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Evidence of ongoing brucellosis in livestock animals in North West Libya

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ABSTRACT

Animal brucellosis is thought to be present in small ruminants, cattle, and camels in Libya, particularly in the west coastal strip. Before the system collapsed due to political unrest in 2011, prevalence of the disease did not exceed 0.2% in cattle, 0.1% in camels, 8.3% in sheep, and 14.8% in goats. The aim of this study was to highlight outbreaks of disease that took place during the 18-month period from November 2014 to April 2016. A total of 1612 serum samples, collected opportunistically from 29 herds in 12 different localities in the northwest region of Libya, were investigated for brucellosis. The samples were screened for *Brucella* antibodies using the Rose Bengal test, and confirmed with either indirect enzyme linked immunosorbent assay in the case of sheep, and/or a serum agglutination test, followed with a complement fixation test, in the case of cattle and camels. Our results showed the highest rates of brucellosis seropositivity in goats (33.4%) and sheep (9.2%). The overall percentage of brucellosis seropositivity was 21%. The high level of brucellosis identified by this study, particularly in small ruminants, strongly suggests re-emergence of the disease in the region. Re-evaluation of intervention measures applied to the control of brucellosis is highly recommended.

1. Introduction

Brucellosis is a contagious, zoonotic, food-borne disease, associated with serious complications in both animals and humans [4], although it is not prioritised by national and international health systems and remains a neglected zoonotic disease [23], particularly in developing countries [9]. It is caused by members of the genus *Brucella*, intracellular Gram-negative pathogens with a range of host species preferences. The species most frequently associated with human disease are *Brucella melitensis*, *Brucella suis*, *Brucella abortus*, and, to a lesser extent, *Brucella canis*. *B. melitensis* biovars 1, 2, and 3 are the most frequently isolated strains in small ruminants in Mediterranean and Middle-East countries [3,17], and may also cause abortion in cattle [13]. In animals, brucellosis can cause significant economic loss due to abortion, premature birth, reduced fertility, and decline in milk production [10]. Animals infected with brucellosis can go through an incubation period of 14–180 days before showing clinical signs. Infected lactating animals shed high amounts of bacteria following parturition or abortion, and high levels are present in the aborted fetus. These represent the most likely reason for the circulation of brucellosis within and between herds [5,24]. Humans acquire the disease through direct contact with livestock and consumption of their products, mostly raw milk and dairy products made from unpasteurised milk [1,2,12]. Brucellosis is also considered an occupational hazard to those engaged in handling infected animals, such as veterinarians, laboratory staff, farmers, and abattoir workers [11]. Brucellosis in humans presents various diagnostic difficulties because it mimics many other diseases, and thus likely remains under-reported and untreated [20], contributing to the chronic nature of the disease [8]. It has been suggested that most cases of human brucellosis remain unrecognised and are treated as a fever of unknown cause [18]. Despite the fact that the disease has been eradicated in many developed countries, it is considered a re-emerging zoonosis in some areas, especially in the Mediterranean region, western Asia, parts of Africa, and Latin America, impacting on human health and livestock production [4,14]. The aim of the current study was to investigate the current status of brucellosis in the northwest region of Libya. The study objectives were: (1) to confirm the ongoing presence of brucellosis in various animal populations in North West Libya; (2) to draw the attention of the government to the need to re-evaluate response and intervention measures applied to control of brucellosis in Libya; and

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(3) to raise public awareness of the risks related to the disease for animals and humans.

2. Methods

2.1. Study design

The study, designed to highlight the disease status, took place during an 18-month period from November 2014 to April 2016. A total of 1612 animals (1322 sheep and goats, 255 cattle, and 35 camels) were sampled. These animals belonged to 29 herds in 12 different localities in the northwest region of Libya (Tajura, Aljmail, Misratah, Algaraboli, Tripoli, Tarhunah, Bani Walid, Al Jfara, AL-Zawiya, Al-Kums, Yefren, and Alzentan). All studied animals had no history of vaccination, as vaccination programmes have been prohibited in Libya since 2007. The methods of sampling were not randomised, and therefore this study is not designed to assess overall prevalence levels but instead it represents case-based reporting in order to highlight the significance of brucellosis in this region. Samples were collected following the report of abortion cases by the owners in cases of cattle and small ruminants (sheep and goats), or based on a request for testing by farmers, such as in the case of the camel herd in this study. Blood samples were collected by government veterinarians and sent in cold containers to the Tripoli branch of the National Centre of Animal Health (NCAH), where they were refrigerated until use within 24 h of collection.

2.2. Laboratory workflow

A simple scheme was proposed by the NCAH for the diagnosis of animal brucellosis in accordance with the procedures in the Manual of Standard Diagnostic Tests and Vaccination, recommended by the World Organisation for Animal Health (OIE) [16]. All serum samples from reported suspected cases were initially screened for *Brucella* antibodies by Rose Bengal plate test (RBPT) antigens. In the case of cattle and camels, positive tests were further examined with the serum agglutination test (SAT) and confirmed by the complement fixation test (CFT). In the case of small ruminants, positive RBPT samples were further confirmed by indirect enzyme linked immunosorbant assay (iELISA). Following confirmation of positive cases, rescreening of whole herds was requested, and they were tested in the same manner.

2.3. Serological testing

Using a commercially bought Rose Bengal*Brucella* antigen (Institut Pourquier, Montpellier, France), 30 μL of plain serum were dispensed onto a plate and carefully mixed with an equal volume of the Rose Bengal test (RBT) antigen. The plate was then agitated for 4 min and read immediately (as per manufacturer's instructions). The SAT *Brucella abortus* antigen and the positive and negative control sera (Animal and Plant Health Agency, Weybridge, UK) were used to detect *Brucella* antibodies following the manufacturers instructions. An SAT titre of 1:40 (final serum dilution) with one + (25% agglutination) was regarded as suspicious, and the test was repeated after 3 weeks, while a titre of 1:40 with two ++ (50% agglutination) or greater was considered positive for brucellosis and was further confirmed with a CFT. Due to the complexity of the CFT, the test was performed periodically and according to the OIE Terrestrial Manual (2012) [16]. Sera giving a titre equivalent to 20 ICFTU/mL (international complement fixation test units per millilitre) or more were considered to be positive. The iELISA for detection of brucellosis was performed according to the manufacturer's instructions (IDvet Innovative Diagnostics, Grabels, France).

3. Results

A total of 1612 samples were collected and analysed from 29 herds distributed over 12 provinces in the northwest region of Libya, and provided clear evidence of the ongoing presence of brucellosis. Table 1 illustrates the percentage of brucellosis cases among different animal species in the northwest region of Libya. The herds were mixed and included four species: cattle, camels, sheep, and goats, regardless of the province of origin. The overall rate of positive cases of brucellosis in the 1612 tested animals was 21%. The rate of brucellosis seropositivity varied among the four species. The rate of brucellosis seropositivity of tested goat samples was 33.4% (n 854). This was considerably higher than the proportion recorded in the tested sheep samples (9.2%, n = 468), (p = 0.005). The positive rate for camel and cattle samples were similar (5.3% and 4.7%, respectively), both of which were noticeably lower than the rate of brucellosis seropositivity recorded in sheep and goats. High numbers of goats testing positive for brucellosis were found in farms in the Tarhunah province (204 tested animals, 38% positive) and the Algaraboli province (583 tested animals, 34% positive). Both regions are periurban regions to the city of Tripoli, and are known to have relatively large intensive breeding farms. Although the proportion of seropositive cases appeared to be low at the Al-Kums province sheep farm (5%), this percentage was considered to be a realistic indicator of prevalence on this farm as the majority of the animals in the field were tested.

4. Discussion

The current status of brucellosis in Libya is unclear, although historically there has been significant testing and surveillance. Between 1997 and mid-1999, a National Bovine Brucellosis Eradication Programme found the prevalence rate of bovine brucellosis was 0.1% (215 positive cases out of 166,388 animals tested) at the end of 1999 [7]. Later surveillance programmes, from 1997 until 2009, suggested a prevalence rate of bovine brucellosis below 0.2%. Camel brucellosis surveillance between 2002 and 2009 revealed prevalence rates between 0.2% and 1%. In contrast, surveillance of sheep/goats in 2008/2009 showed prevalence rates of 8.3% in sheep and 14.8% in goats [6].

Generally speaking, although brucellosis has continued to be present in the country, the incidence of the disease did not exceed 0.2% in cattle, 0.1% in camels, and 14.8% in sheep and goats before the system collapsed due to political unrest in 2011 (unpublished NCAH official reports). At this point, most of the official herds belonging to the government were disbanded and the animals were either sold or destroyed, and financial support for prevention and control programs was ceased. Our preliminary investigation suggests that brucellosis among different animal species in the northwest region of Libya may be on the increase (Table 1) when compared with the history of brucellosis in the country before 2011. However, samples were highly selective and a full

Table 1
The rate of brucellosis seropositivity among domestic animal species in the northwest region of Libya between November 2014 and April 2016.

Animal species	Tested animals (n)	Seropositive animals (n)	Proportion of positive animals (%)
Cattle	255	12	4.7
Camels	35	2	5.7
Goats	854	285	33.4
Sheep	468	43	9.2
Total	1612	342	21.2

epidemiological survey with an appropriate sampling strategy is required to reveal the actual current situation over the whole country, and the extent of the increase in disease in recent years.

The percentage of dairy cattle found to be positive for *Brucella* antibodies with both RBT and SAT reported in the Tajura area was high (5%) when compared to the 0.2% prevalence documented between 1997 and 2009 [6,7]. However, this percentage was recorded from only two adjacent farms in the area and no information exists regarding the source and the extent of the infection. Therefore, further investigation is needed to determine whether this figure reflects a realistic percentage of infection over the whole area.

Two camels out of 35 cases tested positive (6%) using SAT and CFT. The camels were imported from the United Arab Emirates, a camel-racing area, which is considered to be endemic for brucellosis [22]. In fact, with uncontrolled imports, it is difficult to know whether these cases were vaccinated or naturally infected. Furthermore, neither culture nor polymerase chain reaction were performed to confirm brucellosis. Sunaga et al. [19] reported five cases of camels imported into Japan that were tested using CFT and SAT, and found to be positive for *Yersinia enterocolitica* serotype 0:9, which can cause serological cross-reaction after culturing. Strict control policies are needed to control the import of camels from known endemic countries for brucellosis such as the United Arab Emirates, Saudi Arabia, and Sudan [22].

Our finding of infection in 9.2% of sheep and 33.4% of tested goats (24.8% in both species) was remarkably high compared to 14.8%, the reported prevalence for both species before 2011 (NCAH official report). These figures may not reflect the real picture in the whole region as our samples have been taken from suspected diseased herds, but it may again indicate the re-emergence of the disease throughout the region and possibly the whole country.

Recently, the epidemiology of brucellosis in the region appeared to be changing. The possible change in the rate of brucellosis, particularly in cases of goats and sheep, could be attributed to several factors, including in particular, unrestricted trade and movement of animals across borders during this period of political unrest. With uncontrolled distribution of weapons throughout the country, the formal legislation and quarantine services have been replaced with militias that allow the introduction of unlicensed animals to the country. These animals are sold in the open market without any efficient animal registration. The introduction of the animals into a herd can lead to the spread of infection to the whole herd, causing massive economic losses particularly in many parts of the country where sheep and goat flocks are reared together. In this case, cross infection is possible. In 2012, the laboratory records of the National Centre of Animal Health reported a foci of*Brucella* infection in a herd of 217 mixed sheep and goats in the Alkhums region. A rate of brucellosis seropositivity of 47.5% was recorded in the herd. The owner of the farm stated that the animals were brought in from the Bani Walid region. Since then, serious changes in the epidemiology of brucellosis are thought to have taken place in the region. In our study, 340 animals from four farms in the Alkhums province were screened as they were suspected of having brucellosis. The results showed a rate of 5% of infection in small ruminants, indicating the lack of adequate control measures commenced in 2011. Biosecurity measures on most farms are lacking, and most producers do not test animals for brucellosis or other diseases when purchasing replacements. Nielsen [15] suggested that it would be impossible to prevent transmission of*Brucella* to other animals in the surrounding area if actions are not taken to eliminate the source of infection. Refai [17] shares the same view, and thought that failure to apply suitable eradication measures in many countries for many years has led to transmission of *Brucella melitensis* to cattle, buffaloes, and camels. Blasco [3] pointed out the importance of allocating adequate financial resources prior to the selection of any eradication strategy, but pointed out that many countries had succeeded in eradicating*B. melitensis* once adequate economic compensation was introduced. A similar situation was encountered in Egypt. Whilst the animal with the lowest brucellosis seropositivity (0.33%) was recorded in the country before 2011, 'Arab Spring' uprisings since then have led to a reduction of government resources, a decrease of compensation funds for owners, and increased animal movement within and between herds [21].

In light of the current situation in Libya, implementation of control programmes including culling of infected animals is not feasible. Animal health authorities such as the NCAH and veterinary services can only offer advice and instructions to the owners of infected farms. Therefore, selling of suspected infected animals to butchers or to the open market is highly likely. More effort should be made to encourage and support voluntary participation in disease control measures and in enabling veterinary authorities to set up programmes for public awareness of the risks related to the disease for animals and humans. Considering the levels of brucellosis identified by the present study, in particular in small ruminants, appropriate epidemiological studies are required for screening a representative sample of randomly selected farms in the region to determine the exact frequency and distribution of the disease. If the results confirm our suspicions of a rising incidence of disease, steps would have to be taken to change current management practices in the region. A re-evaluation of the interventions applied to control brucellosis in Libya should be prioritised by the government guided by an assessment of the likelihood of success and a realistic cost–benefit analysis.

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