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Cross-border molecular tracing of brucellosis in Europe

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ABSTRACT

To assess the general impact of endemic countries on the re-emergence of brucellosis in non-endemic regions of the European Union, the genetic fingerprints of *Brucella melitensis* strains imported to Germany were compared to ovine strains from Turkey in a molecular epidemiological study.

Genotyping of 66 *Brucella* strains (based on Multiple Locus of Variable number of tandem repeats Analysis) isolated from German travellers and Turkish immigrants living in Germany revealed epidemiological concordance with 20 sheep isolates originating from Eastern Anatolia, Turkey.

In summary, cross-border molecular tracing confirmed brucellosis being a zoonosis of concern for European public health.

1. Introduction

Brucellosis, a severe feverish multi-organ disease, is still one of the most common zoonoses worldwide [1]. The bacterial pathogen is transmitted from various animal reservoirs to humans by direct animal contacts or most often through the consumption of raw animal products such as unpasteurized milk and cheese [2]. Three out of 10 currently known *Brucella* (*B.*) spp. are responsible for the main burden of human disease i.e. *B. melitensis* (transmitted from sheep and goats), *B. abortus* (from cattle and other bovids), and *B. suis* (from pigs). Ovine brucellosis endemically occurs in countries surrounding the Mediterranean Sea, especially along its northern and eastern shores stretching through Central Asia.

The etiologic agent *B. melitensis* is by far the main cause of clinically apparent infections in Turkish natives [3]. In the last decade, the officially reported annual incidence rates in Turkey ranged from 15 to 25/100,000 population summing up more than 10,000 human cases per year [4]. However, only about one third (31.8%) of the serologically diagnosed brucellosis cases are notified to Provincial Health Directorates leading to significant underreporting of the disease [5]. Spatial analysis of human brucellosis cases notified to the Turkish Ministry of Health from 1996 through 2006 identified Eastern and Southeastern Anatolia as high-risk regions with an ongoing uptrend

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of local incidence rates [4]. A major disease cluster was reported from Van province which is located within Eastern Anatolia sharing a border with Iran. Animal migration between Turkey and neighbouring countries such as Iraq, Iran and Syria where brucellosis is known to be highly endemic maintain regional dissemination of the disease. Furthermore, sheep and goats are traditionally kept on small-scale family farms in Turkey which may also hamper the control of ovine/caprine brucellosis. Van is characterized by the highest stock density of the country [4]. Once a year at the Islamic "Festival of Sacrifice" sheep from this province are distributed all over Turkey. In a recently published study from Kars, a province north of Van, a total of 87.5% of the flocks with a history of abortion and about 35% of the unvaccinated sheep were tested positive for anti-*Brucella* antibodies [6]. Furthermore, up to 18% of the farmers and veterinarians were seropositive [7]. In the province of Erzurum neighbouring Kars in East Turkey, a cross-sectional study on human brucellosis in 2005 revealed a seroprevalence of 5.4% [8]. However, these point estimates of brucellosis prevalence both in animals and humans have not been obtained from well designed probabilistic studies which may limit conclusions about the real endemicity of this zoonotic disease in Turkey. In a nationwide survey, conducted in 2010, a herd prevalence of 7.8% and 22.5% in cattle and sheep/goats was ascertained, respectively (National *Brucella* Reference Laboratory, Pendik Veterinary Control and Research Institute, Istanbul, *personal communication*). Hotspots were again identified in the Eastern provinces of the country. Because of these alarmingly high prevalence rates a mass vaccination program was established which will be started in 2012. In preceding disease control programs launched in the early eighties only young animals were vaccinated.

Ovine brucellosis is not known to be enzootic in the United States, Canada, Northern Europe, Australia, New Zealand or Southeast Asia and only sporadic incursions have been notified in these regions (surveillance data can be retrieved from the World Animal Health Information System: http://web.oie.int/wahis/public.php). In contrast to Turkey, Germany has been "officially free from ovine/caprine and bovine brucellosis" since 2000. Nevertheless, about 30 human cases are annually reported and the mean annual incidence of human brucellosis in Germany ranges between 0.02 and 0.04/100,000 population (Robert Koch-Institut: SurvStat, http://www3.rki.de/SurvStat, date of query: 1st August, 2011). The incidence rate is known to be significantly higher in the Turkish segment of the German population with 0.3/100,000 population [9]. Turkish immigrants are mainly at risk to contract *Brucella* infection while visiting their homeland.

Because of its geographic position at the gateway between the Middle East and Europe, Turkey and especially Eastern Anatolia may play a key role in the re-emergence of brucellosis in the European Union [10]. To assess the general impact of this interface the genetic fingerprints of *B. melitensis* strains imported to Germany were compared to ovine strains from Turkey in a molecular epidemiological study.

2. Methods

2.1. Brucella strains

To trace German human brucellosis cases to a potential animal source in Turkey, genotyping data of 20 *Brucella* strains isolated from sheep in the endemic region of Eastern Anatolia, province of Van (Fig. 1), were compared to 66 strains isolated in Germany either from travellers returning from Turkey or more often from Turkish immigrants (data partially published in Al Dahouk et al. [11]). Turkish and "German" strains were isolated in overlapping time periods, 2004–2007 and 1996–2010, respectively.

2.2. Identification of variable number of tandem repeat loci

Genotyping was performed with a Multiple Locus of Variable number of tandem repeats Analysis assay based on 16 markers (MLVA-16) essentially as previously described [11,12]. The assay comprises eight moderately variable minisatellite markers (panel 1) to trace back the geographic origin and eight highly polymorphic microsatellite markers useful for outbreak investigations (divided into two panels, 2a and 2b, according to their diversity index). MLVA-16 has already proven its discriminatory power in *Brucella* strains collected on a global scale [13].

2.3. Data analysis

The cluster analysis (Fig. 2) was based on the categorical coefficient and unweighted pair group method using arithmetic averages (UPGMA). The same weight was given to a large and small number of differences in the repeats at each locus. Three different character data sets were defined and combined using the composite data set tool provided by Bionumerics version 6.5 (Applied-Maths, Belgium). A different weight was given to the markers depending on the panel they belong to i.e. panel 1 markers got an individual weight of 2 (total weight for panel 1: 16), panel 2a markers got a weight of 1 (total weight for panel 2a: 3), and markers of panel 2b got a weight of 0.2 (total weight for panel 2b: 1).

3. Results and discussion

The province of Van which is an area of intensive animal husbandry and a hotspot of ovine brucellosis in Turkey was supposed to be one of the sources of disease distribution within the country, across its borders and potentially throughout Europe by contaminated food products or infected travellers. At the Frankfurt Airport about 17,500 kg of illegally imported animal products are confiscated from arriving airline passengers every year and these figures are probably just the tip of the iceberg. From 1995 to 2005, 74% of the human *Brucella* infections notified to public health authorities in Germany were acquired in endemic regions, most frequently in Turkey [11]. In 26% of the reported cases, the origin of infection was not associated with a travel history and therefore a source within the country has to be assumed [11]. Since Germany is known