Prevalence of Brucellosis in Ruminants and The Risk of Human Exposure in Rural Delta of Egypt

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Abstract

This study aimed to assess seroprevalence of brucellosis in ruminants and the risk of human exposure to Brucella species. A cross-sectional survey was performed in 546 randomly selected households in rural Nile Delta, Egypt. A questionnaire was administered to the household head to collect data about livestock management and risk factors for brucellosis. In addition, serum samples were collected from 699 cattle and 286 sheep for serological investigation against brucellosis. A quantitative exposure assessment model was developed to estimate the probability of human exposure to Brucella spp via contact with ruminants and/or consumption of raw milk and home-made dairy products. The brucellosis seroprevalence in cattle and sheep was 13.87% and 10.84%, respectively. The mean annual probability of human exposure to Brucella spp via contact with cattle (0.98) was the highest followed by the probability of exposure via consumption of home-made dairy products (0.96), processed from cattle milk. The quantitative exposure assessment model demonstrated that the current livestock owners' practices would increase the risk of human and livestock exposure to Brucella infection. In conclusion, contact with cattle and consumption of home-made dairy products were the main routes of human exposure to Brucella species in the rural Nile Delta. Educational campaigns for controlling brucellosis and other zoonoses shall target preferred information channels as field veterinarians. Such campaigns shall be supported by resources that would help disease management at the farm level, thus reducing human exposure.

Keywords: Brucellosis, exposure assessment model, human, livestock, seroprevalence.
**Introduction**

Brucellosis is one of the most widely spread neglected zoonotic diseases with an incidence rate of 500,000 cases per year. However, the true incidence could be 5,000,000 to 12,500,000 new cases per year [1,2]. Neglected zoonotic diseases are often endemic in developing countries, where people in rural and peri-urban areas usually live-in close contact with animals [3]. Similarly, tuberculosis is another significant threat to humans and animal production in Egypt [3,4]. These diseases usually receive less international attention than emerging zoonoses due to underreporting and consequently underestimation of the global burden [5]. Humans are likely to become infected with *Brucella* spp. through the consumption of raw milk and non-heat-treated dairy products or the invasion of the skin and/or mucous membranes during contact with infected animals and/or contaminated materials from infected animals [6–16].

In Egypt, accurate and unbiased estimates of the prevalence and incidence of brucellosis in human and livestock are scarce [17]. In livestock, available data suggested that the prevalence of brucellosis in large and small ruminants is increasing [18–21]. On the other hand, data for the prevalence and incidence of brucellosis in humans are scarce and most of which were hospital-based surveys that had some methodological limitations [8,16,22]. The percentage of positive cases of brucellosis among patients with Acute Febrile Illness (AFI) or cases with pyrexia of unknown origin (PUO) was ranged from 3% to 11% [8,22,23]. The annual incidence of human infections is varied over time and from region to another. It is thought to have increased from 0.5/100,000 in 1994 to 1.9/100,000 population in 1998 and up to 70/100,000 population in 2003 [12,24,25].

Further studies are required about the attribute of human infection with *Brucella* spp. to different routes of exposure and deciding appropriate strategies to mitigate human exposure. Several approaches can be used to determine the relative importance of different routes for human infection with zoonotic pathogens, including microbiological approaches, epidemiological studies, and expert elicitation and risk assessment approaches [26]. The latter work prospectively to predict and/or estimate the risk of infection via different transmission routes using the available data [26]. Developed countries have extensively made use of this approach for improving food safety; however, the scarcity of good-quality data in developing countries may make its use difficult. One of the main steps in risk assessment for human infection with food-borne pathogens such as *Brucella* spp. is the dose-response model for which there are no sufficient quantitative data. However, attempts have been made to use the risk assessment approach in the absence of dose-response data [27].

In Egypt incidence data for brucellosis in humans are not easily obtainable and not considered reliable due to misdiagnosis; often cases are recorded as PUO and not all patients seek medical care from public hospitals and thus likely underreported [8,12,22]. In addition, the multidisciplinary collaborations, communications, and co-operations between the veterinary and the public health authorities (One Health approach) are not yet practiced sufficiently in Egypt. Therefore, developing a risk assessment model based on incidence data for brucellosis in Egypt would result in biased results.

The objectives of this study were to: (i) assess the seroprevalence of brucellosis in cattle and sheep; (ii) estimate the probability of human exposure to *Brucella* spp. in rural Nile Delta, Egypt via different routes and the relative contribution of each exposure route to identify appropriate control strategies.

**Material and Methods**

**Household survey**

A cross-sectional survey was conducted in two of the Nile delta governorates (Al-Gharbia and Kafr El-Sheikh) characterized by a high density of human and livestock population, where humans and animals are living in close proximity, particularly in small-scale farming systems. A two-stage cluster sampling was applied for selection of the villages and households within villages. The sample size - the number of households - was calculated using Win Episcope 2.00 based on a 95% confidence level with an expected proportion of 50% of households having ruminates and accepted error of 5% with unknown population size. The minimum required sample size was 385 households. The design effect was used as 1.2 [15] and the adjusted sample size to be 462 (385*1.2). This number was inflated to 546 households from 60 villages randomly selected from the two governorates. A probability proportion to size method was used to calculate the number of households to be sampled from each village. In each household, the head of the household was interviewed using a questionnaire (Supplimnary matrial) to collect data about livestock management.
and brucellosis. In addition to the questionnaire, blood serum samples were collected from 699 cattle and 286 sheep. All serum samples were tested using Rose Bengal Plate Test (RBPT) and seropositive samples were further confirmed by Complement Fixation Test (CFT). Antigens for the CFT, and RBPT were purchased from the NVSL/DBL, USDA, USA. The RBPT test was carried out according to [28]. The warm fixation (American version) of the CFT was performed as described by Hennager [29]. Animals considered positive to brucellosis if their serum samples gave positive results to both RBPT and CFT (series interpretation). In series combined sensitivity (Cs) and combined specificity (Cp) of both RBPT and CFT (Cs; 78 % and Cp; 99 %) were used in the estimation of seroprevalence [30].

Risk assessment model
A quantitative exposure assessment model was developed to estimate the probability of human exposure to Brucella spp. via different routes. The following subsections describe the structure and input parameters for the exposure assessment model.

Hazard identification and risk question
The hazard of interest was Brucella spp., specifically B. melitensis and B. abortus the most commonly prevalent species in Egypt [17,31]. The risk question was “what is the annual probability of human exposure to Brucella spp. via each of the possible routes, in rural Nile Delta, Egypt?”. The output of this assessment was the probability of human exposure to Brucella spp. per route of exposure per year. Age, sex and occupation were not taken into consideration in this assessment.

Exposure assessment
Risk pathways (exposure routes)
The following exposure routes were considered:

i) Contact with animals (cow, buffalo, sheep or goat) and/or animal excreta (urine, faeces, vaginal discharges, foetal membranes, foetal fluids and aborted foeti). In this study “contact” was used to refer to “direct and/or indirect”.

ii) Consumption of raw “unpasteurised or un-boiled” milk and/or home-made dairy products processed from unpasteurised milk.

Home-made dairy products included cheese, cream and butter. The traditional processing of these products does not involve heat treatment with the only exception of processing of cheese at 20 to 25°C which is not enough to inactivate the Brucella organisms in milk. Sometimes salt is added to some home-made dairy products according to the desired taste. Soft cheeses prepared from contaminated milk without heat treatment are a particularly common source of infection in Mediterranean and Middle Eastern countries [32]. The soft cheese-manufacture process may concentrate the Brucella, which can survive for up to several months in such products [32]. Farmers don’t usually add preservatives to home-made soft cheese, so it has a short life span and usually consumed within few days (3-5 days). Therefore, in this assessment we assumed that Brucella spp. survives the processing steps of these products and the probability of inactivation of Brucella spp. during processing was assumed to be negligible. Given the limited data available for the prevalence of Brucella ovis in each animal species and for the processing and consumption of specific home-made dairy products, it was not possible to develop risk pathways for each species of animals separately or to distinguish between different home-made dairy products. Therefore, within this assessment, “cattle” was used to refer to “cow and/or buffalo”, “sheep” was used to refer to “sheep and/or goat”, “home-made dairy products” was used to refer to “cheese, cream and/or butter”.

Consumption of milk and home-made dairy products refer to those produced and processed at the household not those purchased from other sources. To our knowledge, in households with more than one lactating cattle, milk is usually pooled in one tank before consumption and/or processing of home-made dairy products. Home-made dairy products were usually processed and consumed on daily basis.

Potential routes of exposure and the calculation scheme are detailed bellow and illustrated in Fig. 1 (the probability of exposure to Brucella spp. via each route per day was calculated first, then the annual probability was calculated):

1) The probability of exposure to Brucella spp. via contact with cattle (R1):

\[ R1 = P1 \times P2 \times P3, \]

where: (P1) the probability that cattle was infected with Brucella spp., (P2) the probability that an infected cattle was infectious, and (P3) the probability that a random individual get in contact with cattle.

2) The probability of exposure to Brucella spp. via consumption of raw cattle milk (R2):

\[ R2 = P1 \times P2 \times P4, \]

where: (P1) the probability that cattle was infected with Brucella spp., (P2) the probability that an infected cattle was infectious, and
(P4) the probability that a random individual consumed raw milk from the infected cattle.

3) The probability of exposure to Brucella spp. via consumption of home-made dairy products processed from cattle milk (R3):

\[ R3 = P1 \times P2 \times P5 \]

where: (P1) the probability that cattle was infected with Brucella spp., (P2) the probability that an infected cattle was infectious and (P5) the probability that a random individual consumed home-made dairy products processed from cattle milk.

4) The probability of exposure to Brucella spp. via contact with sheep (R4):

\[ R4 = P6 \times P7 \times P8 \]

where: (P6) the probability that sheep was infected with Brucella spp., (P7) the probability that an infected sheep was infectious and (P8) the probability that a random individual contacted with sheep.

Model inputs and assumptions

The input parameters were derived from the literature, our own results from the cross-sectional survey and expert opinions where there were no data, Table 1.

**TABLE 1. Model input parameters for the assessment of human exposure to Brucella spp. in rural Nile Delta, Egypt.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
<th>Distribution/value</th>
<th>Data sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Probability that cattle was infected with Brucella spp</td>
<td>Risk Beta* (98,603)</td>
<td>Cross-sectional survey, 97 seropositive cows from 699 tested</td>
</tr>
<tr>
<td>P2</td>
<td>Probability that infected cattle is infectious</td>
<td>Risk Beta (61, 481)</td>
<td>Hegazy et al., 2009</td>
</tr>
<tr>
<td>P3</td>
<td>Probability that a random individual get in contact with cattle</td>
<td>Risk Beta (458, 90)</td>
<td>Cross-sectional survey, 457 having cattle at HH from 546 surveyed HH</td>
</tr>
<tr>
<td>P4</td>
<td>Probability that a random individual consumed raw milk from the infected cattle</td>
<td>Risk Beta (13, 535)</td>
<td>Cross-sectional survey, 12 from 546 surveyed HH</td>
</tr>
<tr>
<td>P5</td>
<td>Probability that a random individual consumed home-made dairy products processed from cattle milk</td>
<td>Risk Beta (408,140)</td>
<td>Cross-sectional survey, 407 from 546 surveyed HH</td>
</tr>
<tr>
<td>P6</td>
<td>Probability that sheep was infected with Brucella spp</td>
<td>Risk Beta (32, 256)</td>
<td>Cross-sectional survey, 31 seropositive sheeps from 286 tested</td>
</tr>
<tr>
<td>P7</td>
<td>Probability that infected sheep with Brucella spp. is infectious</td>
<td>Risk Beta (61, 1401)</td>
<td>Hegazy et al., 2009</td>
</tr>
<tr>
<td>P8</td>
<td>Probability that a random individual contacted with sheep</td>
<td>Risk Beta (83, 465)</td>
<td>Cross-sectional survey, 82 having sheep at HH from 546 surveyed HH</td>
</tr>
</tbody>
</table>

\*p ~ Beta (s+1, n-s+1)

Where: p = probability of success, s = number of successes, n = number of trials.

**Prevalence of brucellosis in ruminants**

Results from our survey in the study area indicated that the seroprevalence of brucellosis in cattle and sheep was 13.87% (95% CI: 12.56% to 15.18%) and 10.84% (95% CI: 8.63% to 12.69%), respectively. The number of animals tested and those were serologically positive to brucellosis (series interpretation) were used as inputs for the model using beta distribution, Table 1. Animals infected with Brucella spp. usually shed organisms in vaginal discharges, faeces, semen and milk after abortion and parturition. The shedding period varies from animal species to another. Infected goats may shed Brucella organisms for 2 to 3 months in vaginal discharge but it is less than 3 weeks in sheep [33]. However, the shedding period in milk may be longer especially in goats [33]. The infectious period in sheep and goats was assumed to be 60 days per life span (4 years) [34]. We considered that infectious period as the time during which the infected animal is shedding Brucella organisms in its milk and/or other excreta. Contacting infected animals and/or consumption of home-made dairy products at that time are potential risk factors for human infection with Brucella spp. Based on these data and expert opinion, we assumed that the shedding period in cattle is 60 days per calving interval (18 months) and for sheep/goat is 60 days per life span (4 years) [17].

**Human contact with ruminants and consumption of raw milk and dairy products**

Based on our recent study, we assumed that a person living in a household that kept cattle and/or sheep may contact (directly/indirectly) with these...
animals and/or their excreta at least once per day [16]. Our survey results indicated that 83% and 15% of households kept cattle and sheep, respectively. These data were used to estimate the probability of contact with cattle and sheep using beta distribution. The results also showed that 2.3% of households do not boil milk before consumption and about 75% regularly consume home-made dairy products processed from cattle milk. In this assessment, there was no distinction between different types of home-made dairy products. Based on data from our case-control study [16], we assumed that a person would at least consume one type of home-made dairy product at least once per day. None of the study participants reported consuming raw milk and/or dairy products processed from sheep and/or goats. Therefore, the risk of exposure via these routes was considered negligible in this assessment.

Model outputs and calculations

A simulation model was constructed on Microsoft Excel using @Risk version 7.0 software (Palisade Corporation Inc. Newfield, NY, USA). The model described the variability and uncertainty of the input parameters using probability distributions and Monte Carlo simulations. To estimate the probability of human exposure to Brucella spp. via different routes, the model simulates the probability that any individual selected at random from the study population will come in contact with infected cattle and sheep via different routes. The output of the model was the annual probability of human exposure to Brucella spp. via each exposure route. The probability of infection given exposure was beyond the scope of this assessment due to the lack of data for a dose-response model. The model was run for 10,000 iterations per simulation. The output of the calculation for each exposure route was the probability of exposure to Brucella spp. per person per day ($P_{\text{Exp/day}}$). The annual probability of exposure of a random person living in rural delta (at least once per person per year) via each route was calculated according to [35] the following equation: $P_{\text{Exp/year}} = 1 - (1 - P_{\text{Exp/day}})^{365}$.

Risk mitigation strategies

To reduce the risk of human exposure to Brucella spp. the following risk mitigation strategies were considered:

i) Vaccination to reduce the prevalence of brucellosis in ruminants: A simulation model of the effective control strategies for brucellosis in small ruminants in one of the Nile Delta Governorates indicated that vaccination of 50% of young replacements and 25% of adult sheep every year would reduce the seroprevalence of brucellosis by 75% after 10 years [17]. In Dohuk Governorate, Iraq, the prevalence of brucellosis in small ruminant was predicted to be decline after mass vaccination adopted for all male and female sheep and goats in 20 years from 9.22% to 0.73% [36]. In Mongolia, it has been estimated that the annual mass vaccination of livestock against brucellosis for 10 years would reduce the prevalence of brucellosis by 52% (37,38). We assumed that the implementation of this strategy to large and small ruminants in Egypt would reduce the seroprevalence of brucellosis to 50% of the current situation over 10 years.

ii) Heat treatment of raw milk before processing of home-made dairy products: To our knowledge, almost all home-made dairy products are processed from raw milk without any heat treatment or heated at 60º C for 15 minutes. We assumed that heat treatment such as boiling of milk before processing would inactivate Brucella spp. in contaminated raw milk 90% of the time.

iii) Combination of the previous 2 strategies. For each strategy, the model was run for 10,000 iterations per simulation to simulate the impact of these control strategies on the probability of human exposure to Brucella spp. for each exposure route.

Results

Survey results and the estimated brucellosis seroprevalence among cattle and sheep

In this study, the head of 546 randomly selected households (HH) in rural Nile Delta, Egypt were interviewed. The demographic characteristics of livestock owners are summarized in Table 2. Most of the heads of HH were males with an average age of 48 years. Nearly 44% had no formal education whilst 39% had primary education and more than 80% kept livestock. Livestock production was a major source of income for more than 60% of HH. The seroprevalence of brucellosis in cattle and sheep was 13.87% (95% CI: 12.56% to 15.18%) and 10.84% (95% CI: 8.63% to 12.69%), respectively. These data were utilized in Table 1 above as inputs for the risk assessment model.
TABLE 2. Demographic characteristics of study participants for the risk of brucellosis in the rural Nile Delta, Egypt.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Study participants (n=546)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, n (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>538 (98.50)</td>
</tr>
<tr>
<td>Female</td>
<td>8 (1.50)</td>
</tr>
<tr>
<td>Age, year</td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td>20</td>
</tr>
<tr>
<td>Maximum</td>
<td>89</td>
</tr>
<tr>
<td>Average (SD)</td>
<td>48.17 (9.39)</td>
</tr>
<tr>
<td>Level of education, n (%)</td>
<td></td>
</tr>
<tr>
<td>No formal education</td>
<td>241 (44.14)</td>
</tr>
<tr>
<td>Primary</td>
<td>215 (39.38)</td>
</tr>
<tr>
<td>Preparatory</td>
<td>13 (2.38)</td>
</tr>
<tr>
<td>Secondary</td>
<td>43 (7.88)</td>
</tr>
<tr>
<td>University</td>
<td>33 (6.04)</td>
</tr>
<tr>
<td>Others</td>
<td>1 (0.18)</td>
</tr>
<tr>
<td>Keeping livestock at the household, n (%)</td>
<td>457 (83.70)</td>
</tr>
<tr>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>89 (16.30)</td>
</tr>
</tbody>
</table>

Exposure assessment model

The results of the simulation model (Fig. 2) showed that, the mean annual probability of human exposure to *Brucella* spp. via contact with cattle was the highest followed by the probability of exposure via consumption of homemade dairy products, processed from cattle milk. The probability of exposure via contact with sheep was lower than the probability of exposure via contact with cattle and consumption of raw milk was the lowest. The sensitivity analysis (Fig. 3) showed that the prevalence of infection in ruminants and the shedding period (infectious period) were the most dominating variables for the risk of human exposure to *Brucella* spp. via different routes.
Fig. 1. Risk pathways for the assessment of human exposure to *Brucella* spp. in rural Nile Delta, Egypt via: a) contact with cattle and consumption of milk and home-made dairy products from cattle (P1=Probability of cattle being infected, P2=probability of infected cattle being infectious, P3=Probability of contact with cattle, P4=probability of consumption of raw milk, P5=probability of consumption of homemade dairy products), and b) contact with sheep/goat (P6=Probability of sheep/goat being infected, P7=probability of infected sheep/goat being infectious, P8=Probability of contact with sheep/goat).

Fig. 2. Probability distribution for the annual exposure (at least once a year) of human to *Brucella* spp. via different routes in rural Nile Delta, Egypt.
Fig. 3. Sensitivity ranking for the annual Likelihood that a random person exposed at least once a year to *Brucella* spp. via different exposure routes. (R1=Probability of exposure via contact with cattle, R2=Probability of exposure via consumption of raw cattle milk, R3=Probability of exposure via consumption of dairy products, and R4=Probability of exposure via contact with sheep/goat).

**Risk mitigation**

Results of the effectiveness of the proposed risk mitigation strategies were summarized in Table 3. The results showed that reducing the prevalence of brucellosis in livestock would reduce the probability of human exposure to *Brucella* spp. via all routes. Heat treatment of milk either before drinking or processing homemade dairy products would significantly reduce the probability of exposure via consumption of these products. Implementing both strategies together would reduce the probability of exposure via all routes especially via consumption of home-made dairy products.

<table>
<thead>
<tr>
<th>Routes of exposure</th>
<th>The mean probability of exposure/year</th>
<th>Baseline results</th>
<th>Strategy 1*</th>
<th>Strategy 2**</th>
<th>Strategy 3***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact with cattle</td>
<td></td>
<td>0.99</td>
<td>0.90 (9%)</td>
<td>0.99 (0%)</td>
<td>0.90 (9%)</td>
</tr>
<tr>
<td>Consumption of raw cattle milk</td>
<td></td>
<td>0.13</td>
<td>0.06 (54%)</td>
<td>0.01 (92%)</td>
<td>0.01 (92%)</td>
</tr>
<tr>
<td>Consumption of home-made dairy products</td>
<td></td>
<td>0.98</td>
<td>0.88 (10%)</td>
<td>0.34 (65%)</td>
<td>0.19 (83%)</td>
</tr>
<tr>
<td>Contact with sheep</td>
<td></td>
<td>0.22</td>
<td>0.12 (46%)</td>
<td>0.22 (0%)</td>
<td>0.12 (46%)</td>
</tr>
</tbody>
</table>

**NB:** Percent between practices is the present of exposure reduction with each strategy

*Vaccination of ruminants

**Heat treatment (Boiling/pasteurization) of milk before processing of homemade dairy products

*** A combination of vaccination and heat treatment
Discussion

In the rural Nile Delta, more than 80% of households are keeping livestock and for more than 60%, livestock was the major source of income. This highlights the socio-economic importance of livestock production for people living in this area. On the other hand, this indicated the potential risk of exposure to zoonotic diseases unless hygienic measures are followed as in most of these households' livestock are kept near humans. Raw milk and homemade dairy products were usually sold to milk traders and/or collectors and sometimes directly to consumers without any control or supervision from the authorities for the quality and safety of the products. This behavior may impose a potential risk of health hazards and adulteration. Produced milk, is usually consumed raw or used for processing homemade dairy products such as cheese, butter, and cream. These products are usually consumed by the household inhabitants or sold to neighbors, and consumers at the village market or nearby city.

The results of the exposure assessment showed that the probability of human exposure to \textit{Brucella} spp. via consumption of raw cattle milk was very low as almost all people boiled the milk before consumption. This finding is in the same line with a study performed in Northern Egypt, where the consumption of raw fresh milk wasn't considered a risk factor for human brucellosis as respondents were aware of its danger \cite{39}. The probability of human exposure to \textit{Brucella} spp. via contact with cattle was higher than via contact with sheep. This was probably due to that the probability of contact with cattle was higher than the probability of contact with sheep; more than 80% of households keep cattle while about 15% keep sheep. However, these results were based on our assumptions for the probability of contact, further studies are required to characterize the type and frequency of contact with different species of ruminants. Based on the available data and our assumptions, the results showed that contact with cattle and consumption of homemade dairy products processed from cattle milk were the main routes for human exposure to \textit{Brucella} spp. in the study area followed by contact with sheep. The sensitivity analysis showed that the prevalence of infection in ruminants and the shedding period (infectious period) were the most influential parameters on the probability of human exposure to \textit{Brucella} spp. Therefore, controlling the disease in ruminants would reduce the risk of human exposure to \textit{Brucella} spp. Regardless of this, multidisciplinary co-operations, co-ordinations, and communications between the relevant authorities through justifying the One Health approach are required. Our model showed that vaccination of ruminants as a risk mitigation strategy for human exposure to \textit{Brucella} spp. would reduce the probability of exposure via all routes. However, the impact of vaccination (strategy 1) on reducing the probability of human exposure to \textit{Brucella} spp. was low compared with the impact of boiling milk before processing homemade dairy products (strategy 2). This was probably due to the high frequency of contact with cattle in the study area, given that our model estimates the annual probability, at least once a year, of human exposure to \textit{Brucella} spp. Also, it would take about 10 years to achieve a significant reduction in the prevalence of brucellosis in ruminants, using the vaccination strategy \cite{17,40}. Therefore, controlling the disease in animals should be accompanied by other risk mitigation strategies.

Our results showed that boiling milk before processing it into homemade dairy products would reduce the probability of human exposure via consumption of these products by 50%. This critical finding is matching the results of Celebi \textit{et al.} \cite{41}. However, practical implementation of this strategy may be difficult due to many reasons. The adverse effects of heat treatment on rennet coagulation as heat treatment of milk results in reduced strength of rennet gels upon longer rennet coagulation times as well as heating milk above 60°C leads to denaturation of whey proteins \cite{42}. The probable changes in the taste or flavor of dairy products may not be acceptable to consumers. Processing and consumption of homemade dairy products usually take place in households. In addition, villagers believe that raw milk is more nutritious and boosts immunity. Efforts would be required to convince livestock owners to comply with this strategy to protect themselves and other consumers from brucellosis and any other milk-borne zoonoses. Media could play a very important role in the implementation of this strategy.

In addition to controlling the disease in animals, public health education and awareness of the routes of exposure are very important. Encouraging livestock owners to use protective gloves and other hygienic measures for preventing disease transmission, comply with the veterinary authorities regarding the periodic examination of brucellosis, contact the veterinary authorities if abortions occur among their animals, culling infected or aborted animals rather than selling them or keeping them for continued breeding purposes, and protecting human from exposure via direct contact with animals and/or animal excreta contaminated with \textit{Brucella} spp.

To the best of our knowledge, this is the first study for the assessment of human exposure to \textit{Brucella} spp. in Egypt. Due to data limitations, it was not possible to distinguish between risks associated with each animal species separately and
between different types of homemade dairy products. One of the limitations of this assessment was the type and frequency of contact with different species of ruminants. Despite these limitations, our assessment was worthwhile since we were able to estimate the probability of human exposure to *Brucella* spp. associated with different potential routes. The main data gaps were; the pattern and frequency of shedding of *Brucella* spp. in milk and different discharges, the frequency of human contact with livestock, and the frequency of consumption of home-made dairy products, for which further studies were required. Quantitative data for the concentration of *Brucella* spp. in raw milk and the effect of different processing steps of homemade dairy products on the inactivation of the pathogen were also required to enable a fully quantitative risk assessment from farm to fork.

**Conclusion**

The main routes for human exposure to *Brucella* spp. in the rural Nile Delta were contacting with cattle and the consumption of homemade dairy products processed from cattle milk. Vaccination of ruminants and boiling raw milk before processing homemade dairy products would reduce the risk of human exposure to *Brucella* spp.

**Conflicts of interest**

The authors declared no competing interests.

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**Author’s contributions:**

Conceptualization; M.M.E., N.H.A., and Y.H. Formal analysis; M.E., Y.H., N.H.A. and Y.E. Funding acquisition; M.M.E. and Y.H. Methodology; All authors contributed equally. Software; M.E. N.H.A., and Y.E. Writing - original draft; M.E., Y.H., E.W.A. and N.H.A, review & editing; All authors contributed equally. All authors have agreed to publish the manuscript in its current format. The authors read and approved the final manuscript.

**Ethical approval**

The research ethics committee for experimental and clinical studies, Animal Health Research Institute (No. 165677), approved the protocol of this study. This study follows the guidelines of the Egyptian Network of Research Ethics Committees and the international laws and regulations concerning ethical considerations in research. The scientific research ethics committee, Faculty of Medicine, Fayoum University, Egypt (No. 186/79) approved the study design and the questionnaire of the human participant in this study. We obtained written informed consent from all the participants.

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