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Seroprevalence of Brucellosis and Coxiellosis in a Linked Study Population in Egypt

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MG, MEA and AEG conceived and designed the study. Authors MG, MEA and AA performed the laboratory work. Authors MG and IK wrote the first draft of the manuscript and interpreted the data. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To estimate the prevalence of brucellosis and coxiellosis in different household livestock where the current epidemiological data are still limited.
Study Design: A cross sectional study.
Place and Duration of Study: Hygiene and Zoonosis laboratory, Faculty of Veterinary Medicine, Mansoura University, during 2018.
Methodology: The study included 1400 female animals, consisting of buffaloes (*n*=500), cattle (*n*=500), sheep (*n*= 250), camels (*n* = 100), goats (*n*= 50) as well as their contact owners (*n*= 25). A

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blood sample was drawn from each animal as well as their contact owners and was tested for *Brucella* (using Rose Bengal *Brucella* antigen and i-ELISA) and for *Coxiella burnetii* antibodies (using i-ELISA).

Results: The results demonstrated different rates of seropositivity among the examined animals. In total, 308 (22%) out of 1400 serum samples were tested positive for *Brucella* antibody in both RBT and i-ELISA. Of those, 99/500 (19.8%) came from buffaloes, 110/500 (22%) from cows, 70/250 (28%) from sheep, 19/100 (19%) from camels and 10/50 (20%) from goats. *C. burnetii* antibodies were detected in 104/ 500 (20.8%) buffalo samples, 80/500 (16%) of cattle, 50/250 (20%) of sheep, 20/100 (20%) of camels and 5/50 (10%) of goats. One hundred thirty nine cases out of 1400 (9.9%) harboured antibodies against *Brucella* spp. and *C. burnetii*. Only five cases (20%) among contact owners were tested positive for *Brucella* infection by using RBT and IgG ELISA; however, all tested human sera were negative for *C. burnetii* antibodies.

Conclusion: The study indicated a wide distribution of both infections in the study area and demonstrated an intense transmission within the studied livestock population.

Keywords: Brucellosis; Egypt; i-ELISA; livestock; coxiellosis; public health.

1. INTRODUCTION

In rural areas of Egypt, livestock species play a pivotal role in the livelihoods, and are considered as a valuable source of high-quality protein, as well as being a profitable mean of household wealth storage [1]. Approximately, 85% of the livestock species in Egypt are household animals kept in small herds i.e. less than five animals forming a small-scale family farming [2]. This kind of farming system has become an important factor in agricultural and rural development in the country which is characterized by coexistence of different animal species within the same flock and the close contact between the owners and their animals as well as a potential consumption of unpasteurized milk and dairy products [3]. Those factors could represent a potential risk for the transmission of some zoonotic infections such as brucellosis and coxiellosis which considered a global threat to public health and animal welfare [4].

Brucellosis is an endemic zoonotic disease in Egypt causing significant economic losses for the livestock sector and elicits considerable impact on international trading and has dramatic consequence on producers and livestock industry [5]. Q fever, caused by *Coxiella burnetii* which is an obligatory intracellular Gram-negative bacterium, is a ubiquitous zoonotic contaminant infecting a wide range of animal species particularly domestic ruminants which remain the common reservoirs and main source of human infections [6]. This bacterium is being stable in the environment and can survive for a long time in milk, birth products, animal excretions, animal feed, wool, equipment's and even in the clothes [4]. Both pathogens receive a growing interest from several researchers worldwide because of their zoonotic significance [7,8]. There is a need to address the current status of *Brucell*osis and *Coxiellosis* in the small-scale farming animals and their owners in Egypt because these pathogens did not receive sufficient attention from the policy makers and researchers in accordance with their actual merits. Given that camels are not routinely monitored for brucellosis and Q fever in Egypt as a part of national control program, the present study was delineated to estimate the prevalence of brucellosis and *Coxiellosis* in different livestock species in Egypt where the current epidemiological data are yet to be elucidated.

2. MATERIALS AND METHODS

2.1 Study Population

During 2018, a total of 1400 female animals as well as their contact owners (n = 25) were included in this study. The investigated animals were buffaloes (n=500), cattle (n=500), sheep (n= 250), camels (n = 100), and goats (n= 50), being older than 6 months of age, and have had a previous history of abortion. The examined animals were not previously vaccinated against brucellosis or Q fever. All livestock animals (except camels) and their keepers were belonged to a rural-based farming community at Aga District in Dakahlia Governorate in Nile Delta region of Egypt, while the investigated camels (*Camelus dromedaries)* were collected from Kirdasa District, Giza Governorate. The study area was selected based on convenience of sample collection and the lack of epidemiological surveys regarding the studied diseases.

2.2 Sampling

Ten ml of blood was drawn from each animal via jugular vein puncture beside five ml of blood collected from the radial vein of contact owners. The collected blood samples were added to sterile plain vacutainer tubes, then allowed for clotting to yield sera which were collected and transported in coolers to the Hygiene and Zoonosis laboratory, Faculty of Veterinary Medicine, Mansoura University. All sera were initially tested by Rose Bengal, which will be discussed later, and then kept frozen at –20°C for further serological analyses.

2.3 Diagnostic Tests

For animal sera, a commercially available Rose Bengal *Brucella* antigen (Ubio, quick vet, India) was used according to the standard protocol recommended by OIE [9]. In addition, all collected sera were tested for whole antibodies against *Brucella* spp. using an indirect multi species commercial ELISA test kit (ID®-Screen Brucellosis Serum Indirect Multispecies, ID-VET, Montpellier, France) following the manufacturer's instructions as a confirmatory test. The results were expressed as a percentage of the optical density (% OD) which was calculated as % OD = 100 * (S-N)/ (P-N), where S are the values of the sample, N and P are the OD of negative and positive controls, respectively. The optical densities were measured by Stat Fax 2100 Microplate Reader (Awareness Technology INC, Fl, USA) at 450 nm. Samples gave positive results in both tests were considered seropositive, while those yielded negative results to either RBT or indirect ELISA were considered negative. On the other side, sera were tested for the whole antibodies against *C. burnetii* inactivated phase I and II antigen using a commercially available kit supplied by ID Screen® Q Fever Indirect Multi-species (ID-VET innovative diagnostics, Grabels, France) according to the manufacturer's recommended cut-off ranges. The interpretation of the results was given as follow: value of S/P < 40% were considered negative; values between 40 and 50% were considered inconclusive; values between 50 and 80% were considered positive; while values >80% were considered strong positive.

For human samples, the collected sera were initially tested by RBT (Spinreact, Girona, Spain) according to Dean et al. [10]. In addition, all sera were tested for brucellosis and Q fever using indirect IgG ELISA (Serion ELISA classic *Brucella* IgG, Institut Virion\Serion GmbH, Würzburg, Germany) and *C. burnetii* indirect IgG Phase II ELISA (*Coxiella burnetii* ELISA IgG, Vircell SL, Granada, Spain). The results were interpreted as positive or negative according to the manufacturer's guidelines.

2.4 Statistical Analysis

Data were analyzed using SPSS crosstab (Chi-square test) to test the potential differences in frequencies of positive samples in all tested animal species.

3. RESULTS AND DISCUSSION

The present study was set to evaluate the current status and the extent of *brucellosis* and *coxiell*osis in domestic household livestock because insufficient monitoring of such zoonotic agents could be the rationale for persistence of infections in Egypt. *Brucella* antibodies were proved by using two different serological tests. In this regards, different rates of seropositivity were reported among the examined animal species. In total, 308 out of 1400 serum samples (22%) tested positive for *Brucella* antibody using RBT and i-ELISA. From these, 99/500 (19.8%), 110/500 (22%), 70/250 (28%), 19/100 (19%), and 10/50 (20%) corresponded to samples from buffaloes, cows, sheep, camels and goats, respectively (Table 2). By using RBT alone, the seropositivity in buffaloes, cattle, sheep, camels, and goats were 24.4%, 29%, 28%, 19 %, and 20%, respectively, with an overall prevalence of 26%; while by using i-ELISA, the respective rates of seropositivity were 22%, 30%, 34%, 28% and 20% with an overall prevalence of 27.4% (Table 1). These findings were proven by the statistical analysis where the frequencies of positive samples were significantly different (P = 0.0031) when using i-ELISA for the detection of *Brucella* infections among the investigated livestock species, while other tests showed no significant variation (Table 1). Information of this seropositivity was comparable to those reported in previous studies in Egypt. For instance, some researchers screened 1670 serum samples from different Egyptian Governorates by using RBT and i-ELISA [11]. The authors found a prevalence of 26.6%, 18.8% and 17.2% in sheep, goats and cattle, respectively. Similar

detection rates (i.e. 29.3% and 28.7 using the same tests) were also reported by other researchers in sheep flock in Kafr El-Sheikh and Gharbia Governorates, respectively [12]. On the contrary, low detection rates were reported in several animal species [13,14]. In the former study, the authors found a detection rate of 5.4% in cattle, 4.1% in buffaloes, 5.4% in sheep and 3.5% in goats from different Governorates of Egypt; while in the later study, the researchers adopted a large-scale control campaign during 2005 and 2008 in seven Upper Egyptian Governorates and found a seroprevalence of 1.16% in sheep, 0.44% in goats, 0.79% in cows and 0.13% in buffaloes. On the other side, a higher rate of seropositivity (52.3%) was previously detected using RBT [15]. In that study, the researchers examined 1685 serum samples collected from cattle (n=660), buffaloes (n= 482), sheep (n =194) and goats (n=198) from different private farms in El-Menofia, Gharbia, Behira and Fayoum Governorates and found incidences of 45.8%, 66.6%, 37.6% and 61.1%, respectively. In the same trend, a seroprevalence of brucellosis among sheep (*n* = 791) and goats (*n* = 383) in Kafr El Sheikh Governorate was previously given by Hegazy et al. [16]. The authors have found a prevalence of 12.2% and 11.3% in the respective animals, but they detected a higher prevalence of infection in sheep (41.3%) and goats (32.2%) from the infected villages as well. In less developed nations of Africa and South/South East Asia, an average range of prevalence (0-88.8%) was detected in sheep and goats, while in cattle it ranged between 0-68.8% [7]. A high detection rate of brucellosis was also detected among aborted sheep and goat flocks in Jordan [17].

Table 1. Percentages of brucellosis and Q fever cases in various livestock species

Species	RBT		ELISA (*Brucella*)		ELISA (Q fever)		Chi-square (χ^2)*	*P* -value
	+ve	-ve	+ve	-ve	- ve	+ve		
Buffalo (*n*=500)	122 (24.4%)	378 (75.6%)	110 (22%)	390 (78%)	396 (79.2%)	104 (20.8%)	430.08	≤ 0.0001
Cattle (*n*=500)	145 (29%)	355 (71%)	150 (30%)	350 (70%)	420 (84%)	80 (16%)	396.99	≤ 0.0001
Sheep (*n*=250)	70 (28%)	180 (72%)	85 (34%)	165 (66%)	200 (80%)	50 (20%)	162.33	≤ 0.0001
Camel (*n*=100)	19 (19 %)	81 (81%)	28 (28%)	72 (72%)	80 (80%)	20 (20%)	88.83	≤ 0.0001
Goats (*n*=50)	10 (20%)	40 (80%)	10 (20%)	40 (80%)	45 (90%)	5 (10%)	1130.8	≤ 0.0001
Total (*n*)	366 (26%)	1034 (73.8)	383 (27.4%)	1017 (72.6%)	1141 (81.5%)	259 (18.5%)		
Chi-square (χ^2)**	6.96		15.911		6.74			
P-value	6.137 ns		0.0031**		0.15ns			

* *The frequencies of postive and negative samples of the three used tests for each animal species.*
** *The frequencies of positive and negative samples of each test in all animal species (Total χ^2 = 11.004, P -value = 0.201)*

Table 2. The prevalence of brucellosis in different livestock species using RBT and i-ELISA

Number of examined animals	RBT		i-ELISA (*Brucella*)		*Brucella* antibodies ELISA+RBT	Chi-square (χ^2)*	*P* -value
	+	-	-	+	Positive in both		
Buffaloes (*n*=500)	99	367	367	99	99 (19.8%)	305.69	≤ 0.0001
	23	11	23	11			
Cattle (*n* =500)	110	320	320	110	110 (22%)	203.17	≤ 0.0001
	35	35	30	40			
Sheep (*n* =250)	70	165	165	70	70 (28%)	75.2	≤ 0.0001
	0	15	0	15			
Camels (*n* =100)	19	72	72	19	19 (19%)	74.42	≤ 0.0001
		9		9			
Goats (*n* =50)	10	40	40	10	10 (20%)	33.64	≤ 0.0001

* *The frequencies of positive and negative samples of both tests for each animal species (total χ^2 = 8.61, P- value = 0.071),*
ns: non significant

The present study throws light on the potential occurence of brucellosis in household camels. Our findings showed that 19% of investigated camels were seropositive. A comparatively low seroprevalence rates were previously reported in Egypt. In this context, some researchers have investigated 500 camels from different abattoirs of Sharkia and Kaluobia Governorates and have found a seroprevalence of 14% and 11.6%, using buffered acidified plat agglutination test (BAPAT) and tube agglutination test (TAT), respectively [18]. In another study, a seroprevalence of 8.7% and 9.4% was also found in slaughterhouse camels at Behira Governorate; while in resident contact camels the prevalence was 9.2% and 10.1% using RBT and BAPAT [19]. Some other authors have detected *B. abortus* and *B. melitensis* among 340 camels raised in an open yard in a Government quarantine station (Nobaria City) with a rate of 4.1% and 3.2%, respectively using the agglutination and CFT tests [20]. In a recent study, a seroprevalence of 4.1% and 3.7% was detected in 1126 apparently healthy resident dromedary camels using RBT and c-ELISA [21]. A higher seroprevalence rate was also detected from Sudan using c-ELISA (40.5%) and RBT (39.9%) [22]. The high prevalence of brucellosis among the investigated camels could raise the suspicious of a potential occupational and public health concern. As camels are not regularly vaccinated against *Brucella*, the presence of *Brucella* antibodies reflects prior infection.

For the neighbor countries, variable seroprevalences of camel brucellosis were reported in Kenya (6.0- 38%), Libya (4.1%), Jordan (15.8%), Ethiopia (0.73–11.9%) and Pakistan (21%) [23,24,25,26,27]. The differences between the studies could attribute to the variations in animal management and production systems, mixed farming, importation of animals from endemic area and the continues lacking of a national eradication program for camel brucellosis including periodical testing and slaughtering of reactors.

C. burnetii antibodies were detected in 104/500 (20.8%) buffalo samples, 80/500 (16%) of cattle, 50/250 (20%) sheep, 20 (20/100) camels and 5/50 (10%) of goat samples (Table 1). 139 samples out of 1400 (9.9%) harbored antibodies against *Brucella* spp. and *C. burnetii* [35 cases of buffaloes (7%), 75 cows (15%), 25 sheep (10%), two camels (2%) and two goats (4%)] (Table 3). For human sera, only five cases (20%) out 25 samples were tested positive in both RBT and IgG ELISA, but all samples were negative for *C. burnetii* antibodies. Our data were comparable to the results obtained from various studies in Egypt. For example, in 2018, Klemmer et al. reported a seroprevalence of 19.3% (162/840) in cattle, 11.2% (34/304) in buffaloes, 8.9% (64/716) in sheep, 40.7% (215/528) in camels, and 6.8% (21/311) in goats from different Egyptian Governorates [28]. Other researchers have examined 184 apparently healthy ruminants from three Egyptian Governorates (Giza, Cairo and El-Fayum) and reported higher prevalence rates in sheep (32.7%, 18/55), and goats (23.3%, 7/30) and comparable detection rate in cattle (13%, 7/54), but the authors failed to determine *C. burnetii* specific antibodies among the examined buffalo samples [29]. In another study, some researchers have detected *C. burnetii* specific antibodies in 158 samples of cattle out of 1,194 (13.2%) from nine farms from Dakahlia, Damietta and Port Said Governorates [30]. A base line serosurvey was also conducted at the Muneeb abattoir in Giza Governorate in central Egypt [8]. In that study, *C. burnetii* specific antibodies were not detected in 161 slaughtered cattle but a lower detection rate (8%, 14/174 and 4%, 6/153) was found in slaughtered sheep and buffaloes. In 2017, Abushahba and others reported a high prevalence rate in sheep (25.68%, 28/109) and in goats (28.20%, 11/39) from different villages in El Minya Governorate [31].

Table 3. The percentage of animals co-infected with both brucellosis and Q fever

Species	Number of positive animals
Buffaloes (*n*=500)	35 (7%)
Cattle (*n* =500)	75 (15%)
Sheep (*n* =250)	25 (10%)
Camels (*n* =100)	2 (2%)
Goats (*n* =50)	2 (4%)
Total (*n* =)	139 (9.9%)
Chi-squar (χ^2)*	28.17
P -value	≤ 0.0001

**The frequencies of positive samples of both diseases in all tested livestock species*

According to a previous report, the seroprevalence of *C. burnetii* in dromedary camels was reported to range from 0% to 80% [32]. Likewise, a wide range of prevalence rates (18.6% to 51.6%) were also reported by other authors [33,34,35,36]. In Egypt, high detection rates of *C. burnettii* were determined in 71% and 70% of the examined camels [8]. The presence of antibodies in ELISA-positive camels indicated exposure to *C. burnetii* in the past and the possibility of chronically harboring the infection by the animals. Consequently, camels may play a role in the maintenance of infection in nature. The discrepancies among these studies could be attributed to the differences in geographic distribution, divergence in sampling, health status of the examined livestock, laboratory methods and interpretation. The numbers of positive cases either for brucellosis or coxiellosis are relatively high among the previously aborted animals. It might be attributed to several factors including keeping the animals in shelters during the night, grazing mixing herd at common pasture, absence of vaccination strategy for these groups of animals and livestock farmers do not cull or dispose the aborted animals.

The seroprevalence of brucellosis among the investigated animal owners` was 20%. A similar detection rate (21%, 62/295) was previously reported in Sharkia Governorate [36]. Low different seroprevalence rates (5.1%, 11%, 14% and 1.25%) were also reported from Egypt [37,38,39,40], respectively. Surprisingly, none of the contact owners had *C. burnetii* specific antibodies; however different detection rates were previously reported among humans in close contact with animals [31,41,42].

4. CONCLUSION

The results from this study demonstrated high seroprevalences of both infections among all investigated ruminant species in the study area, with a potential transmission within the livestock population and alarming public health hazards in the study area.

5. RECOMMENDATION

There is an urgent need for collaboration between human and veterinary sectors especially in such poor resource setting and raising the community awareness of such zoonotic diseases to help provide efficient surveillance and strengthen the health system in general.

CONSENT AND ETHICAL APPROVAL

The study complies with national and international ethical guidelines and with that of Mansoura University. An informed consent was received from most of owners after explaining the rationale for sample collection and the potential public health concern of the studied diseases. However due to the Egyptian cultural settings, obtaining a written consent from some participants was not possible either from literate or illiterate participants to sign a written consent. Therefore, the objectives of the study were explained in the local language and verbal consent was obtained from all owners prior to samples collection. The animals were also handled in a manner to minimize stress and suffering.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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