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## **A multiple risk model for brucellosis at the human-animal interface in Egypt**

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## Abstract

Brucellosis is a highly contagious zoonosis affecting humans and a wide range of domesticated and wild animal species. An important element for effective disease containment is to improve knowledge, attitudes and practices (KAP) of afflicted communities. This study aimed to assess the KAP related to brucellosis at the human-animal interface in an endemic area of Egypt and to identify the risk factors for human infection. A matched case-control study was conducted at the central fever hospitals located in six governorates in northern Egypt. Face-to-face interviews with cases and controls were conducted using a structured questionnaire. In total, 40.7% of the participants owned farm animals in their households. The overall mean practice score regarding animal husbandry, processing and consumption of milk and dairy products was significantly lower among cases compared to controls [ $-12.7 \pm 18.1$  vs  $0.68 \pm 14.2$  respectively;  $p < 0.001$ ]. Perceived barriers for notification of animal infection/abortion were predominate among cases and positively correlated with participants' education. The predictors of having brucellosis infection were consumption of unpasteurized milk or raw dairy products and practicing animal husbandry. Applying protective measures against infection significantly reduced its risk. A model predicting risk factors for brucellosis among those who own animal showed that frequent abortions per animal increased the chance for brucellosis infection among human cases by 50-fold (95% CI: 8.8 – 276.9), whereas the use of protective measures in animal care reduced the odds [OR= 0.11 (95% CI: 0.03 – 0.45)]. In conclusion, consumption of unprocessed dairy products was equally important as contact with infected/aborted animals as major risk factors for *Brucella* spp. infection among humans in Egypt. There is poor knowledge, negative attitudes and risky behaviors among villagers which can perpetuate the risk of brucellosis transmission at the human-animal interface. This supports the need for integrating health education into the national brucellosis control program.

**Keywords:** brucellosis; KAP; risk factors; human-animal interface; Egypt

**Running title:** Brucellosis at the human-animal interface in Egypt

## Author summary

Zoonotic brucellosis has a vast global burden and remains neglected in many areas of the world despite notable advances in disease containment strategies. Despite the implementation of a national brucellosis control program in Egypt, the challenges for disease eradication are intractable and multifaceted. In this study, we modelled multiple risk factors for brucellosis persistence in Egypt and found that populations across a wide region of the country lacked basic understanding of the disease nature and unknowingly engaged in risky behaviours and traditional practices on farms and within households. The predominant behaviours putting them at risk included consumption of dairy products from unregulated sources; underreporting animal infection and abortion; underutilization of animal vaccination service; unsanitary disposal of abortus and animal waste; use of milk from infected/aborted ruminants; and lack of protective measures during husbandry and handling animal wastes. Together, these practices negate disease intervention strategies by contributing to disease spread and re-emergence. The proposed model provides a framework for future containment

strategies that should be adopted to support and enhance the adherence to the current national brucellosis control program.

## Introduction

Brucellosis is a neglected zoonosis of public health and economic significance in most developing countries. Although the disease is well controlled in some countries, it remains endemic in others with the highest records in the Middle East and central Asia (Kirk et al., 2015; Pappas, Papadimitriou, Akritidis, Christou, & Tsianos, 2006). In most of these countries, the primary source of human infection is cattle, buffaloes, sheep and goats infected with *Brucella* spp. (Marcotty et al., 2009; Refai, 2002). Therefore, measures and strategies aimed to reduce the prevalence of brucellosis in animals are considered the most effective means of controlling human infection (Glynn & Lynn, 2008).

In animals, the disease is highly contagious affecting almost all domestic species, leading to severe economic losses due to abortion, infertility, loss of milk production and cost of veterinary care. Reliable estimates of the frequency of brucellosis among ruminants in Egypt are lacking due to inability to test all eligible animals periodically and properly (Hegazy, Ridler, & Guitian, 2009). However, recent studies addressing the occurrence of brucellosis in ruminants indicate that the disease is endemic in all ruminant species with a prevalence mounting to 26.6% (Kaoud, Zaki, El-Dahshan, & Nasr, 2010; Hegazy, Moawad, Osman, Ridler, & Guitian, 2011; Hegazy, Molina-Flores, Shafik, Ridler, & Guitian, 2011; Holt et al., 2011; Wareth et al., 2014; Hegazy, Elmonir, Abdel-Hamid, & Elbauomy, 2016). This magnitude questions the efficacy of the applied national control programme for brucellosis which was established in the early 1980s. The program is based on serological surveys that rely on the test and slaughter strategy where compensations are paid for livestock owner, milk ring testing for pooled milk, and optional vaccination of ruminants using S19

vaccine for calves and Rev1 vaccine for young sheep and goats (Wareth et al., 2014; Eltholth, Abd El-Wahab, Hegazy, & El-Tras, 2015).

Human infection with brucellosis has been reported in different studies in different geographical areas. The median number of foodborne illnesses, deaths, and Disability Adjusted Life Years (DALYs) were reported by the WHO in 2010 to be 832,633 (95% CI=337,929–19,560,440); 4,145 (95% CI=1,557–95,894); 264,073 (95% CI=100,540–6,187,148) (Kirk et al., 2015). In Egypt, the rate of human infection is greatly affected by the rate of disease in animals (Afifi et al., 2005; Jennings et al., 2007; El-Ghitany, Omar, Abaza, Hassan, & Abd El-Wahab, 2014; Eltholth et al., 2015). Direct contact with infected animals, aborted foeti, foetal membranes, and vaginal discharges of infected animals are risk factors for human infection with brucellosis. Further, humans can be exposed to infection through ingestion of un-pasteurised milk and raw milk products, such as soft cheeses and yogurt, which are commonly consumed in Egypt. Establishing the relative contribution of occupational and food-borne risk factors will inspire more targeted public health programmes. In this context, Jennings and co-workers recommended that further studies are needed to assess the risk of human exposure to brucellosis via different exposure routes (Jennings et al., 2007).

So far in Egypt, no specific study had tackled the economic and logistic causes for the failure of the control programme on small livestock holders and on the commercial level as well. However, the lack of compliance of the farmers with this programme due to the weak compensation for the slaughtered infected/aborted ruminant which is usually the key incentive used for test-and-slaughter strategy was suggested as one of the major causes of program inadequacy (Holt et al., 2011). In addition to poor farmer engagement, the program relies on serological testing of ruminants although culture positive/seronegative cows and intrauterinely infected calves that seroconvert after birth is well documented (El-Diasty & Wareth, 2018). This might perpetuate the spread of infection to susceptible hosts and the environment (El-Diasty & Wareth, 2018). Further, the program ignores the surveillance of infection in non-specific hosts including pet animals on farms as reservoirs of infection (Wareth et al., 2017) despite that that spillover from livestock to wildlife has

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been reported (Truong et al., 2011). This is becoming particularly relevant, as stray dog and cat populations are increasing. Importantly, the program did not establish designated disposal sites for animal wastes. As such, the livestock owners manage their own contaminated materials depending on their own knowledge and resources and consequently biohazards are not safely discarded in ways that ensure biosecurity.

Therefore, the aims of this study are to explore the risk factors of *Brucella* spp. infection among humans and to study the specific KAP components that contribute to the poor response to brucellosis control at the human-animal interface in Egypt. Further, we sought to identify the critical points which need to be addressed and managed in future interventions. This will build baseline data to design a framework for identifying problems facing the current national brucellosis control programme for smallholders and at the national-level and help scientists and policy-makers to develop more effective control strategies.

## Methods

### Study design, setting and population

A case-control study was conducted between June 2014 and June 2016 in the central fever hospitals serving 6 governorates in north Egypt (Al-Beheira, Al-Gharbia, Kafr el-Sheikh, Al-Daqahliyah) and two neighbouring cities (Alexandria and Matrouh) (Figure 1). These governorates have a high density of people and livestock, where human and animals are living in close proximity, particularly in small-scale farming systems. An electronic map of Egypt was provided by the General Organization of Veterinary Services (GOVS) in Egypt. A choropleth map (Figure 1) was built for the geographic distribution of different study locations within Egypt using Quantum GIS (Quantum GIS Development Team 2017), [www.qgis.org](http://www.qgis.org).

The sample size was calculated using Win Episcopes 2.00 for a matched case-control study based on 80% power and 95% confidence interval with 40% estimated exposure rate for controls and 2.2 minimal Odds Ratio (OR) to be significantly detected. The minimal required sample size was

206 (103 cases and 103 controls). However, we included in the study 217 cases and the number of controls was doubled to have 434 controls, 2 controls for each case. Cases were defined as individuals seeking medical care at the fever hospitals within the study area. The case definition of the Centers for Disease Control and Prevention (CDC, 2010) was applied: *“acute or insidious onset of fever and one or more of the following: night sweats, arthralgia, headache, fatigue, anorexia, myalgia, weight loss, arthritis/spondylitis, meningitis, or focal organ involvement (endocarditis, orchitis/epididymitis, hepatomegaly, splenomegaly)”*. The diagnostic work-up of suspected cases included Rose Bengal Test (RBT). Positive RBT results were confirmed by Standard Tube Agglutination Test (SAT) with titre > 1:160. Cases were included in the study once they are identified and controls were sampled over the same time period.

To reduce selection bias, for each enrolled case, two controls matched for age, gender and residency (rural or urban) were selected from persons seeking medical care for other health conditions at the same hospitals. Controls were confirmed as serologically free of brucellosis using SAT with titre > 1:160. No incentives to participate in the study were provided.

#### **Data collection**

A structured questionnaire was used for collecting sociodemographic (for the study participants and other family members in the same houses) and epidemiological data on potential risk factors for an individual being seropositive against brucellosis. These included dairy product consumption habits, animal husbandry practices and history of exposure of humans and animals to brucellosis in the same household. Further, information on the cooperation with health services in case of human or animal infection with brucellosis was gathered. Knowledge, attitudes and practices regarding brucellosis were assessed through the use of open-ended questions (S1 File). The questionnaire was developed, pre-tested and validated to have a good insight in the small-scale dairy farming sector in the study area. All interviewers were trained to standardise the interviewing

method. During the interviews, the questions were continuously evaluated to make sure that the farmers understood them correctly.

#### **Data curation and storage**

Collected data was checked for integrity and completeness, coded and fed to computer software. All paper forms were stored in a dedicated storage place (filing cabinet) after erasing any identifying information. Data were electronically stored in files and copies were saved on CDs, a computer drive and a cloud drive.

#### **Calculation of scores**

Knowledge of participants towards brucellosis was assessed through 5 open-ended questions. Correct complete answers were scored 3 points, incomplete answers were scored 1 point and wrong/do not know answers were scored 0 point. Attitude (perceived barrier, perceived risk, perceived susceptibility) were assessed through 7 open questions. For perceived barriers, 1 point was given for each response. For perceived risk and perceived susceptibility, responders were asked to rate their responses as none, low, mild, moderate and high. Accordingly, responses were rated on a 5-point Likert scale [1 low-5 high]. Hygienic practices were assessed via 26 practice statements measured by yes/no answers or through a three-point frequency rating with the options “always”, “sometimes” and “never”. Safe practices were given score of 1 and risky ones were scored -1. The overall KAPs of the study participants were analysed using the sum score of each outcome based on the modified Bloom’s cut-off point. The total score was qualified as “good” if exceeded 75% of the total score, “average” for scores between 50-75% and poor for scores < 50% to -100%. Milk consumption score was calculated as number of days of milk consumption per person per week; 0= no consumption, 1= consumed 1-3 times in a week, 2= consumed >3 times in a week.

## Statistical analysis

Data were analysed using IBM® SPSS® Statistics version 21.0 and SAS 9.2 (SAS Institute Inc. 2008). Differences in proportions among cases and controls were evaluated by Pearson's Chi-square test. Differences in the total mean KAP scores were analysed using student t- test. A  $p$  value  $< 0.05$  was set as a level of significance. The association between the potential risk factors and a brucellosis case was examined using a multivariable conditional logistic regression model, with individual status being a control or a case as the response variable. The selection of variables to be included in the multivariable model was carried out in two steps. Initially, a univariate analysis was performed to determine the association between each of the examined variables and disease status of each individual where variables for which  $P > 0.2$  were excluded from further analysis. The collinearity between pairs of variables with a  $P < 0.05$  in the previous step was assessed by calculating the Phi coefficient. The significance of this collinear association was examined using chi square test. In the case of a pair of variables with a significant association ( $P < 0.05$ ), the variable judged as the most biologically plausible was used as a candidate in the multivariable analysis. All variables passed the previous 2 steps were incorporated in the final multivariable conditional logistic regression model. A manual stepwise selection approach was used for the selection of variables in that model to keep only variables with  $P < 0.05$  in the final model. All two-way interactions between variables retained in the model were assessed. Testing for confounders was carried out by monitoring the change of logit of factors by removing a suspected factor from the model.

## Results

### 1. Socio-demographic characteristics of the study participants

The study comprised 217 cases of confirmed human brucellosis and 434 matched controls with an overall mean age of  $35.2 \pm 13.9$  and  $35.6 \pm 14.5$  years, respectively. There were no statistically significant differences between cases and controls regarding other socio-demographic characteristics including their occupation, education and income (Table 1). Infected individuals



reported a significantly higher number of household members infected with *Brucella* spp in the 3 months preceding the test compared to controls [(4.6% vs 1.8%) ( $P= 0.015$ )] (Table S1). Infected individuals and their household members generally sought medical advice in private clinics (96.1%) and 96.8% of them were then referred to fever hospital for admission. About 95% of infected participants initially purchased medicine from the pharmacy before visiting a doctor.

## **2. Owned farm animals in households and human exposure to *Brucella* spp.**

In total, 40.7% of the study participants reported having farm animals in their households [48.8% of cases vs 36.9% of controls; ( $P= 0.003$ )]. Of those households having animals, 91.7%, 74.1%, 62.8%, 41.0% and 35.7% reported having cows, buffaloes, sheep, goats and donkeys/camels, respectively. The percentage of households having sheep and goats was significantly higher among cases compared to controls ( $P< 0.001$ ) Figure 2. The majority of livestock owners (78.1%) accommodate large ruminants (cows and buffaloes) and small ruminants (sheep and goats) together with no significant difference between cases and controls ( $P= 0.33$ ) (Table 1).

Cases of human brucellosis were significantly associated with a history of animal abortion in the household within the 12 months preceding the incidence of human infections compared to the controls [(23.5% vs 9.7%, respectively),  $P= 0.0003$ ] (Table 1). All ruminant species were reported to have experienced abortions (Table S2). The majority of participants claimed that they did not know the cause of abortion, while others reported trauma (17.0%), fever (8.5%), brucellosis (6.4%) and poor feeding (2.1%) as possible causes (Table S2). When their livestock was aborted, livestock owners, tended to call a private veterinarian (83.2%) rather than a government veterinarian (12.8%) when their livestock aborted (Table S3). Biological samples were collected to identify the causes of the abortion in utmost 10.9% of abortion incidents, resulting in no significant difference between controls and cases in such practice ( $P= 0.34$ ). The proportion of aborted cows and goats that were seropositive for brucellosis belonging to human cases was significantly higher than those among control participants. The majority of the participants (82.4%) did not notify authorities of aborted

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animals with no significant difference between cases and controls. Most of the study participants (65.8%) admitted that they do not know that they have to notify authorities in case of animal abortion, while some of them expressed concerns that health authority would slaughter the affected animals without sufficient compensation (Table S3). Data about how participants handle aborted animals, dispose aborted foeti and process milk from aborted animals is displayed in Tables S4 and S5. Most of households kept aborted animals for fattening, reproduction, or sell them for reproduction. Almost 49% of livestock owners reported throwing the foetal membranes and aborted foeti in water canals. Importantly, about half of participants consumed or sold milk and dairy products for consumption either with or without heat treatment.

Utmost, 6 (2.3%) human cases and none of the controls declared that they had brucellosis infected animals, as proved by private laboratory tests (50.0%) or across the national brucellosis control campaigns (33.3%), within 12 months before the study (Table S6). Reasons for not notifying and the outcomes following a confirmed animal case are summarized in Table S6. One of the main causes of denying notification is that the participant did not know that he should notify authorities. Half of participants admitted that they discard the milk of aborted lactating animals. On the other hand, cases and controls indifferently reported consumption of heat-treated milk, selling raw milk and processing homemade cheese, cream, butter, and ghee from milk of infected animals (Table S7). The number of control participants that used measures for protecting their owned household ruminants from infection with *Brucella* spp. was significantly higher than number of the infected cases (Table 2). The practice and the positive attitude towards animal vaccination were significantly higher among controls comparing to infected cases ( $P < 0.001$ ) (Table S8). The latter reported the adoption of some measures for protecting household members from exposure to *Brucella* spp. more than controls [54.8% of cases vs 29.3% of controls;  $p < 0.001$ ]. These included boiling milk before consumption [30.9% of cases vs 28.3% of controls], buying pasteurised milk [14.3% of cases vs 2.1% of controls], not involving in parturition/abortion of animals [14.3% of cases vs 5.5% of controls], and using personal protective equipment (PPE) against occupational hazards [6.0% of cases vs 4.6% of

controls]. On the other hand, vaccinating animals was more frequently specified by controls [1.8% of cases vs 8.3% of controls] (Table 3).

Human brucellosis cases tended to be more frequently involved in activities in which they come in contact with animals, particularly when helping in animal parturition, abortion, disposing placental and aborted foeti and slaughtering (Table S9). Furthermore, infected individuals were more frequently involved in dairy product processing (Table S10). Control participants were more likely to sell/buy livestock products in markets and neighbouring villages than cases (Table S11). The proportion of cases consuming dairy products from small ruminants was higher than controls, but the frequency of consumption of dairy products did not differ significantly (Table S12). Cases admitted the use of PPE such as gloves and mask and emphasized hand hygiene more frequently when compared to controls (Table S13).

### 3. Knowledge

More than two thirds (67.4%) of the participants had not heard about a disease called brucellosis with no significant difference between cases and controls [(70.0% of the cases vs 66.1% of the controls),  $P= 0.315$ ] (Table S14). The source of knowledge was mainly through communications with neighbours (74.6%), veterinarians (48.8%) and relatives (7.4%). About 82% of the participants did not know which animal species were more susceptible to brucellosis. According to 17.4%, 17.1%, 13.5%, 11.4%, 3.1% and 2.8% of participants the following animal species are susceptible to infection with *Brucella* spp.: cows, sheep, goat, buffaloes, poultry/duck - and “all animal types”, respectively (Table S14). Controls were significantly more knowledgeable than cases that sheep, goats and cows are susceptible to *Brucella* spp. infection (Table S14). The age at which the animals are most susceptible to *Brucella* spp. infection as stated by the study participants is displayed in Table S14. Almost one third of the participants believed that *Brucella* spp. can be transmitted to humans, with cases being more acquainted with that than controls [43.4% of cases vs 28.6% of controls; ( $P< 0.001$ )] (Table 4). Data about the perceived risk of the mentioned routes of

transmission are displayed in Table S15. Despite that cases were more knowledgeable than controls about the danger of consuming infected dairy products and having contact with infected animals, there was significantly lower understanding among cases than controls of how importance are these routes in transmission of *Brucella* spp. to humans (Table S15).

The results showed that cases were less knowledgeable about brucellosis and the risk of infection. Moreover, they reported more risky attitudes and practices than controls (Table 5). The overall mean practice score was significantly lower among cases comparing to controls ( $-12.7 \pm 18.1$  vs  $0.68 \pm 14.2$  respectively;  $P < 0.001$ ). Perceived barrier for notification of animal infection and/or abortion was significantly higher among cases ( $p = 0.034$ ) and positively correlated with participants' education (Table 5). Knowledge was strongly associated with participants' practice, perceived barriers, as well as perceived susceptibility and risk (Figure 3).

#### **4. Risk factors for human brucellosis infection**

The results of the univariable relationships between independent variables and infection status of the participants are shown in Table 6. The following activities were associated with an increased risk of contracting brucellosis: keeping i) animals in households, especially small ruminants, non-vaccinated and aborted animals, ii) processing dairy products, iii) consuming different dairy products, iv) not following protective measures to protect animals from infection, v) applying low number of protective measures to protect human from *Brucella* spp. infection and opting out notifying authorities for animal infection or abortion. The odds of having human brucellosis infection was 9.65 times higher among participants reporting the occurrence of animal abortion [(95% CI: 3.37 – 27.64);  $P < 0.001$ ]. This risk mounted to 52.7 times more among cases who have high number of aborted animals compared to those having a single aborted animal [95% CI: 11.32 – 245.4);  $P < 0.001$ ]. On the other hand, people who have vaccinated animals [OR= 0.1 (95% CI: 0.04 – 0.24)], follow protective measures for animals against *Brucella* spp. infection [OR= 0.11 (95%

CI: 0.04 – 0.31)] and notify authorities of aborted animals [OR= 0.14 (95% CI: 0.04 – 0.45)] were significantly at lower risk of getting human brucellosis infection. Nevertheless, these 5 variables were not incorporated in the final multivariable analysis because in each variable, 385 out of 651 respondents had no animals in their households.

The presence of animals in the households of the respondent, number of owned animals, presence of small ruminants and the number of small ruminants were found to have strong collinearity at  $P < 0.05$ . Therefore, in the final multivariate logistic regression model, these variables were detected as confounders to each other. Thus only “the presence of small ruminants in respondent household” was included in the final model as it was the only variable with constant OR (95% CI) and  $P$  value comparing to other variables. Likewise, strong collinearity was found between the use of protective measures and number of protective measures used by the livestock owners when they come in contact with animals, with the first variable being used in the final multivariable analysis. On the other hand, the number of protective measures taken to prevent human infection was considered in the final model instead of if the person follows these protective measures or not for the same last reason. Milk score consumption was removed from the multivariable analysis for the same reason since they had significantly collinearity with: consumption of dairy products in last 3 months, consumption of unpasteurized milk, consumption of yoghurt and consumption of home-made cheeses. Having infected animals in the households and notification of authorities for of a case of brucellosis, were not significantly associated with human infection and were not included in the multivariable analysis as their  $P$  value was  $> 0.2$ . However, this may be due to type II error, as there were few responses to this question, rather than a true lack of association.

The following variables were used in the multivariable model: presence of small ruminants in the households, contact with animals, following protective measures during contact with animals, number of protective measures the respondent uses to protect himself and household members from exposure to *Brucella* spp., having consumed dairy products in the 3 months preceding the test, regular consumption of unpasteurized milk, fermented milk, cream, home-made cheese or yoghurt,

being involved in processing milk products and having a household member infected with *Brucella* spp. in the 3 months preceding the test. As involvement in animal husbandry was found as confounding factor for presence of small ruminant, it was therefore removed from the analysis. Testing for confounder was carried out by monitoring the change of logit of factors by removing a suspected factor from the model. Out of the 11 variables, 5 were removed before building the final model because they did not meet the 0.05 significance level for consideration. These included consumption of fermented milk and cream, involvement in dairy products processing, infected household member in last 3 months, and protective measures followed when the participant come in contact with animals.

Results of multivariable logistic regression analysis are shown in Table 7. Consumption of dairy products in the last 3 months preceding the test [OR= 2.71 (95% CI: 1.06 – 6.93);  $p < 0.038$ ] and consumption of unpasteurized milk [OR= 4.12 (95% CI: 1.62 – 10.75);  $p < 0.003$ ] or home-made cheeses [OR= 1.96 ( 95% CI: 1.17 – 3.30);  $p < 0.011$ ] and yoghurt [OR= 2.51 (95% CI: 1.21 – 5.24);  $p < 0.014$ ] were associated with higher odds of having brucellosis infection. Participants who were involved in activities where they came in contact with animals had more than 4.97 times greater odds of having brucellosis infection [95% CI: 2.84 – 8.72;  $p < 0.001$ ]. Finally, participants who take more protective actions for themselves against brucellosis are almost 5 times less likely to have been diagnosed with brucellosis [OR=0.23 (95% CI: 0.10 – 0.58);  $p < 0.001$ ].

We developed another model to test the association between selected potential risk factors and individual *Brucella* spp. positive status among people who owned animals at their households. The same variables including the aforementioned 5 variables deleted from the first model were used (Table 8). For participants that owned animals, vaccination of animals, if the owner has animals that aborted, and whether or not they notified the authorities were removed from the final model as they were confounders for the variables: protective measures respondents apply to prevent their animals from *Brucella* spp. infection and the number of abortion per animal, respectively. Increase in the number of abortions per animal increased the odds of brucellosis infection among human cases

by 49.33-fold (95% CI: 1.5 – 155.7;  $P < 0.001$ ) while the practice of protective measures with animals significantly reduced the odds [OR= 0.11 (95% CI: 0.03 – 0.45);  $P < 0.002$ ].

## **Discussion**

### **Livestock ownership**

Despite its high burden in many parts of the world, brucellosis is rarely prioritized by health systems in developing countries and therefore it is considered a neglected zoonosis (Nicoletti, 2010; WHO, 2006). Our study adopted an integrated approach in attempt to identify knowledge gaps, attitude and practices associated with brucellosis from both veterinary and human health perspectives in Egypt, where the disease remains endemic (El-Metwally et al., 2011; Moawad, et al., 2011; Holt et al., 2011; Hotez, Savioli, & Fenwick, 2012; El-Ghitany et al., 2014; Eltholth et al., 2015; Hegazy et al., 2016). In this major agrarian region, most of residents rely on agriculture, with a large proportion entirely depending on livestock production for their livelihood and this could pose a public health threat. The present study revealed that 40.7% of the study population kept animals in their households and they house cows and buffaloes together with sheep and goats. This represents a great risk factor for brucellosis transmission between animals and to humans since small ruminants are the primary hosts of *B. melitensis*, the predominant *Brucella* spp. circulating in Egypt, which can cross species barriers and establish a permanent reservoir in cattle and buffaloes (Holt et al., 2011).

### **Knowledge regarding brucellosis and modes of its transmission**

Knowledge about the disease and preventive herd management practices have previously been identified as the most important factors required for minimizing the risk of disease in animals (Diez & Coelho, 2013). In this report, more than two thirds of the participants had not heard of brucellosis or its possible transmission, indicating that knowledge is a major barrier to disease prevention in the area. This is consistent with previous reports in Egypt (Safaan & Mohsen, 2016) and Nigeria (Adesokan, Alabi, Stack, & Cadmus, 2013; Buhari, Saidu, Mohammed, & Raji, 2015), but

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differed from others studies in Egypt (Holt et al., 2011; Hegazy et al., 2016) and other countries such as Kenya (Obonyo & Gufu, 2015), Jordan (Musallam, Abo-Shehada, Omar, & Guitian, 2015), India (Mantur & Amarnath, 2008), Tajikistan (Lindahl, Sattorov, Boqvist, & Magnusson, 2015), Nigeria (Buhari et al., 2015) and some neighbouring countries including Sudan (Madut et al., 2017) and Palestine (Awwad et al., 2017) where the majority of the study respondents had heard of brucellosis and correctly believed that brucellosis is transmissible from animals to humans. This could be attributed to different population composition since these studies tended to interview pastoralist, shepherds and livestock keepers whereas more than half of our study population had no contact with animals whatsoever.

Poor knowledge and misconception on brucellosis determinants could negatively impact individuals' preventive and disease control methods, particularly, at the humans-animal interface. This was evident in the current study, since participants' knowledge significantly reflected their attitudes, practices, perceived barriers and perceived susceptibility and risk. However, high levels of awareness do not necessarily lead to appropriate behaviour and practices, as the perception of a risk is influenced by many factors such as life experience and culture (Sjoberg, 2000). Contrary to findings in Tajikistan (Lindahl et al., 2015) and Yemen (Al-Shamahy, Whitty, & Wright, 2000) and some other countries (Zhang, Zhou, Huang, & Guan, 2019), participants awareness about the disease did not correlate with the educational level, although participants with a low level of literacy were more likely to have risky practices and high perceived barriers towards the disease control. Those with a lower level of education are thus likely to be at higher risk of contracting brucellosis.

Similar to reports from Uganda (Kansiime et al., 2014) and Tajikistan (Lindahl et al., 2015), the main sources of information on brucellosis was through communication with neighbours and veterinarians. Few of our study participants mentioned media, such as radio or television as a source of information about the disease, although prior interventions used media as the main approach for disseminating information on brucellosis disease in Jordan (Musallam, Abo-Shehada, & Guitian, 2015). From our experience, in Egypt, the media typically disseminates health information only in



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cases of epidemics, but rarely addresses diseases of endemic nature. Together, these highlight the powerful role that veterinarians and the community health workers play in terms of conveying important health messages to livestock owners in the study area on preventative biosecurity practices, particularly that in most circumstances, most of them have faced barriers in accessing basic health care services particularly. Deliberate actions should therefore be taken to incorporate all aspects of health care education for the livestock owners.

Kozukeev et al., found that having knowledge about the transmission of brucellosis from animals to humans had a protective effect towards human infection (Ajeilat, Maes, & Favorov, 2006). In our study, consumption of raw contaminated milk and milk products, contact with infected ruminants and involvement in infected animal abortion or parturition were the most frequently listed modes of brucellosis transmission. The participants' responses regarding involvement in animal husbandry and consumption of milk as a mode of transmission were comparable to earlier findings in Egypt (Hegazy et al., 2016; Holt et al., 2011), Kenya (Obonyo & Gufu, 2015) and Uganda (Kansiime et al., 2014; Asiimwe, Kansiime, & Rwego, 2015). Other additional routes were mentioned, most of which have been previously identified in many studies as major risk factors for transmission of brucellosis at human-animal interface (Cooper, 1992; Al-Shamahy et al., 2000; Kozukeev et al., 2006; Glynn & Lynn, 2008; Earhart et al., 2009; Abo-Shehada & Abu-Halaweh, 2013; Calistri et al., 2013).

In a recent meta-analysis of 79 observational studies, the total pooled awareness level of brucellosis regarding its zoonotic nature, modes of transmission, signs of human or animal disease was 55.5% that was obviously higher among health workers (human health workers and veterinarians) compared to livestock owners, farmers, herders and abattoir workers (Zhang et al., 2019).

### **Perceived susceptibility of animals to brucellosis**

Knowledge of the animal species affected and symptoms of brucellosis in animals is crucial because it positively impacts livestock owners' practices towards prevention and control measures of brucellosis in both animals and humans. In this regard, the basic knowledge of interviewed participants about the animal species that could be affected by brucellosis was poor. This contrasts with the findings of studies in Tajikistan (Lindahl et al., 2015) and an earlier study in Egypt (Holt et al., 2011) where the majority of respondents knew that cattle, sheep and goats could be affected. Few mentioned fish as a susceptible host to brucellosis. Although this may appear incorrect, Nile catfish have been found to be infected with *B. melitensis* in small tributaries of Nile canals in Kafr el-sheikh, Al-Gharbiya, Al-Menoufia, and Al-Daqahliya governorates in the Nile Delta region, where it was isolated from liver, kidney, spleen samples and skin swabs of wild fish; but not from samples of farmed fish (El-Tras et al., 2010). This indicates that the heavy contamination of water by animal waste presents a new potential route of human infection. Other respondents listed cats, dogs and rats as susceptible host for brucellosis. In fact, *B. melitensis* biovar 1 and 3 had previously been isolated from stray dogs, cats and rats trapped near dairy farms and water canals in Egypt; at levels higher than seropositive herds (El-Sherif & El-Sheary, 2002; Wareth et al., 2017). It is likely that respondents who stated these rare brucellosis susceptible animal species were not acquainted with what have been recently published in the literature and reported that by chance. However, these results clearly imply that there is a need for increasing the knowledge and awareness of the community regarding these emerging issues.

### **Perceived severity of brucellosis and barriers to disease notification**

Only a small proportion of the study respondents perceived that brucellosis was a serious disease in both animals and humans and that animal husbandry is a risky practice. Accordingly, they had unfavourable attitude towards good practices preventing brucellosis. Several known high-risk behaviours were common self-reported practices among the study participants, particularly among

infected individuals. They were more likely to engage in risky practices that could expose them to infection. This was evident from actions most of them would take when confronted with an aborting animal in their herd, where the majority would not seek governmental veterinary services and thus would not notify the disease. Some manage animal infection or abortion on their own, while others called private veterinarians. Holt and co-workers stated that private veterinarians decline to report brucellosis to local health authority as they are unlikely to be penalized for that and sometime get benefits from livestock owners for not reporting. Indeed, livestock owners fear of economic losses caused by governmental tracing and culling of their livestock. In contrast to government officials, private veterinarians are usually local community members with social or personal loyalties to livestock owners. Instead of testing, they advise livestock owners to fatten animals suspected to have infection, so that the livestock owner secures profits, thus facilitating the underreporting and disease surveillance (Holt et al., 2011). Livestock owners find tactic easier and more profitable to than notifying the veterinary authorities which may cause delays in sales. While the test and slaughter strategy implemented in Egypt guarantees compensation for livestock owners requiring testing, the amount paid is less than 50% of the market value of the animal and this payment is often delayed (Holt et al., 2011; Hassanain & Ahmed, 2012). This results in underreporting of the diseases and hence hinders brucellosis control in Egypt. Similar barriers to effective disease management were reported in Greece where 44% of patients with brucellosis would not allow veterinary investigation as they were worried about the effects on their herd (Minas, Minas, Gourgulianis, & Stournara, 2007). The government should approach private veterinarians and work more closely with them in order to improve the flow of information and disease notification. Furthermore, adequate compensation or replacement animals should be considered (Holt et al., 2011).

### **Risky versus safety practices**

Instead of calling a veterinarian to deal with abortions or parturitions, livestock owners and others in the village assist with calving, usually by pulling the calf out or removing placenta and foetal membranes. Most farmers bury or dispose of placentas, aborted foeti and carcasses in local water canals. As *Brucella* spp. can survive in aborted fetuses and humid environment (manure and soil) for a period up to 8 months (Saegerman, Berkvens, Godfroid, & Walravens, 2010), these methods are ineffective at containing pathogens. Most villagers come in contact with this potentially contaminated water through daily routines such as bathing, irrigation of fields, washing of utensils, fishing and other activities. Therefore, lack of effective carcass disposal and unrestricted local husbandry methods could result in significant environmental contamination with *Brucella* and increases the risk of disease transmission to human and livestock populations (El-Tras, Tayel, Eltholth, & Guitian, 2010).

Our proposed model revealed that those who practice 2 or more protective measures were at lower risk of getting brucellosis. However, infected cases in the present study were more likely to claim the use of protective measures including gloves and mask and emphasized washing their hands more often compared to controls, suggesting a reverse causality. This result contrasts with a previous study in Egypt where villagers admitted that they never wear protective gloves or masks when assisting with the parturition or abortion of animals or whilst handling placentas and aborted fetuses (Holt et al., 2011). It is plausible that human brucellosis cases may have previously experienced a major illnesses, and consequently increased vigilance to protect themselves. This could otherwise be explained by the “Hawthorne effect” that is, “a behavioural tendency of subjects to provide information consistent with their perception of the study objectives that positively value hygienic behaviours” (Sedgwick & Greenwood, 2015). The deficient use of protective equipment is attributed not only to poor knowledge of the risks associated with this practice but also the lack of access to protective clothing like gloves, or prohibitive costs.

### **Management of animals and animal products**

Unregulated buying and selling of animals and animal products are great hazards as they facilitate transmission between new animals and to people (Holt et al., 2011; Jackson et al., 2007). When the animals abort or become infected, a considerable number of livestock owners in the current study sold the aborted or infected animal either to other livestock owners for reproduction for or to butchers for slaughtering. This imposes a great risk of infection among abattoir workers and butchers. These findings were in the same line with a study conducted by Holt et al., in Egypt (Holt et al., 2011) who explained that animals purchased at a market can move without restriction to anywhere in Egypt. This may increase the transmission of brucellosis between households in the same village as well as between villages and even larger geographical areas (Safaan & Mohsen, 2016).

### **Practices related to consumption, processing and commercialization of dairy products**

Female animals infected with *Brucella* spp. excrete high concentrations of the organism in their milk (Corbel, 1997; WHO, 2006). Therefore, there is a risk of humans becoming infected through consumption of dairy products. In fact, consumption of raw milk has been previously described as one the riskiest practices (Young, 1995). In the present study, drinking raw fresh milk was an uncommon practice as respondents were aware of its danger. Nevertheless, some traditionally believed that consuming raw milk is healthier, boosts immunity and have a cooling effect in the summer while heating affects its nutritive value. Consumption of unpasteurized milk was reported by more cases comparing to controls. This risk appeared negligible in an earlier Egyptian study (Hegazy et al., 2016). However, nearly all respondents in the Kenya study consumed raw milk (Obonyo & Gufu, 2015). More education to explain these risks to villagers is needed to alter practices that facilitate the transmission of brucellosis.

Consistent with findings from Tajikistan (Lindahl et al., 2015), the majority of households admitted their involvement in dairy product processing and sold unpasteurized dairy products from farms directly to consumers on regular basis. Such system contributes around 72% of total milk produced in Egypt. Even though the legislation in Egypt imposes the pasteurization of milk before processing, only modern large-scale dairy plants and 27% of the municipal dairy plants follow the law instructions (Soliman & Mashhour, 2011). Further, the local commercialization of these homemade products is not restricted. There is little surveillance and consequently, these products are often remotely transported and sold without proper refrigeration, preservation, packaging or storage. There is a high demand for these dairy products, particularly for home consumption in big urban cities, including Cairo and Alexandria. Consumers typically heat or boil raw milk before its consumption. Thus, practice of trading with unpasteurized milk and home-made animal products could constitute a risk to public health and threaten food safety.

#### **Modelled brucellosis risk factors at the human-animal interface**

Previous studies in the Nile Delta region identified that the main risk factors of contracting brucellosis [adjusted odds ratio (AOR)] are: having sheep (6.2), being a farmer or butcher (4.5), having aborted animal (3.5) and being older 1.04 per year (El Sherbini et al., 2007). In our proposed logistic regression model, although having infected animal in the households, and notification of authorities for of a case of *Brucella* were disqualified as predictors for brucellosis infection, we do not think that these are not considered as risk factors for brucellosis infection but the results we had may be attributed to the fewer number of respondents to these questions.

Greater numbers of abortions per animal increased the chance for infection among human cases. It is crucial that this finding is communicated to livestock owners who may otherwise insist on keeping aborted animals (for fattening or reproduction) rather than slaughtering them. It is worth noting that livestock owners deny having animal abortion to be able to sell them or their milk (Hegazy et al., 2016).

Animal vaccination appeared as a protective factor in our proposed model for risk factors of human infection. This finding supports the benefits of animal vaccination in reducing the occurrence of human brucellosis (Roth et al., 2003). Interestingly, a significant number among those practicing animal vaccination did not list this practice among the measures that they adopt for protecting themselves, their household member or their animals against brucellosis. This means that villagers can follow a preventive measure without being aware of its benefits probably because this was not properly explained to them.

### ***Conclusion and Perspectives***

In conclusion, there is a poor understanding of brucellosis and a high level of risky practices being undertaken on farms and at households across a wide region of the country. These all hijack disease intervention strategies and contribute to the risk of humans to contract brucellosis. The lack of success of the current national control program for brucellosis in Egypt can be attributed to lack of compliance with disease control measures, particularly: underreporting animal infection and abortion; lack of vaccination; unsanitary disposal of abortus and wastes; consumption and sales of milk from infected ruminants; and lack of protective equipment when practicing animal husbandry.. Understanding the KAP is crucial for assessing the feasibility, acceptability and barriers of potential measures that might be instituted. This strongly supports the need for including health education as part of brucellosis control programs in rural communities with a special emphasis on hygienic animal husbandry, disease notification and the benefits of animal vaccination. This can be achieved by targeted messages in local FM radios and television, besides integrating the community health volunteers in the control and prevention efforts.

The prospective of this work is to collect information from different community sections to assess the economic impacts of brucellosis on small livestock holders and on the national level. The results of this study will be the corner stone for building a more realistic, feasible and economically efficient model for brucellosis control in Egypt to substitute the current national brucellosis control strategy.

### **Limitations of the study**

The key limitation of the present work is self-reporting on practices by the respondents that was subject to recall bias, Hawthorne effect and the face-to-face interview situation. Observational checklist could have enhanced this type of bias in assessing attitudes and behaviours.

### **Ethical considerations**

#### **Compliance with Ethical Standards**

The study was approved by the institutional review board and the Ethics Committee of the High Institute of Public Health-Alexandria University [no. 14-2014/6]. Permission to conduct the study was obtained from the Ministry of Health. The research was conducted in accordance with the ethical guidelines of Helsinki's Declaration (2013) and the International Conference on Harmonization Guidelines for Good Clinical Practice. All participants were given verbal information about the study and were assured about the confidentiality, protection and anonymity of their data. Informed written consent was obtained from the individuals participating voluntarily. Data sheets were coded with numbers to ensure anonymity and confidentiality of patient's data.

This article does not contain any studies with animals performed by any of the authors.

### **Conflict of Interest**

All authors declare no conflict of interest.

### **Data availability**

All data are fully available without restriction by the corresponding author at [ekramwassim@mail.com](mailto:ekramwassim@mail.com) and through the public data repository Harvard Dataverse at <https://dataverse.harvard.edu/dataverse/BrucellosisEgypt>

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### **Supporting Information Legends**

S1 File: questionnaire tool developed by the authors for data collection

S2 Checklist: STROBE Checklist

### **Figure legend**

Figure 1: A choropleth map of Egypt showing the administrative boundaries of the governorates: the dotted governorates represent the study area. The map was created using Quantum GIS (Quantum GIS Development Team 2017)

Figure 2: Distribution of animal species at the household level of study participants (cases and controls)

Figure 3: A corplot visualizing a correlation matrix of the different KAP components

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## List of Tables

Table 1: Sociodemographic characteristics of the study participants

	Total (n=651)		Cases (n=217)		Control (434)		<i>p</i> - value <sup>*</sup>
	No.	%	No.	%	No.	%	
Governorate							
Al Behira	167	27.7	56	25.8	111	25.6	1.0
Alexandria	137	20	45	20.7	92	21.2	
Al-Gharbiya	123	18.9	41	18.9	82	18.9	
Kafr el-Sheikh	123	18.9	41	18.9	82	18.9	
Al-Daqahliya	96	14.7	32	14.7	64	14.7	
Matrouh	5	0.8	2	0.9	3	0.7	
Age (Years)							
4 to < 10	13	2	4	1.8	9	2.1	0.95
10 to <20	68	10.4	23	10.6	45	10.4	
20 to <40	315	48.4	106	48.8	209	48.2	
40 to <60	215	33	73	33.6	142	32.7	
60+	40	6.1	11	5.1	23	5.3	
Gender							
Male	426	65.4	142	65.4	284	65.4	1.0
Female	225	34.6	75	34.6	150	34.6	
Residence							
Rural	481	73.9	163	75.1	318	73.3	0.61
Urban	170	26.1	54	24.9	116	26.7	
Education							
None	174	26.7	63	29.0	111	25.6	0.9
Primary	105	16.1	32	14.7	73	16.8	
Preparatory	68	10.4	21	9.7	47	10.8	
Secondary	165	25.3	53	24.4	111	25.6	
University	139	21.4	48	22.1	90	20.7	
Occupation							



Crop farming	87	13.4	34	15.7	53	12.2	0.45
Animal keeping	46	7.1	24	11.1	22	5.1	
Trading in animals/products	25	3.8	13	6.0	12	2.8	
Trading in agricultural products	37	5.7	7	3.2	30	6.9	
Formal salaried employee	115	17.7	45	20.7	70	16.1	
not working/unemployed	153	23.5	52	24.0	101	23.3	
old/retired	17	2.6	4	1.8	13	3.0	
Infant (<6 years)	16	2.5	3	1.4	13	3.0	
Student/Pupil	61	9.4	25	11.5	36	8.3	
Disabled	3	0.5	2	0.9	1	0.2	
others	142	21.8	36	16.6	106	24.4	
Having animals at the household							
No	385	59.3	111	51.2	274	63.1	0.003
Yes	265	40.7	106	48.8	160	36.9	
Accommodate cows and buffaloes with sheep and goats							
No	58	21.9	20	18.9	38	23.9	0.33
Yes	207	78.1	86	81.1	121	76.1	
Importance of livestock as a source of income for the household							
NA	386	59.3	111	51.2	274	63.1	0.05
Only income source	21	3.2	11	5.1	10	2.3	
Major income source	98	15.1	37	17.1	61	14.1	
same importance as other income source	73	11.2	24	11.1	49	11.3	
Minor income source	52	8	23	10.6	29	6.7	
Negligible	11	1.7	6	2.8	6	1.4	
Do Not Know	10	1.5	5	2.3	5	1.2	
Source of income other than farming**							
Yes	95	30.4	31	29.8	64	30.6	0.93
No	218	69.3	73	70.2	145	69.4	
What overseas family members do**							
NA	216	69.9	73	68.22	143	68.42	0.4

Crop trading	9	2.9	3	2.80	6	2.87	
Animal/poultry trading	3	1	2	1.90	1	0.49	
Trading	24	7.8	11	10.28	13	6.22	
Employee	37	12	14	13.10	23	11.00	
Auxiliary worker	18	5.8	3	2.80	15	7.18	
others (barber, plumber, carpenter, painter, electrician, mechanics, waiter, butcher, smith, tailor, dresser, cashier, clerk, driver, mechanic, seller, cabbies, photographer)	9	2.9	1	0.9	8	3.82	
<b>Other sources of contribution to the household expenses</b>							
NA	465	71.4	155	71.4	310	71.4	0.65
Yes	81	12.4	30	13.8	51	11.8	
No	105	16.1	32	14.7	73	16.8	
<b>History of animal's abortion in the last 12 months (n=266) **</b>							
Yes	93	35	51	48.11	42	26.25	<b>0.0003</b>
No	173	65	55	51.89	118	73.75	

\*  $p$ -value is for chi square or Fisher's exact tests

\*\* the total number not = 651 because of no response of some participants

Table 2: Protective measures taken to protect household ruminants from exposure to *Brucella* spp

	Total		Cases (n=217)		Control (434)	
	No.	%	No.	%	No.	%
None	117	43.9	68	64.2	49	30.6
Yes	149	56.1	38	35.8	111	69.4
$p < 0.001$ *						
		**		**		**
Vaccination	97	36.5	13	6.0	84	19.4
Regular check-up at vet clinic	72	27.1	20	9.2	52	12.0
Never mix or contact foreign animals with other animals/never rear goat with other ruminants	8	3	2	0.9	6	1.4
Do not buy except after being sure it is free of disease	10	3.8	2	0.9	8	1.8
Isolate sick animals and investigate the cause/void contact between sick and healthy ruminants	9	3.4	0	0.0	9	2.1
Isolate new bought animal and verify that it is free of disease	3	1.1	0	0.0	3	0.7
Cleaning animal house/keep it well ventilated	42	15.8	12	5.5	30	6.9
Not breeding poultry with animals	5	1.9	1	0.5	4	0.9
Do not involve in parturition	6	2.3	0	0.0	6	1.4
Treatment of infected ruminants	28	10.5	3	1.4	25	5.8
Slaughter infected ruminants	2	0.8	1	0.5	1	0.2
Sell infected ruminants	4	1.5	1	0.5	3	0.7
Never rear goats / sheep with big ruminants (cows and buffalos)	8	3	2	0.9	6	1.4
Spraying (wetting) with water in the summer/cover its back in the winter	3	1.1	3	1.4	0	0.0
Provide good and clean food/water (at regular times) for ruminants	11	4.1	5	2.3	6	1.4
Never leave clover under legs of the ruminants	2	0.8	1	0.5	1	0.2
Hand washing and ruminant udder washing before milking	3	1.1	1	0.5	2	0.5
Making cement floor for the animal house and not muddy (sandy)	1	0.4	0	0.0	1	0.2

\* $p$ -value is for chi square or Fisher's exact tests

\*\* the total percentage is not 100% because of multiple response answers

Table 3: Protective measures taken to protect household members from exposure to *Brucella* spp.

	Total		Cases (n=217)		Control (434)	
	No.	%	No.	%	No.	%
None	405	62.2	98	45.2	307	70.7
Yes	246	37.8	119	54.8	127	29.3
$p < 0.0001^*$						
	**	***		***		***
Animal vaccination	40	6.1	4	1.8	36	8.3
Boiling milk	190	29.2	67	30.9	123	28.3
Do not involve in parturition/parturition of infected animals	55	8.4	31	14.3	24	5.5
Do not involve in disposing placenta	2	0.3	0	0.0	2	0.5
Regular animal checkup	14	2.2	1	0.5	13	3.0
Let vet doctor responsible for parturition and abortion	7	1.1	0	0.0	7	1.6
Animal slaughter if infected	2	0.3	1	0.5	1	0.2
Do not involve in abortion	8	1.2	0	0.0	8	1.8
Do not be very close to ruminants (breathing)	3	0.5	2	0.9	1	0.2
Do not drink milk/eat homemade cheese or fermented milk of infected ruminant/buy from secure source	24	3.7	9	4.1	15	3.5
Treatment of infected ruminants	10	1.5	0	0.0	10	2.3
Not coming in contact with infected ruminant	19	2.9	6	2.8	13	3.0
Sell infected ruminant	2	0.3	0	0.0	2	0.5
Using personal protective equipment against occupational hazards/wearing gloves, long boot	33	5.1	13	6.0	20	4.6
Not drinking goat milk	2	0.3	1	0.5	1	0.2
Personal cleaning/cleaning animal house, applying sand on ground of animal house	29	4.5	6	2.8	23	5.3
Separate animal house from own house/avoid going to animal house	3	0.5	1	0.5	2	0.5
Do not rear goats and sheep with other ruminants (cows and buffalos)	1	0.2	0	0.0	1	0.2
Prepare/eat cheese and milk products from healthy ruminants	4	0.6	0	0.0	4	0.9
Avoid animal contact when having hand wound	1	0.2	1	0.5	0	0.0
Buying animals from secure source	2	0.3	1	0.5	1	0.2
Eating only milk and milk products from one's own ruminants	1	0.2	0	0.0	1	0.2
Being careful during handling poultry/ deal with animals	5	0.8	1	0.5	4	0.9

Cost of buying milk from secure source (neighbours you know their animal status)	1	0.2	0	0.0	1	0.2
Fighting flies and mosquitos	3	0.5	0	0.0	3	0.7
Provide clean clover, animal food, water	2	0.3	1	0.5	1	0.2
Avoid that children play with sheep	1	0.2	1	0.5	0	0.0
Do not rear animal at all	3	0.5	1	0.5	2	0.5
Buying pasteurized milk	40	6.1	31	14.3	9	2.1
Hand washing	3	0.5	2	0.9	1	0.2
Not take milk from aborted ruminant	1	0.2	1	0.5	0	0.0

\* $p$ -value is for chi square test

\*\* the total number not = 651 because of no response of some participants

\*\*\* the total percentage is not 100% because of multiple response answers

Table 4: Study participants' knowledge regarding *Brucella* spp. infection and its modes of transmission

	Total		Cases (n=217)		Control (434)	
	No.	%	No.	%	No.	%
<b>Do you think that <i>Brucella</i> spp. can be transmitted to humans?</b>						
Yes, I think so	219	33.6	95	43.8	124	28.6
No, I do not think so	235	36.1	60	27.6	175	40.3
I do not Know	197	30.3	62	28.6	135	31.1
<b><i>p</i> &lt; 0.0001*</b>						
<b>Potential routes of transmission of <i>Brucella</i> spp. to humans (n=219)</b>						
	**	***		***		***
Do not know	15	6.8	7	3.2	8	1.8
Parturition	25	11.4	6	2.8	19	4.4
Animal abortion / of infected ruminant	44	20.1	11	5.1	33	7.6
Drinking milk of infected animal	72	32.9	45	20.7	27	6.2
Drinking unboiled milk	78	35.6	19	8.8	59	13.6
Help in getting placenta out	4	1.8	1	0.5	3	0.7
Drinking fermented milk/fermented milk of infected ruminant	76	34.7	37	17.1	39	9.0
Parturition of infected ruminants	8	3.7	0	0.0	8	1.8
Eating homemade cheese/cheese prepared from of infected ruminants	83	37.9	42	19.4	41	9.4
Contact/daily dealing with ruminants/infected ruminants	87	39.7	52	24.0	35	8.1
Breathing/animal odour	14	6.4	4	1.8	10	2.3
Accidental exposure to animal vaccine	2	0.9	1	0.5	1	0.2
Drinking unboiled goat milk	1	0.5	1	0.5	0	0.0
Exposure to animal/birds secretions/ cleaning poultry	7	3.2	1	0.5	6	1.4
Contacting goats/goat come near children food (smell it) and so infect them	3	1.4	1	0.5	2	0.5
By flies and mosquitos	4	1.8	0	0.0	4	0.9
By birds (poultry)/poultry odor/handling sick poultry/cleaning poultry	5	2.3	1	0.5	4	0.9
Through hand wound	2	0.9	2	0.9	0	0.0
Contaminated animal house/cleaning animal house	5	2.3	1	0.5	4	0.9
Exposure to ruminants' excreta (urine and stool)/ saliva	5	2.3	2	0.9	3	0.7

Having the animal house in the same own house	2	0.9	0	0.0	2	0.5
Handling raw milk and milk product	1	0.5	0	0.0	1	0.2
Milking infected ruminants	1	0.5	0	0.0	1	0.2
Passing into animal house without wearing long boots	2	0.9	0	0.0	2	0.5
Not washing hand after daily dealing with ruminants	2	0.9	0	0.0	2	0.5
Eating fatty meat	1	0.5	0	0.0	1	0.2
Eating dinner very late and direct sleeping after	1	0.5	1	0.5	0	0.0
If children play with sheep	1	0.5	1	0.5	0	0.0
Rearing cats and dogs	1	0.5	0	0.0	1	0.2
Rats	1	0.5	0	0.0	1	0.2

\**p* –value is for chi square test \*\* the total number not = 219 because of no response of some participants \*\*\* the total percentage is not 100% because of multiple response answers

Table 5: The mean scores of knowledge, attitude and practice among study participants, and by the level of education

Score	Group		t- test	p-value	Education		t- test	p-value
	Cases	Control			Low Literacy	High Literacy		
	Mean±SD	Mean±SD			Mean±SD	Mean±SD		
Knowledge	4.3±7.2	4.8±10.6	-0.8	0.418	4.9±9.7	4.4±9.5	0.55	0.58
Practice	12.7±18.1	0.68±14.2	-9.5	<b>0.0001</b>	-5.5±18.0	-1.9±15.2	-2.72	<b>0.007</b>
Attitude	3.4±7.6	4.6±11.0	-1.7	0.093	4.5±10.0	4.0±9.8	0.59	0.56
Perceived barrier	-0.3±0.7	-0.2±0.64	-2.13	<b>0.034</b>	-0.28±0.75	-0.12±0.53	-3.25	<b>0.001</b>
Perceived susceptibility	1.5±3.2	1.9±4.0	-1.2	0.24	1.6±3.24	1.9±4.1	-0.82	0.414
Perceived risk	2.5±4.9	2.8±7.3	-0.682	0.496	3.1±46.9	2.3±6.1	1.53	0.127
Perceived susceptibility and risk	4.0±7.0	4.7±10.5	-0.961	0.337	4.7±9.6	4.2±9.3	0.73	0.47



Table 6: Univariable analysis of the association between selected potential risk factors and individual *Brucella* spp. seropositive status

Variable	Categories	Number		OR	P <	95% CI
		Case	Control			
Have animals in the house	Yes	106	160	2.7	0.001	1.68 – 4.5
	No	111	274	-	-	-
Number of animals in the house	0	111	274	-	-	-
	1-5	49	100	2.21	0.26	1.32- 3.70
	>5	57	60	8.2	0.001	3.88 – 17.32
Have small ruminant in the house	Yes	85	105	3.57	0.0001	2.1 – 6.03
	No	132	329	-	-	-
Number of small ruminants	0	132	329	-	-	-
	1-5	58	89	2.65	0.274	1.52 - 4.61
	>5	27	16	14.83	0.001	4.86 – 45.23
Have aborted animals in last 12 months	Yes	51	42	9.65	0.001	3.37 – 27.64
	No	55	118	-	-	-
Number of aborted animals in last 12 months	0	55	118	-	-	-
	1	26	39	6.17	0.715	2.06 – 18.48
	>1	25	3	52.70	0.001	11.32– 245.4
Notification of authorities of a case of abortion	Yes	7	28	0.14	0.002	0.04 – 0.45
	No	44	27	-	-	-
Have infected animals with <i>Brucella</i> spp. in last 12 months	Yes	6	0	>99.9	0.98	<0.01- >99.9
	No	100	160	-	-	-
Notification of authorities of a case of <i>Brucella</i> spp.	Yes	8	46	<0.001	0.99	<0.0- >99.9
	No	2	0	-	-	-
Protective measures for animals against <i>Brucella</i> spp. infection	Yes	38	111	0.11	0.0001	0.04 - 0.31
	No	68	49	-	-	-
Vaccinate animals against brucellosis	Yes	19	93	0.1	0.0001	0.04- 0.24
	No	87	67	-	-	-
Involve in activities where you come in contact with animals	Yes	136	148	6.85	0.0001	4.13 – 11.35
	No	81	286	-	-	-
protective measures applied if come in contact with animals	Yes	119	127	6.15	0.0001	3.70 – 10.25
	No	98	307	-	-	-

Number of protective measures you apply when come in contact with animals	0	98	307	-	-	-
	1-2	23	71	1.23	0.008	0.59 – 2.57
	>2	96	56	20.08	0.0001	9.03 – 44.64
Participating in dairy product processing	Yes	76	141	4.06	0.037	1.09 – 15.08
	No	141	293	-	-	-
Have consumed dairy products over the last 3 months	Yes	210	378	5.28	0.002	2.12 – 12.62
	No	7	56	-	-	-
Score of milk consumption	0	9	58	-	-	-
	1-2	75	234	2.52	0.18	1.15 – 5.54
	>2	133	142	12.49	0.0001	5.28 – 29.57
Have consumed unpasteurized milk over the last 3 months	Yes	21	7	6.17	0.0001	2.62 – 14.55
	No	196	427	-	-	-
Have consumed fermented milk over the last 3 months	Yes	163	279	1.7	0.006	1.15 – 2.4
	No	54	155	-	-	-
Have consumed homemade cheese over the last 3 months	Yes	141	189	3.61	0.0001	2.33 – 5.67
	No	76	245	-	-	-
Have consumed yoghurt over the last 3 months	Yes	63	71	4.32	0.0001	2.3 – 8.13
	No	154	363	-	-	-
Have consumed cream over the last 3 months	Yes	84	131	1.44	0.035	1.03 – 2.01
	No	133	303	-	-	-
Protective measures take to protect humans from infection	Yes	92	153	1.53	0.037	1.03 – 2.30
	No	125	281	-	-	-
Number of protective measures take to protect humans from infection	0	125	281	-	-	-
	1-2	83	104	2.30	0.0001	1.46 – 3.85
	>2	9	49	0.35	0.0004	0.15 – 0.79
Infected household in last 3 months	Yes	10	8	2.68	0.048	1.01 – 7.09
	No	207	426	-	-	-

Table 7: A multivariable model for the association between selected potential risk factors and individual *Brucella* spp. positive status

Variable	Categories	Number		OR	P-value	95% CI
		Case	Control			
Have consumed unpasteurized milk	Yes	21	7	4.12	0.003	1.62 – 10.75
	No	196	427	-	-	-
Have consumed homemade cheese	Yes	141	189	1.96	0.011	1.17 – 3.30
	No	76	245	-	-	-
Have consumed yoghurt	Yes	63	71	2.51	0.014	1.21 – 5.24
	No	154	363	-	-	-
Consumption of dairy products in the last 3 months before the test	Yes	210	378	2.71	0.038	1.06 – 6.93
	No	7	56	-	-	-
Involve in activities where you come in contact with animals	Yes	136	148	4.97	0.001	2.84 – 8.72
	No	81	286	-	-	-
Number of protective measures you apply to protect yourself and household members from <i>Brucella</i> spp. infection	0	125	281	-	-	-
	1	83	104	1.27	0.003	0.71 – 2.29
	2	9	49	0.23	0.001	0.10 – 0.58

Table 8: A multivariable model for the association between selected potential risk factors and individual *Brucella* spp. seropositive status among people who owned animals

Variable	Categories	Number		OR	<i>P</i> - value <	95% CI
		Case	Control			
Number of abortions per animal	2	25	3	49.33	0.001	8.79 – 276.91
	1	26	39	4.80	0.46	1.5 – 15.57
	0	55	118	-	-	-
Protective measures you apply to protect animals from <i>Brucella</i> spp. infection	Yes	38	111	0.11	0.002	0.03 – 0.45
	No	68	48	-	-	-



