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Research Article

Investigation of an outbreak of brucellosis in a mixed dairy farm and evaluation of a test and slaughter strategy to release the herd out of the quarantine

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Abstract

An outbreak of brucellosis in a mixed dairy farm with 508 animals (370 cows, 120 sheep, and 18 camels) at Fayoum governorate, North Upper Egypt, was investigated. A storm of abortion and several cases of retained placenta were reported among cows and ewes in April 2020. Serodiagnosis of brucellosis was done using Rose Bengal Test (RBT) and Buffered Acidified Plate Antigen Test (BAPAT). The Milk Ring Test (MRT) was applied to the milk of seropositive animals. A total of 89 samples were used for isolation of *Brucella* and isolates were confirmed using Abortus, Melitensis, Ovis, Suis-PCR (AMOS-PCR). Test and slaughter strategy was applied to eradicate brucellosis from the farm based on RBT every month until three successive negative tests were obtained. Results showed that the seroprevalences of brucellosis based on RBT and BAPAT were 9.5%, 35% and 50% in cattle, sheep, and camels, respectively. Despite 50% of male camels being seropositive, no clinical signs have been reported. The MRT identified fewer positive cases than BAPAT and RBT, thus, it cannot be used alone to eliminate the infection from the farm. A total of 31 *Brucella* isolates were recovered from cows and sheep on the farm. All isolates were confirmed as *Brucella melitensis* biovar 3 based on bacteriological examination and Brucella AMOS-PCR confirmed all isolates as *Brucella melitensis*. No positive reactors at the 6^{th}, 7^{th}, 8^{th}, 9^{th}, and 10^{th} examinations were reported after the implementation of test and slaughter strategy. In conclusion, extensive animal farming and mixed breeding are potential risk factors for interspecies transmission of brucellosis. Additionally, the test and slaughter strategy could be helpful to release the herd out of quarantine, however, application of biosecurity practices and fair compensation policy for owners should be implemented.

Keywords: Bovine, Sheep, Abortion, Brucellosis, *B. melitensis*

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Introduction

Brucellosis is a zoonotic bacterial disease of high prevalence in countries of the Middle East, the Mediterranean region, Central and South America, Africa, and Asia (Moreno, 2014; Georgi et al., 2017; Sayour et al., 2020). *Brucella* is a Gram-negative facultative intracellular pathogen causing infection in sheep and goats (*B. melitensis*), rams (*B. ovis*), bovines (*B. abortus*), canines (*B. canis*), and pigs (*B. suis*) (Morgan, 1984; Shome et al., 2018). Brucellosis also affects terrestrial wildlife (*B. microti*) and marine mammals (*B. ceti* and *B. pinnipedialis*) (Miller et al., 2017). However, cross-species infection is also possible (Richomme et al., 2006; Saeed et al., 2019). Brucellosis in animals is causing high economic losses to the livestock industry due to abortions, loss of milk production, stillbirth, retained placenta, infertility in both males and females, in addition to poor health, debility, and poor quality livestock products (Maadi et al., 2011; Singh et al., 2015). In humans, brucellosis causes a severe acute febrile illness with a high cost of treatment and becomes chronic if left untreated, resulting in significant public health concerns (Godfroid, 2017).

Brucellosis is an endemic disease in Egypt causing annual economic losses estimated at 60 million Egyptian pounds, and incidence differs between researchers

according to the season of work, the number of examined animals, and the used serological tests (El-Diasty, 2004). In the period from 1985 to 2005, the highest prevalence rate was 34% and the lowest prevalence rate was 9.2% in cattle while in sheep the highest prevalence was 31.7% and the lowest prevalence was 2.8% (Refai, 2002). Despite several studies have been carried out on brucellosis, it remains endemic in Egypt, thus, the test and slaughter program was established in addition to the use of vaccination, whether RB51 in cattle and Rev1 in sheep (El-Diasty, 2009; Wareth et al., 2017).

Shedding of *Brucella* occurs through the milk and aborted material (Corbel, 1997), and abomasal contents of the aborted fetus are considered the best sampling site for isolation of *Brucella* (Abd Eltawab et al., 2020). In pregnant animals, *Brucella* displays a strong tropism for placental trophoblasts (Anderson et al., 1986; Tobias et al., 1993) and also for mammary glands, in which it replicates extensively, causing placentitis and abortion in the last trimester of pregnancy (Moreno and Barquero-Calvo, 2020). Secretions from the female genital tract form the main source of infection and spill over the microorganisms to other animals and men. Therefore, in most circumstances, the primary route of dissemination of *Brucella* is the placenta, fetal fluids, and vaginal discharges expelled by infected animals after abortion or full-term parturition. A very high number of bacteria is shedding during parturition or abortion (Songer and Post, 2004).

Diagnosis of brucellosis is still challenging and usually relies on serological tests (El-Diasty et al., 2018), which are applied *in-vitro* using milk or blood. Exceptionally, *in-vivo* (allergic tests) are used. The isolation of brucellae and detection of *Brucella* DNA by PCR is the method that allow definitive diagnosis (Godfroid et al., 2010). Although bacterial culture and identification confirm the disease, *Brucella* is difficult to grow, and bacterial culturing is time-consuming. Additionally, this method poses a risk to laboratory personnel and requires specific biosafety measures (Mathew et al., 2015). Hence, culture and biochemical typing remain the "gold standard" for diagnosing *Brucella* infection (Vicente et al., 2014).

A comparatively new method like Matrix-Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) has been applied as the first line of diagnosis in several laboratories globally for microbial identification (Angeletti, 2017). It is an economic tool and considered an easy, rapid, and accurate method at the genus level, and based on the automated analysis of the mass distribution of bacterial proteins (Patel, 2015). A recently published study indicates that MALDI-TOF MS can accurately identify 99.5% and 97% of *Brucella* strains at the genus and species level, respectively, minimizing laboratory hazards. However, there are limitations in terms of sub-species level and biovars identification (Sali et al., 2018). *Brucella* identification and species differentiation can be accomplished using genus-specific *Brucella* PCR (B4/B5), *Brucella* AMOS-PCR, and Bruceladder PCR (Hinić et al., 2008).

The present study aimed to investigate brucellosis in a dairy farm with a mixed rearing system (cattle, sheep, and camels) suffered from a storm of abortions and other typical symptoms of brucellosis, to determine the predominant *Brucella* strain causing abortion using diagnostic serological, bacteriological, and molecular procedures. Moreover, we evaluated the test and slaughter strategy on a small scale to eradicate brucellosis from the farm.

Materials and Methods

Study area and animal population

The study was carried out on a farm that is raising Holstein dairy cows, sheep, and camels at Fayoum governorate, one of the North Upper Egypt governorates, and located in the southwest of Cairo (29°21'48"N 30'44'45"E). The dairy farm follows a mixed breeding system consisting of 370 Holstein dairy cows, 120 sheep, and 18 male camels. Farm construction has no fence, so the ability of stray dogs and foxes to get into the farm was found. The management system and biosecurity are not secured; however, introducing newly purchased animals without examination is forbidden. Moreover, no vaccination program for brucellosis has been applied to the farm since 2012, despite the farm having a history of an outbreak of brucellosis in 2004. Suddenly, in April 2020, several cases of abortion have been observed. Abortion occurred in 6.8% (17 out of 250) of pregnant cows between the 5^{th} and 8^{th} months of gestation, and 37.5% (30 out of 80) of pregnant ewes in the last third trimester of pregnancy. All cases of abortion in cows and ewes occurred within 3-6 weeks.

Ethical approval

This study was carried out according to the guidelines of ethical committees of the Faculty of Veterinary Medicine, Benha University, and Animal Health Research Institute, Dokki, Egypt.

Sample collection

Blood samples were collected from all animals (370 cows, 120 ewes, and 18 camels) for serological examination of brucellosis. All blood samples were centrifuged, and sera were collected to be examined for brucellosis by Rose Bengal Test (RBT) and Buffered Acidified Plate Antigen Test (BAPAT). Eighty milk samples were collected from seropositive cows (n=35) and seropositive ewes (n=45). Milk samples were investigated using the Milk Ring Test (MRT), and only positive samples were sent for bacteriological examination to isolate *Brucella* spp. Additionally, tissue and abomasal contents from aborted foeti accidentally present in the farm during the investigation were collected for bacteriological examination Three abomasal contents and 5 retained placenta from cows, and 5 abomasal contents, 4 retained placenta, and 11 uterine discharge from ewes were collected for bacteriological examination.

Serological tests

All serum samples were examined by RBT and BAPAT according to Alton (Alton et al., 1988). Antigens and tests materials were obtained from Veterinary Serum and Vaccine Research Institute (VSVRI), Abbassia, Cairo, Egypt. Milk samples of seropositive animals were examined using the MRT according to Alton (Alton et al., 1988). The antigen was obtained from the Animal Health and Veterinary Laboratories Agency (AHVLA, New Haw, Addlestone, Surrey KT15 3NB, UK).

Bacteriological isolation and identification of *Brucella* organisms

Isolation, identification, and bio-typing of *Brucella* organisms from abomasal contents (n= 8), MRT positive milk samples (n=61), retained placenta (N=9), and uterine discharge (n=11) were carried out according to the FAO/WHO Expert Committee's recommendations on brucellosis (Alton et al., 1988; OIE, 2018). All suspect colonies were identified using classical biotyping methods according to colony morphology, biochemical tests (oxidase, catalase, urease), CO2 requirement, H2S production, growth in the presence of thionin and fuchsine dyes, reaction with mono-specific anti-sera "A, M, R" (Animal health Research Institute (AHRI), Giza, Egypt) and agglutination with acriflavine and crystal-violet. Isolates were stored at -20°C for further processing.

Molecular identification and differentiation

Brucella isolates were further molecularly characterized at the species level using Abortus, Melitensis, Ovis, Suis-PCR (AMOS-PCR) as previously described by (Bricker and Halling, 1994). Briefly, 25 µl of a reaction mixture containing 10× PCR buffer, 10mM of deoxynucleotide triphosphates (dNTPs) and 10 pmol/µl of primers, (0.2 µM each) of *B. abortus*, *B. melitensis*, *B. ovis*, *B. suis*, and IS711-specific primers (Friedrich-Loeffler-Institut,Germany), 0.2 µl of 5U/µl of Taq DNA polymerase was used. HPLC was used to complete the 25 µL. A total of 1 µl DNA extraction template was added to the 24 µl reaction mixture. The PCR was performed with Thermocycler. Amplification was performed with an initial temperature of 95°C for 5 minutes followed by 30 cycles of denaturation at 95°C for 1 minute, annealing at 58°C for 2 minutes, and elongation at 72°C for 2 minutes. The PCR products were incubated for five minutes at 72°C to allow elongation of products before storage at 4°C. The PCR products were separated by electrophoresis using 1.5% agarose gel (w/v). Gels were stained with ethidium bromide and photographed using a gene snap camera (Syngene Pvt Ltd., Cambridge, UK). Visible bands were considered positive reactions of appropriate sizes of (498 bp) for *B. abortus*, (731 bp) for *B. melitensis*, (976 bp) for *B. ovis*, and (285 bp) for *B. suis*.

Evaluation of test and slaughter program based on RBT

The test-and-slaughter strategy was evaluated on the farm by testing all animals every month using RBT as a survey test for ten months. All positive cases were removed immediately and sent for obligatory slaughtering. The farm was subjected to quarantine, and the biosecurity regulations were strictly applied during the examination. In addition, prevention of stray dogs and cats and wildlife species was applied, and the introduction of new animals was stopped.

Results

Health status of the farm and serology

Abortion is the main clinical sign that threatened the animals on this farm, but the severity of the abortion differed between cows and sheep, as it was noted that the rate of abortion in sheep was much higher than in cows. In total, 37.5% (30 out of 80) of pregnant ewes were aborted in the last trimester, while the percentage was 6.8% (17 out of 250) among pregnant cows that were aborted between 5-7 months of pregnancy (Figure 1). All aborted animals suffered from the retained placenta and different degrees of endometritis. The farm also contains 18 male camels that do not have any disease symptoms and were used inside the farm to transport fodder, equipment and were fattened as a source of meat. The prevalence of brucellosis on the farm by BAPAT and RBT were 9.5%, 35%, and 50% in cows, sheep, and camels, respectively. While MRT was applied on the milk of seropositive animals and was 80% in cows (compatible with the results of BAPAT and RBT) and 76.3% in sheep (compatible with the results of BAPAT and RBT) (Table 1 and Table 2).

Bacteriological identification and molecular confirmation of the isolates

A total of 89 samples have been used for the isolation of *Brucella*, 36 from cows and 53 from sheep. 31 samples (34.8%) were culture positive, among them 13 from cows and 18 from sheep, and the rest 58 (65.2%) failed to grow any *Brucella* isolates (Table 3). All isolated strains showed typical characteristics for the genus *Brucella*; round, glistening, pinpoint, translucent colonies with a pale honey color when viewed in the daylight, convex and pearly white when viewed from above, stained with Gram and modified Ziehl-Neelsen (MZN) staining procedures. Based on bacteriological and biochemical characteristics, all isolated strains were typed by classical bacteriology as *B. melitensis* biovar 3. *Brucella* DNA of 13 isolates from cattle and 18 from sheep were amplified with the genus-specific assay. AMOS-PCR differentiated these 31 isolates as *B. melitensis*, with visible bands at a size of 731 bp.

Evaluation of test and slaughter program based on RBT

All animals were serologically examined every month until three successive negative tests were obtained. However, the *Brucella* is a notorious microorganism and ubiquitous disease; therefore, we continue to investigate the animals more than two times after the three

Figure 1: A case of abortion in a Holstein cow in the 6th month of gestation.

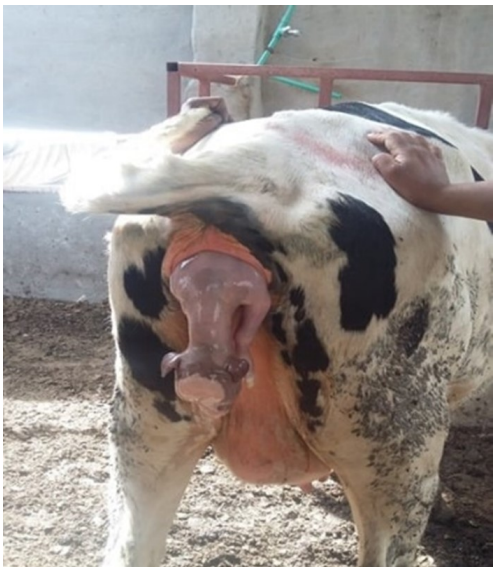

Table 1: Prevalence of brucellosis in serum of cattle, sheep, and camels.

Species	No. of serum samples	BAPAT[1] positive (%)	RBT[2] positive (%)
Cattle	370	35 (9.5%)	35 (9.5%)
Sheep	120	42 (35%)	42 (35%)
Camels	18	9 (50%)	9 (50%)
Total	508	86 (16.9%)	86 (16.9%)

[1]BAPAT: Buffered Acidified Plate Antigen Test.

[2]RBT: Rose Bengal Test.

successive negative tests (Table 4). Concerning the results obtained from the evaluation of test and slaughter in cows depending on RBT results, 9.5% serologically positive cattle could be detected at first examination. These animals were immediately removed from the herd for slaughtering. At the 2nd examination, 3.9% of positive reactors were detected and removed. At the 3rd examination, 3.1% of positive animals were detected and removed. Also, at the 4th examination, 2.2% reactors were detected and removed, while at the 5th examination, 0.9% positive animals could be detected. Finally, there were no positive reactors at the 6th, 7th, 8th, 9th, and 10th examinations.

Discussion

Brucellosis is a highly zoonotic disease that threatens animal health and production, resulting in huge economic losses worldwide since ancient times (Manish et al., 2013; Khurana et al., 2021). In the present study, serum samples from 508 animals in a dairy farm suffering from abortion with a mixed rearing system at Fayoum governorate were examined for brucellosis after the occurrence of a storm of abortion in April 2020. Two serological tests, including BAPAT and RBT, were used as screening tests, while MRT was employed on the seropositive cows and ewes. This study was conducted to determine the predominant *Brucella* strains causing abortion using diagnostic serological, bacteriological, and molecular procedures.

Mixed farming of cows and sheep has increased the risk of brucellosis, where the sheep act as the primary hosts for *B. melitensis* and cattle as an instance of overflowing to other hosts, including humans (Abd Eltawab et al., 2020; Hashem et al., 2020). Although BAPAT is more sensitive as a screening test and RBT is more specific than BAPAT due to its (El-Diasty, 2004), the results showed that the seroprevalence of brucellosis using RBT and BABAT was equal in cattle (9.5%), sheep (35%) and camels (50%). This may be due to the high infection rate with brucellosis on the farm. This explanation was supported by (Mustafa, 2010) who mentioned that extensive animal farming had been documented as a potential risk factor for brucellosis. Furthermore, the MRT revealed that the prevalence of brucellosis in cow's milk samples was 80%, and in ewe's milk samples was 73.3% (Table 2).

The MRT identified fewer positive cases than BAPAT and RBT because the MRT may be less sensitive to detect antibodies in milk containing low concentrations of antibodies or due to fat clustering factors (Cadmus et al., 2008). Therefore, MRT cannot be applied alone to eradicate the infection from the farm. In the same context, no single serological test was capable of conclusive diagnosis of positive cases in all examined

Table 2: Prevalence of brucellosis using MRT among seropositive cattle and sheep.

Species	No. of milk samples	MRT[1] positive (%)	MRT negative (%)
7 (20%)			
Ewe	45	33 (73.3%)	12 (26.7%)
Total	80	61 (76.3%)	19 (23.7%)

[1]MRT: Milk Ring Test.

Table 3: Result of *Brucella* bacterial isolation from different specimens.

Samples	Cows			Sheep			
	Milk samples	Abomasal content	Retained placenta	Milk samples	Abomasal content	Retained placenta	Uterine discharge
Total no. of samples	28	3	5	33	5	4	11
Positive samples	6 (21.4%)	3 (100%)	4 (80%)	3 (9.1%)	5 (100%)	2 (50%)	8 (72.7%)
Negative samples	22 (82.9%)	0 (0%)	1 (20%)	30 (90.9%)	0 (0%)	2 (50%)	3 (27.3%)

animals (El-Diasty, 2004). Careful analysis of the results of the serological tests and linking those results to the clinical signs of brucellosis that were observed on the farm, such as abortions, endometritis, and placenta retention, a quick and accurate decision was taken to remove all positive animals to stop new infections and new cases of abortion. Thus, applying other confirmatory tests was not achieved. However, in endemic areas, especially in the presence of severe symptoms of brucellosis, when the results of two serological tests are symmetric, the positive animal must be excluded.

Following up on the camel's health status inside the farm, they did not show any clinical signs of brucellosis such as orchitis, arthritis, and joint swelling, despite 50% of camels being seropositive (Table 1). Hence, brucellosis is considered an insidious disease of the camel, but it does not show the usual clinical signs. The danger here lies in the ability of the camel to maintain and increase the spread of infection among other animals (Musa and Shigidi, 2001).

The isolation and typing of *Brucella* species from clinical samples is the gold standard diagnostic method for brucellosis (Bricker, 2002; Al Dahouk et al., 2003). It is also an essential tool for the confirmation of *Brucella* infection and to trace back the sources of infection. The PCR assays were capable to confirm isolated *Brucella* spp. In the current study, 31 *B. melitensis* isolates from cattle and sheep at the biovar level were characterized. The obtained results showed that all isolates of *B. melitensis* were typed as biovar 3 by classical bacteriological tools. This biovar was previously identified and considered the prevalent pathovar in Egypt (El-Khatib, 1989; Sancho et al., 2014; El-Diasty et al., 2016; El-Sayed et al., 2017; El-Diasty et al., 2021).

The isolation rate of brucellosis was 34.8%, and *Brucella* was isolated from 6 retained placenta out of 9 (66.7%) (Table 4). Such a high rate of *Brucella* isolation may be attributed to the number of organisms that tend to be very high in the placental cotyledons, as reported by (da Silva Mol et al., 2012). On the other hand, the lower rate of *Brucella* isolation from 9 milk samples out of 61 (14.7%) is probably due to several limiting factors such as the fastidious nature of the organism, the low number of viable organisms, intermittent descent of *Brucella* in milk and milk contamination during collection which is considered as complicating factor for *Brucella* isolation (Alton et al., 1988; Seleem et al., 2010). Fetal abomasal contents and vaginal secretions are among the samples of choice for *Brucella* isolation (Alton, 1975).

In this study, *Brucella* was isolated from all samples (8/8) collected from stomach contents of aborted calves and lambs. Stomach contents consider the "preferred site" for nesting *Brucella*, however, it was recovered from 72.7% (8/11) obtained from uterine discharges of aborted ewes. These are attributed to the fact that brucellae propagate in the gravid uterus, amniotic fluid, and fetal membranes of pregnant cows in large numbers as predilection sites due to erythritol affinity, as reported by (Poester et al., 2013). Such preferential multiplication is related to the ability of the genus *Brucella* to induce abortions in ruminants (Yaeger and Holler, 2007). The reduced recovery rate obtained from uterine discharges of seropositive ewes may be attributed to the possibility of contamination of the samples and the fastidious nature of *Brucella* organisms from contaminated samples (Salem and Hosein, 1990).

Bacteriological characterization and genotyping of the 31 *Brucella* isolates revealed that all isolates were undoubtedly *B. melitensis*. This explains the higher percentage of abortion in ewes (37.5%) than cows (6.8%) because sheep are the primary host for *B. melitensis* (OIE, 2018). *B. melitensis* is the predomi-

Table 4: Relapsed time to get rid of cow brucellosis inside the farm depending on the serological investigation.

Test	Number of samples collected monthly[1]	No. of positive samples	No. of negative samples
1st	370	35 (9.5%)	335 (90.5%)
2nd	335	13 (3.9%)	322 (96.1%)
3rd	322	10 (3.1%)	312 (96.9%)
4th	312	7 (2.2%)	305 (97.8%)
5th	305	3 (0.9%)	302 (99.01%)
6th	302	-	302 (100%)
7th	302	-	302 (100%)
8th	302	-	302 (100%)
9th	302	-	302 (100%)
10th	302	-	302 (100%)

[1]Animals were tested monthly, and positive animals were removed from the farm for slaughtering.

nant *Brucella* spp. circulating in humans and livestock in the Middle East and Mediterranean countries, including Egypt (Abedi et al., 2020; Al-Sherida et al., 2020; Dadar et al., 2021; Ebid et al., 2020; Wareth et al., 2020). In Egypt, it was isolated from cattle, buffalo, sheep, goats, camels, and humans (Abdel-Hamid et al., 2017; Sayour et al., 2020; Wareth et al., 2020; El-Diasty et al., 2021). Several previous studies described the prevailing of *Brucella* spp. infection among cattle in Egypt (Khoudair and Sarfenaz, 2007; Rehab, 2011; Menshawy et al., 2014; Hosein et al., 2017, 2018). *B. melitensis* biovar 3 was previously considered the prevalent type in Egypt (El-Diasty et al., 2018). In this study, the isolation of *B. melitensis* from cattle may be attributed to mixed farming of large and small ruminants, as previously explained by (Wareth et al., 2014; Hosein et al., 2016).

Cross-species infections frequently occur when different species are raised together. In the current study, AMOS-PCR confirmed the 31 *Brucella* isolates as *B. melitensis*. PCR was used for the direct detection of *Brucella* in serum samples collected from sheep and goats (Wareth et al., 2015), and tissue and milk samples (Abdali et al., 2020), and the results revealed that the sheep shed *Brucella* spp. in their milk more frequently than cattle. Furthermore, Ilhan et al. (2008) examined 102 milk samples taken from sheep after abortion using bacteriological culture, PCR, and MRT. PCR found *B. melitensis* DNA in 24 (23.5%) out of 102 milk samples, while only 8 (7.8%) samples were positive in culture. MRT found 28 (27.4%) positive milk samples. The detection limit for PCR in sheep milk inoculated with *B. melitensis* strain 16 M was 1.7×10^3–1.7×10^4 CFU/ml.

With the current situation and previous history of *Brucella* on the farm, a probable scenario was expected for the continued *Brucella* infection. Many gaps in the management system of the farm as the ability of stray dogs, cats, and foxes to access easily to the farm, and these wild animals are probably the primary source of infection (Wareth et al., 2017). Additionally, the common mistake in dairy farms in Egypt is introducing new animals from the market without serological examination which may contribute to the introduction of *Brucella* infection. Moreover, vaccination program has not been applied nationwide, and the rearing of different domestic animals (cattle, sheep, and camel) in close vicinity may aggravate the virulence, interspecies transmission of *Brucella* infection (Moreno, 2014; El-Diasty et al., 2018).

In this study, eradicating brucellosis inside the investigated farm was impossible due to the difficulties in changing the mixed breeding system. Eradication of brucellosis inside a farm is only possible when positive animals are culled associated with the prohibition of movement of *Brucella*-positive animals and additionally, application of biosecurity practices as well establishing a fair compensation policy for owners (Hosein et al., 2018; Musallam et al., 2019). Depending on RBT as a screening tool to eliminate the positive reactors, the results showed that the periodical testing of cattle with quick elimination of positive cases could virtually eliminate the source of infection among cattle that result in a gradual decrease of prevalence of reactors till it reached (zero%) beginning of the 6th examination up to 10th examination using RBT (Table 4).

In endemic areas, the early release of the herd out of the quarantine should be avoided, especially under unhygienic conditions and lack of controlled movement of animals. However, the animal population must be subjected to successive serological examinations to discover the disease incubating animals. It is known that the rules of veterinary authorities allow the release of the quarantined herds after three successive negative

serological examinations (Yagupsky et al., 2019). Periodical serological testing of brucellosis may help manage outbreaks, especially when implementing a stamping out policy is unfeasible. However, it didn't reduce the prevalence (Caetano et al., 2016).

Conclusion and Recommendations

Brucellosis is a worldwide and still a major threat to livestock production in Egypt. It affects both animals and humans and has a very high economic and public health impact. Isolation and typing of *Brucella* are mainly based on both bacteriological and molecular bases represent the essential operation toward evaluating *Brucella* herd infection status and tracing back the sources of infection. Our study revealed that *B. melitensis* biovar 3 is the prevalent type circulating in that farm.

The implemented test and slaughter strategy could be an effective tool to release the herd out of quarantine. Additionally, for eradication of *Brucella* from Egypt, the following recommendations are required i) Implementation of a strategy to control brucellosis through regular examination of all farm animals. ii) Efforts should be made to develop a new vaccine against brucellosis in cattle, sheep, and goats based on rough strains devoid of the disadvantages of the current smooth vaccines iii) Coordination of the governmental, public health officers, and veterinarians' efforts toward reducing the economic and zoonotic impact of brucellosis. iv) Application of biosecurity practices and fair compensation policy for owners should be also implemented.

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