellosis surveillance in France

France has been officially free of brucellosis in cattle since 2005, and the last outbreak of brucellosis in sheep and goats was reported in 2003. In order to detect and prevent any re-emergence of the disease, annual screening using Rose Bengale test or complement fixation test is carried out in all cattle, sheep and goat farms producing raw milk as well as in all cattle herds, and every one to three years in small ruminant, according to EU regulations [1-4]. Moreover, abortion in ruminants is mandatorily notifiable and the investigation of abortion includes examination for brucellosis.

Human brucellosis in France is mandatorily notifiable. The National Reference Centre (NRC) determines the characteristics of *Brucella* strains isolated from patients [5,6]. Serological suspicions also have to be confirmed by the NRC, as the low specificity of available tests can be responsible for false-positive results. The confirmation is carried out using a combination of in-house tests including Rose Bengale test, immunoassay, complement fixation test, and specific detection of antibodies against *Yersinia enterocolitica*.

Veterinary investigation

All animals were tested serologically (Rose Bengale test, complement fixation test and indirect enzyme linked immunosorbent assay) before slaughter in April [5]. Following French regulations, all animals in the infected herd were immediately slaughtered, and three pairs of lymph nodes (retro-pharyngeal, retro-mammary and internal iliac) were sampled from all animals for *Brucella* culture [5] and PCR [7]. All animals were seronegative with the exception of the index animal which showed a very strong reaction in all three tests. However, *Brucella* was isolated from a second animal in the herd, and PCR-positive results were obtained for

four further animals, in addition to the index animal and the second cow with an isolation of *Brucella*.

Following the confirmation of brucellosis in the cow, a trace-back investigation was implemented by the veterinary services to determine the origin of the contamination of the herd. The animals of the infected herd had not taken part in a transhumance nor did they graze with other herds on the same pastures. Other neighbouring farms as well as farms that had traded animals with the infected farm in the year before the outbreak were investigated. All tested negative in serology [5].

A trace-forward investigation was also carried out to determine the places of distribution of cheese produced at the affected farm since the abortion of the cow.

Reblochon cheese is a raw milk soft cheese, requiring a maturation period of three weeks to one month. The cheese from the affected farm had been commercialised after the abortion in seven districts. Cheese was sold directly at the farm, and as whole pieces or in parts in supermarkets. Cheese produced by the affected farm had not been exported to other countries but might have been bought by foreign tourists during their winter holidays in several ski resorts in the area. For this reason, the European rapid alert system for food and feed (RASFF) was informed.

Human investigations

After the identification of the first bovine case, the human case was interviewed again to investigate any direct or indirect epidemiological link with the infected herd. During the second interview, it became clear that the patient and their family had visited the infected farm in autumn 2011, although it was not possible to determine the exact date. During this visit, the family had bought *Tome Blanche* cheese, a fresh cheese obtained during the first step of Reblochon production. The four family members had shared the *Tome Blanche* on the same day, but the index case was the only one who later presented with symptoms. The other three family members were serologically investigated in May 2012 and only one presented with a positive high titre in agglutination (1,600). The farm reported no other visitors during that period, apart from neighbours.

Microbiological investigations

The strain isolated from the human case and from the two cows both belonged to *Brucella melitensis* biovar 3. The strains had the same genotype as determined by multilocus variable number tandem repeat analysis (MLVA) [8].

Control measures

All cheese pieces produced by the affected farm and still within the shelf life were withdrawn from retailers. In addition, a recall of already sold products was carried out via a national press release by the cheese producer and by posters in the sale points. Medical doctors in the concerned districts were informed by the regional health authorities. Consumers of these products were advised to seek medical attention should they present symptoms consistent with brucellosis.

The release of cheese from the affected farm was immediately stopped. The movements of animals from other herds that had epidemiological links with the infected herd (those that were geographically close to the infected herd, or had been bought from the infected herd) have been restricted until the end of the investigation. Furthermore, raw cheese products from farms with epidemiological links to the infected farm were put on sale only after negative bacteriological tests results had been obtained.

Reinforcement of human surveillance

Notification of human brucellosis is mandatory in France. All notified human cases in France have to be confirmed by the national reference laboratory. From 2002 to 2011, 219 human cases were confirmed in France. Among them, 183 (84%) were patients infected through the consumption of raw milk products or direct contact with animals in (or from) countries with enzootic brucellosis, 14 (6%) were laboratory workers infected through the handling of *Brucella* strains, 17 (8%) were relapses in people with past infection, while the origin of contamination could not be determined for five patients (2%) [9].

Because the investigation of the origin of the human case diagnosed in January 2012 had been inconclusive, it was decided to reinforce the surveillance immediately. Since January 2012, all notified suspected cases have been interviewed with a trawling questionnaire before the diagnosis was confirmed. Since April 2012, any epidemiological link with the infected herd has been systematically investigated. No other related human cases have been identified so far.

Discussion

At this time, several hypotheses can be proposed to explain the re-emergence of brucellosis in cattle in France. One explanation is contact with an infected cattle or small ruminant. Knowing that the affected herd had not received any imported animals, it needs to be investigated whether animals had been introduced in one of the herds that sold animals to the affected farm or whether the affected herd had been in contact with animals of neighbouring farms. Another hypothesis would be a contamination of cattle by wildlife. Some chamois (*Rupicapra rupicapra*) were found infected with *B. melitensis* biovar 3 in 1988 in the Alps, and some of these animals may have become chronically infected and not display symptoms [10]. However, no infected chamois has been identified in the last 10 years, despite several serological surveys (Garin-Bastuji, personal communication, July 2012). *B. melitensis* biovar 3 is the most common biovar isolated in ruminants worldwide, and therefore the identification of this biovar in a district like the French Alps

with many different ruminant species cannot contribute to a more precise hypothesis.

The veterinary investigations are still ongoing to determine the origin of the contamination of the herd, to investigate the possible spread of the infection to other herds and to take control measures to avoid the infection of new herds and consequently the occurrence of additional human cases.

However, the absence of infected animals in the herds that are epidemiologically linked with the infected herd, and the absence of other autochthonous human cases argue in favour of a single outbreak and a limited episode. The index animal on the farm was born from a dam that itself was born in 1999 before the last outbreak in the area and died in 2006. The lifetime of the mother of the index infected animal is therefore consistent with the hypothesis of a congenital case of bovine brucellosis [11].

In addition to the investigations already carried out, all herds coming back from transhumance in the concerned district will be serologically screened during the fall. Serological tests lack specificity but they have a good sensitivity and are of good value to detect recent or active infections. The index animal had an active infection demonstrated by *Brucella* excretion in milk. This animal displayed a high level of antibodies in relation with the active although possibly chronic infection. During the early investigation, a *Brucella* strain and *Brucella* DNA were detected in ganglions of seronegative animals, demonstrating chronic latent infections, with no antibodies. Strengthened surveillance of human and animal brucellosis will be maintained until the end of the investigations.

The surveillance of human brucellosis in non-endemic countries is complicated by the lack of specificity of serological tests [12-16]. In our experience, all available tests still may cross-react with other bacteria (mainly *Y. enterocolitica*, but not only), and can also give false positive results in patients presenting with immune disorders. In countries with low prevalence and incidence of the disease, this low specificity contributes to the low positive predictive value of serology. A positive diagnosis has important consequences for the patients (long antimicrobial therapy with possible adverse effects and ecological consequences on intestinal bacteria), and for the dairy animals (culling of the entire herd in our country). It is therefore important to obtain as much evidence as possible to confirm a serological diagnosis.

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