Assessing brucellosis incidence among Djibouti's livestock and exploring plant remediation approaches

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ABSTRACT
Livestock farming plays a pivotal role in Djibouti's meat and milk production, but it frequently faces the threat of diseases that significantly impact both food security and public health. Brucellosis, a formidable obstacle to livestock production and public well-being, is one such disease. While Djibouti has witnessed previous epidemiological studies on brucellosis, they are relatively limited in scope and not recent. To address this gap, we conducted a comprehensive study across fifty-three livestock farms in Djibouti's six regions or districts (Djibouti City, Arta, Ali Sabieh, Dikhil, Tadjourah, and Obock) to ascertain the prevalence of brucellosis among herds. This study involved the collection of 500 blood samples from farm animals, with the detection of anti-Brucella antibodies performed in sera through three diagnostic methods: the immune-chromatographic test, the Brucella Wright test, and the Brucella Rose Bengal test, which specifically identifies anti-Brucella antibodies for brucellosis diagnosis. Our findings revealed 12 cases of brucellosis among cattle, representing 4.76% of cattle samples, 6 cases in goats (3.40%), and a single case in camels (1.38%). This data establishes an overall brucellosis prevalence rate of 3.8%. Brucellosis stands as a significant zoonotic disease, posing potential threats to consumer health. Consequently, its prevalence necessitates the attention of livestock producers, consumers, and all stakeholders involved in animal health management. Furthermore, this study highlights the availability of herbal treatment options concentrated within Djibouti's Day National Park for combating this disease.

1. Introduction:
Livestock production stands as a crucial livelihood for African communities, including Djibouti's population. However, the development of livestock is hindered by various factors, with diseases being a primary concern. Some of these diseases not only impact animal health but also pose significant public health risks. Brucellosis, a frequently overlooked zoonotic disease, has a global distribution, posing threats to both human health and animal production [1]. It ranks among the most highly pathogenic zoonotic diseases affecting animals and humans and is caused by the genus Brucella [2]. Brucellosis holds historical significance, being recognized worldwide since ancient times. The emergence of brucellosis in new regions and its transmission between wild and domestic animals represent substantial epidemiological challenges [3]. The consumption of unpasteurized milk and dairy products from unsanitary farms in endemic areas makes brucellosis a serious

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public health threat [4]. The distribution of brucellosis varies across regions, with new infection areas emerging and previously infected regions experiencing a resurgence [5]. The primary Brucella species responsible for human infections include B. melitensis, B. abortus, and B. suis, underscoring the ongoing global threat posed by this zoonotic disease [6]. Brucella is a small intracellular coccobacillus capable of causing abortion and infertility in host animals, affecting individuals of all ages and genders [7]. Transmission to humans can occur directly or indirectly through contaminated food ingestion, direct contact with infected animal tissue, or inhalation of contaminated aerosols [8]. Brucella species exhibit a propensity to adapt to new hosts, leading to natural transmission to primary hosts through direct or indirect contact and, at times, accidental transmission to other susceptible hosts [9]. The extensive use of antibiotics in livestock farming fosters the emergence of new drug-resistant strains, some of which pose zoonotic risks, contributing to the rise of foodborne illnesses in humans [10].

Medicinal plants have been a traditional resource for treating infectious diseases in both livestock and humans for centuries [11-13]. Plant-based veterinary medicines play a vital role in disease prevention and herd health enhancement [14-16]. The ability to store dried leaves and seeds for extended periods at room temperature without compromising recombinant proteins makes plant expression systems promising for developing edible vaccines and prophylactics. These innovations eliminate the need for cold storage and transportation, allowing for bulk administration with minimal processing. Despite successful eradication efforts in many developed nations, brucellosis remains a major global public health concern due to its status as the most common zoonotic infection, even though humans are incidental hosts [17].

The underreporting of the disease in domestic animals in developing countries is primarily due to the absence of national surveillance programs, limited diagnostic capabilities, and unreliable data [18]. Although Djibouti has witnessed a few prior epidemiological studies on this matter [19], these studies are relatively scarce and outdated. The primary goal of the present study was to rejuvenate our understanding of the epidemiology of brucellosis in Djibouti. It was conducted under the auspices of the Center for Research and Study of Djibouti (CERD) with the specific aim of identifying infected animals that could serve as potential vectors of brucellosis transmission to humans. Moreover, this research sheds light on the accessibility of herbal treatment alternatives concentrated within Djibouti’s Day National Park as a means to combat this disease.

2. Materials and methods:

2.1. Study area and sample collection:

Fifty-three livestock farms in the six regions or districts of Djibouti (Djibouti City, Arta, Ali Sabieh, Dikhil, Tadjourah, and Obock) were selected for this study. A total of 500 blood samples were collected from livestock for the qualitative detection of Brucella abortus (cow), Brucella melitensis (goat), and Brucella abortus, melitensis, or suis (camel) in the serum.

2.2. Diagnosis of brucellosis:

Three different tests were employed for the serological diagnosis of brucellosis, caused by Brucella abortus, Brucella melitensis, or Brucella suis.

2.2.1. Immuno-chromatographic test:

The immuno-chromatographic test is a rapid and straightforward method, making it highly suitable for field applications [20]. This test relies on the migration of liquid across a nitrocellulose membrane’s surface and has gained popularity in the past decade due to its convenience and versatility. These tests typically take about 20 minutes to yield results and require only a small serum volume (<200 μL) to be applied to the test strip. The immuno-chromatographic test kits utilized include:

- Antigen Rapid B. Brucella Ab for detecting Brucella abortus in cattle.
- Antigen Rapid GS (Goat and Sheep) Ab for detecting Brucella melitensis in goats and sheep.
- Antigen Rapid Camel Brucella Ab for detecting Brucella melitensis, abortus, or suis in camels. These kits were provided by BIONOTE (Bionote Inc) based in South Korea (2-9 Seogu-dong, Hwaseong-si, Gyeonggi-do), a company specializing in the development and production of diagnostic kits for human and animal diseases since 2003.

2.2.2. Brucella Wright test (detection of anti-brucella antibodies):

This quantitative test yields positive results early, as early as day 10 or 12, in cases of acute brucellosis. However, it quickly turns negative because it detects IgM antibodies. The WRIGHT sero-diagnostic is an agglutination test that employs a suspension of Brucella, inactivated by formalin and heat, as the antigen. A titer of 1/80 (equivalent to 120 I.U/ml) or higher indicates an active brucellosis infection, while a lower titer (1/40 or even 1/20) is highly suspicious [21].

2.2.3. Brucella Rose Bengal (detection of anti-brucella antibodies):

This qualitative test, which turns positive shortly after the Wright sero-diagnosis, serves as a valuable tool for brucellosis screening, diagnosis, and surveillance, particularly in epidemiological surveys. The Rose Bengal antigen test enables the
serological diagnosis of brucellosis caused by Brucella melitensis, Brucella abortus, Brucella bovis, or Brucella suis by detecting IgG antibodies. After mixing equal parts of rose bengal antigen and serum, the presence of colored agglutinates indicates the presence of brucellosis. The test's sensitivity limit is set at 25 IU/ml [22].

2.3. Survey on Herbal Treatments:
To gather herbal treatment recommendations for brucellosis, a survey was conducted to document the use of herbs within the scope of this research. A comprehensive questionnaire and interview guide were developed, targeting both traditional healers and ethnobotanists. The analysis of the collected data was carried out by the CERD team, who carefully examined the responses to identify prevalent trends and recurring themes. These findings were then interpreted to better understand the underlying motivations and beliefs that guide the use of herbal treatments. The conclusions drawn from this research were shared with the scientific community through collaborations with national and international researchers, and the results were disseminated through a collection of articles focusing on the topic of medicinal plants in Djibouti.

3. Results:
The prevalence of brucellosis in Djibouti was assessed through the examination of 500 blood samples collected from various livestock species. The results, as presented in Tables 1 and 2, reveal the following key findings:

Among 252 cattle samples, 12 tested positive for Brucella abortus, representing a prevalence rate of 4.76%. These positive cases were exclusively identified in Djibouti City.

Out of 176 goat blood samples, 6 were found to be positive for Brucella melitensis, accounting for 3.40% of the goat samples. These positive cases were concentrated in the WALWALA locality within the Tadjourah Region.

In contrast, only one positive case of Brucella, which could be Brucella melitensis, abortus, or suis, was detected among 72 camel samples in the WALWALA locality of the Tadjourah Region, constituting 1.38% of the camel samples analyzed. Remarkably, the study indicates that the four other regions, namely ARTA, ALI-SABIEH, DIKHL, and OBOCK, appear to be free from the brucellosis infection, as no positive cases were reported in these areas (Figure 1). These findings provide valuable insights into the distribution of brucellosis in Djibouti's livestock populations, aiding in disease management and control efforts.

Table 1. Distribution of brucellosis prevalence by farms, tests, and grouping by locality.

<table>
<thead>
<tr>
<th>Localities</th>
<th>Type of herd</th>
<th>Number (Serum)</th>
<th>Positive animals</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Test IC</td>
<td>Test BW</td>
</tr>
<tr>
<td>Djibouti</td>
<td>Cattle</td>
<td>114</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>41</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Camels</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Arta</td>
<td>Cattle</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>29</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Camels</td>
<td>43</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ali Sabieh</td>
<td>Cattle</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>24</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Camels</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dikhil</td>
<td>Cattle</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>26</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Camels</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tadjourah</td>
<td>Cattle</td>
<td>111</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>32</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Camels</td>
<td>21</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Obock</td>
<td>Cattle</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Goats</td>
<td>24</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Camels</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>500</td>
<td>19</td>
<td>17</td>
</tr>
</tbody>
</table>
The analysis reveals that out of the 500 farm animals tested, 19 were found to be positive for brucellosis, accounting for a prevalence rate of 3.8% among farm animals in Djibouti. This indicates that the risk of brucellosis in livestock is presently quite low and is primarily concentrated in two regions, Djibouti City and Tadjourah. Consequently, it becomes imperative to implement measures for eradicating the disease from these two regions to mitigate the potential of it evolving into a significant public health concern.

**Figure 1.** Percentages of overall brucellosis prevalence by herd type.

![Overall prevalence (%)](image)

**Figure 2.** Distribution of brucellosis in Djibouti.

4. **Discussion:**
Brucellosis, classified as a notifiable occupational disease, exhibits a wide range of clinical symptoms, underscoring the imperative need for early identification, prompting subsequent confirmatory examinations. The severity of brucellosis is intrinsically linked to the emergence of secondary complications, some of which can prove fatal, necessitating prolonged antibiotic treatments and, at times, surgical interventions [23].

In the context of Djibouti, the impact of these zoonotic diseases on livestock productivity and public health remains currently limited. Nevertheless, their presence increases the potential risk to human populations, especially with the
intensification of agricultural practices. Implementing control measures involves eradicating disease reservoirs while closely monitoring the introduction of animals into disease-free herds, which is particularly relevant in the context of peri-urban milk production. Further investigations should be conducted within Djibouti's animal collectives [24-25]. Prevention emerges as the predominant strategy in the fight against brucellosis. This proactive approach relies on principles of rigorous hygiene practices, heightened public awareness, abstaining from the consumption of unpasteurized dairy products, and systematic livestock vaccination. The epidemiological landscape of brucellosis outlines a divergence between low individual incidence rates and higher herd prevalence. Consequently, these diseases persist at attenuated levels in numerous herds due to management practices that inhibit the spread of infection within contaminated animal collectives. Throughout Africa, including Djibouti, livestock farmers have harnessed the therapeutic potential of medicinal flora for countless generations, elevating herbal remedies to increased prominence. Moreover, contemporary consumer preferences for antibiotic-free meat are driving market demand [26]. As a result, researchers have meticulously collected data from farmers across Africa and Asia for many years, studying the effects of using these botanical resources for animal well-being (Table 2).

Medicinal plants, bearing a multitude of biologically active compounds, have served as global antidotes for various ailments [27-31]. The composition of these compounds varies depending on plant species, soil composition, and microbial associations [32]. Given the persistent burden of infectious diseases on public health, pharmaceutical companies are actively pursuing research into new antimicrobial agents, given the ongoing emergence of resistant microorganisms. The quest for innovative antimicrobial agents remains paramount, especially in addressing challenges posed by traditional antibiotics, such as resistance and therapeutic ineffectiveness, particularly in diseases like brucellosis. Consequently, the search for novel antimicrobials remains a major priority in the field of infectious disease management [33].

Table 2. List of Medicinal plants showing the antibrucellosis activity.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Plant part</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Callistemon Citrinus</td>
<td>Leaf</td>
<td>[34]</td>
</tr>
<tr>
<td>Caryopteris mongolica</td>
<td>Root</td>
<td>[35]</td>
</tr>
<tr>
<td>Cinnamomum zeylanicum</td>
<td>Bark</td>
<td>[36]</td>
</tr>
<tr>
<td>Crocus sativus</td>
<td>Leaf</td>
<td>[37]</td>
</tr>
<tr>
<td>Cyathula auncinidata</td>
<td>Leaf</td>
<td>[38]</td>
</tr>
<tr>
<td>Juniperus oxycedrus L.</td>
<td>Leaf</td>
<td>[39]</td>
</tr>
<tr>
<td>Moringa oleifera</td>
<td>Leaf</td>
<td>[40]</td>
</tr>
<tr>
<td>Oliveria decumbens</td>
<td>Leaf</td>
<td>[41]</td>
</tr>
<tr>
<td>Origanum syriacum</td>
<td>Leaf</td>
<td>[42]</td>
</tr>
<tr>
<td>Petroserinum crispum</td>
<td>Seed</td>
<td>[43]</td>
</tr>
<tr>
<td>Scrophularia desertii</td>
<td>Leaf</td>
<td>[45]</td>
</tr>
</tbody>
</table>

5. Conclusion:
This study has revealed the presence of brucellosis in Djibouti's cattle herds, with an overall prevalence of 3.8%. Nevertheless, it is imperative to validate these findings by isolating the bacteria from cattle lesions or during abortions in animals affected by brucellosis. While these estimates offer valuable insights into the significance of brucellosis in the Republic of Djibouti, it is essential to bear in mind that they are based on pre-existing sera, potentially influenced by the method of collection. Furthermore, it is worth noting that the Bayesian approach considers prior information, which can affect the actual prevalence estimate. Thus, relying on the most credible expert opinion becomes crucial. In the Djiboutian context, the impact of these zoonotic diseases on livestock productivity and public health is currently limited. However, their presence heightens the potential risk to human populations, particularly with the intensification of agricultural practices. Implementing control measures entails eradicating disease reservoirs and vigilantly monitoring the introduction of animals into disease-free herds, particularly relevant in peri-urban dairy production settings. Further investigations within Djibouti's animal collectives are warranted to deepen our understanding of these diseases and refine control strategies.

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References:


