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Some Risk Factors Associated with Animal Brucellosis in Aswan Governorate, Egypt

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Abstract

A CROSS-SECTIONAL study was carried out during 2022 to evaluate the current status of bovine brucellosis at Aswan governorate as a border governorate and where an extensive process of live animal importation occurs through Abu Simbel quarantine. The present study estimated the seroprevalence of brucellosis among local and imported cows, identified the currently field circulating Brucella strains in Aswan governorate and described the most important risk factors associated with the dynamics of bovine brucellosis. A total of 932 cows including 145 imported cows and 787 local cows from different localities, 319 ewes and 581 does at Aswan governorate were sero-tested for brucellosis using Rose Bengal plate test (RBPT), and Buffered acidified antigen plate test (BAAPT) as screening tests and positive samples were confirmed by complement fixation test (CFT). The seroprevalence of brucellosis was estimated as 2 (1.4%) in imported cattle and 20 (2.5 %) in local cattle with overall prevalence of 22 (2.4%) in cattle, 4 (1.2%) ewes and 6 (1%) in does. Different clinical specimens including 10 cow's milk samples, 5 cow's aborted material and 5cow's manure samples were collected. Clinical samples were processed for bacterial isolation and PCR testing. A total eight *Brucella melitensis* biovar 3 and two *Brucella abortus* biovar1 isolates were recovered and identified on bacteriological and molecular bases. *Brucella melitensis* biovar 3 could be isolated from two manure samples of infected cows that suggests that manure could be a vehicle for indirect dissemination of infection in both animals and man and could be an important potential risk factor.

Keywords: Brucellosis, Manure, PCR, Risk factors, Sero-prevalence.

Introduction

Brucellosis is a highly contagious bacterial disease of sexually mature animals caused by different members of the genus Brucella [1]. Different types of Brucellae have their own specific and preferred natural hosts [2, 3, 4]. These hosts act as the most significant link maintaining the chain of infection of brucellosis through shedding of the pathogen by different routes achieving the persistence of the disease in infected regions. It is of importance to recognize that Brucellae are excreted significantly from both aborted animals and cases of normal parturition of previously infected animals [5]. This leads to spreading the infection among herds especially in non-vaccinated cows.

As an intercellular pathogen, Brucella is a facultative intracellular pathogen that can multiply in macrophages avoiding the killing mechanisms by inhibition of phagosome lysososme fusion [6]. Additionally, Brucellae have an efficient adaptation that prevents their recognition by the immune system [7].

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As the organism is capable of replicating inside macrophages [8, 9, 10], the multiplying Brucellae are then disseminated to other host cells infecting other organs. Brucella organisms have a predilection for placentas and fetal fluids in females and the testes of males [11]. Moreover, *Brucella melitensis, Brucella abortus* and *Brucella suis* are important cause of human illness representing one of the most serious neglected zoonotic diseases especially in developing countries [12, 13].

The epidemiology of bovine brucellosis is influenced by those factors that influence the maintenance of infection within both the infected animal and the surrounding environment. In addition, it is also influenced by the factors associated with disease transmission that lead to spread of infection among animals and between herds [14].

Brucella organisms have been confirmed to be excreted through the urine, milk, and abortion products of infected animals and up to 1×10^{13} organisms/gram have been associated with placenta and fluids as reported by [15]. Moreover, [16] found that Brucellae can be excreted in vaginal fluids associated with aborted fetuses, thereby contaminating feces and bedding of animals. Consequently, Brucellae can exist in the manure and environment of Brucella-infected animals and could be a vehicle for indirect dissemination and could be an important potential risk factor. Interestingly, [17] described an outbreak of *Brucella melitensis* in humans in Argentina that was attributed to contact with the manure of infected goats. In addition, high prevalence of Brucella-infection related with diminished proper manure removal, cleaning and disinfection procedures has been described by [18].

In animals, the main symptoms and sequelae suggesting brucellosis are late abortion, long inter-calving interval, infertility, premature birth and retained placenta [19]. However, following chronic infection, orchitis, swelling of the testes and hygromas can also be observed [20]. Clinical symptoms are not specific to brucellosis and must be confirmed by laboratory diagnosis.

Animal brucellosis mostly occurs due to the addition of infected animals to a susceptible herd, un-controlled movement of animals especially in markets as well as mixing of different animal species [21, 22, 23, 24].

For the development of an effective control program for bovine brucellosis, it is essential firstly to recognize the potential risk factors that preserve the disease in both animals and the environment. Reservoirs that include Brucella-infected animals and contaminated environment especially the manure constitute the most important link of the chain of infection of brucellosis.

Therefore, the objectives of this study were focused on estimating the seroprevalence of brucellosis in imported and local cattle, ewes and does in Aswan governorate, identifying the currently field circulating strains of Brucella among cattle, describing the most important risk factors associated with brucellosis and discuss the role of Brucella infected animals and environmental reservoirs in the dynamics of animal brucellosis.

Material and Methods

Study areas and investigated animals

Different animal populations at different localities in Aswan governorate, Egypt, during 2022 including both imported and local cows, ewes and does were employed in this investigation (Figure 1). All animals were Brucella non-vaccinated and all populations were following the mixed breeding system especially with small ruminants.

Samples:

Ethical approval

The present study was approved by the “Ethical Committee", Beni-Suef University (BSU-IACUC/ 022-510), Egypt.

Blood sera

A total of 932 cows, 319 ewes and 581 does belonging to three districts (Kalabsha, Kom Ombo, and Sahari) at Aswan governorate, Figure (1) were selected and employed for serological diagnosis of brucellosis. Ten ml of blood were obtained from the jugular vein of each animal and left at room temperature to clot for serum separation. Blood sera were collected with plastic pipettes and transported in cold ice box to the laboratory stored at −20 °C until tested.

Aborted material

Vaginal discharges and placentas of 5 aborted cows with retained placenta were collected. Cotyledons were separated and wrapped in a greaseproof paper, wrapped in strong plastic sacks, kept on ice and sent to the laboratory without delay for Brucella isolation.

Milk samples

Last streaks of milk were collected from 10 cows, and centrifuged at (6000 g) for 15 minutes. Cream and deposit mixture was used for Brucella isolation and PCR.

Manure samples

Five cow’s manure samples were collected and processed from Brucella infected populations in private farms where Brucella infection was serologically confirmed in recently aborted cows. Samples were collected in sterilized plastic bottles directly from the rectum of each animal according to

the method described by [25]. Manure samples were then filtered using a syringe with a sterile cotton ball to remove solid debris twice, and the liquid was centrifuged at 3,000 x g for 30 min. Ten grams were added to 200 ml of tryptic soy broth, Becton Dickinson, 0.5% yeast extract, 5% horse serum, 1% glucose, and Brucella selective supplement), and incubated at 37 °C for 48 h in a shaking incubator. After centrifugation, 0.5 ml of the resulting pellet was used for Brucella isolation and PCR testing.

Serological examination

Blood sera were screened using Roe Bengal plate test (RBPT), and Buffered acidified antigen plate test (BAAPT) according to [26] and positive samples were confirmed by complement fixation test (CFT). Positive results were considered in samples that gave positive results in both screening and confirmatory tests.

Bacteriological examination

Milk cream and sediment mixture, vaginal discharges, placentas as well as pellets of manure samples were cultured on tryptose agar medium with selective antibiotic supplement (Oxoid), [27], according to [26] and the OIE [1] guidelines. Cultured plates were incubated in a CO_2 incubator with 10% CO_2. Vaginal discharges and the cream and deposit mixture of milk samples were spread on culture plates of the selective medium. Cotyledons were macerated and scrubbed onto the surface of the medium with and without antibiotics. The criteria used for Brucella identification included the requirement for additional atmospheric 10% CO_2, production of hydrogen sulfide gas, production of urease, growth on media containing the thionine and fuchsin , agglutination with monospecific antisera A, M and R and lysis by Tbilisi (Tb) and Izatnagar (Iz1) according to OIE [1] guidelines. Reference Brucella strains, *B. melitensis* Ether, *B. abortus* 544, and *Brucella suis* 1330 were supplied by the Central Veterinary Laboratory, Weybridge, Surrey KT15 3NB, UK and used as positive controls .

Molecular typing of Brucella isolates

DNA extraction

Milk, and manure samples were conveyed into new RNase-free Eppendorf tubes. Extraction was performed according to the instruction manual of Geneaid DNA extraction kit (New Taipei City, 22180 Taiwan, Cat. No. GS 100).

Conventional PCR

PCR amplification was carried out for molecular identification of *Brucella* spp. in DNA extracts from milk and manure at the genus level using primer sequences flanked the Immunodominant antigen, gene bp26 and Primer sequences flanked Erythritol catabolism, gene eryC according to [28] (Table 1).

Multiplex PCR

Multiplex PCR was performed for molecular identification of Brucella in DNA extracts from milk, and manure at the species level using five primers according to [29] (Table 1). The amplification was performed in Multigene thermal cycler (Labnet, USA).

The conventional and multiplex PCR thermal profiles were five minute at 94°C for initial heating followed by 30 cycles of 30 s at 94°C،45 s at 62°C, 1 min at 72°C, and final extension for10 min at 72°C. The PCR amplicons were analyzed by running 7 µl of the PCR product in 1% agarose gel stained with ethidium bromide (0.5µg/mL). Gel documentation were used to visualize the gel under UV illumination. Control positive in conventional and multiplex PCR were illustrated in table (2)

Results and discussion

The seroprevalence of bovine brucellosis in the current study was estimated as 2 (1.4%) in imported cows and 20 (2.5 %) in local cows with an overall prevalence of 22 (2.4%) in cows, 4 (1.2%) in ewes and 6 (1%) in does based on RBPT and BAPAT as screening tests and CFT as a confirmatory test (Table 3). The results of this study are nearly comparable with other previous findings reported in different parts of Egypt. The prevalence of animal brucellosis in Egypt in the last few years ranged from 0.33% to 1.32 % [30]. Several reports have described the bovine brucellosis prevalence in Egypt with variable figures, 4.64% [31], 5.7% [32], 5.33% [33]. In sheep, the prevalence of brucellosis has been previously reported as 8.61% [34], 3.05% [35], 8.52% [31] and 8.2% [32]. Among goats, the prevalence of brucellosis was 11.3% [34], 3.64% [35], 8.51% [31] and 6.96% [32].

The difference between the prevalence obtained in this study and other previous studies could be due to the variation in management and breeding systems, the use of different diagnostic procedures, the presence of infected animals in the herds as sources of infection, introduction of infected animals, animals movement, and mixing of different animal's species.

An important factor responsible for the spreading of brucellosis is the uncontrolled importation from some brucellosis enzootic countries [22]. This was the common pattern in this study because Aswan is a border governorate through which many animals pass through the Abu Simbel quarantine where the introduction of live animals continuously occurs as a result of the urgent need for animal protein in Egypt. This is in addition to the illegal movement of animals across the open southern borders from neighboring enzootic countries including Sudan and Libya that is considered one of the major risk factors.

Moreover, movement of cattle between different animal's markets was observed in this study. This is considered as major risk factor and principal cause of failure of brucellosis eradication programs as previously discussed by [22, 24]. Moreover, [36] concluded that addition of animals in the herd is an important critical control point of brucellosis.

In this study mixed farming was observed as a criterion in the management system of animals in Aswan governorate where cattle were kept closely with small ruminants. Mixed farming of large and small ruminants has been reported to be a risk factor for Brucella transmission among different animal species as reported by [3, 37]. Cross-species infections frequently occur when different species are raised together. *Brucella melitensis* cross-infection from sheep and goats to cattle has been reported in southern Europe [38], the Middle East [39] and in Egypt [24].

The obtained results revealed isolation of 10 Brucella isolates. Among the 10 field Brucella isolates obtained from clinical specimens of cattle, eight were definitely *Brucella melitensis* biovar 3, Table 4 (80%) and two (20%) were bacteriologically identified as *Brucella abortus* biovar1.

Brucella melitensis biovar 3 was previously described as the prevalent brucella type in Egypt as reported by [24, 39, 40, 41, 42].

Concerning *Brucella abortus* biovar 1, [40, 43] reported isolation of this type from 2 cows and 8 cows respectively in Egypt.

Isolation of Brucella organisms from aborted material (80%) is attributed to colonization of the reticuloendothelial system and genital organs causing chronic infection, reproductive disorders, abortion, stillbirth and infertility as explained by [44]. Moreover [45] concluded that pregnant cattle above five months of gestation are more likely susceptible to infection due to the preferential localization of Brucella in the uterus in which allantoic fluid factors such as erythritol stimulate the growth of Brucella organisms.

High association of Brucella seropositivity and abortion was observed in this study. To explain this association, it is important to recognize that Brucella infection is characterized by persistent infection in lymphoid tissues and inflammatory lesions in the reproductive tract of pregnant animals [44, 46]. Trophoblastic cells are the main target of Brucella infection during late gestation [44].

Retained placenta in this study was reported in all examined five aborted cows. Retention of the placenta can be explained on the basis of severe hemorrhagic necrotizing placentitis as described by [47].

Brucella abortus could be isolated from milk samples of 2 native cows. On the other hand, Brucella melitensis was isolated from milk samples of 2 native cows, 4 aborted materials of 4 imported cows and manure samples of 2 local cows. Isolation of Brucella organisms 4 (40%) from milk samples of cows coincides with our knowledge of considering the udder as an important portal of exit where the pathogen is shed. Cattle infected with Brucella spp. excrete high concentrations of the organism in their milk [48]. The recovery of Brucella from milk samples is of great public health significance as previously reported by [41].

Bacteriological examination revealed isolation of *Brucella melitensis* biovar3 from two manure samples after exhaustive bacteriological work. Using the PCR assay the two manure samples revealed *Brucella melitensis* biovar3 (Figure 2). Inappropriately, manure collected from animal farms is used as a fertilizer in most of the developing countries. Mitscherlich & Marth [49] reported the survival of Brucella in cow feces at room temperature for 122 days and 10 days in animal's environment. Remarkably, high humidity, low temperatures and lack of direct sunlight are the main factors leading to prolonged survival for Brucellae for months in water, aborted fetuses, placentas, liquid manure and hay as reported by [50]. The results obtained in this study suggest and add another mean of Brucella dissemination that complicates the infection chain of Brucellosis and may represent a potential risk for both animals and human health.

The results of conventional PCR confirmed the identification of Brucella in DNA extracts from milk, and manure on genus level using the genus-specific PCR with amplification of the fragment of 450 bp (Figure 2), as well as a fragment of 587 bp, specific for pathogenic Brucella strains of Erythritol catabolism, gene eryC (Figure 3).

Multiplex PCR in this study amplified the amplicons of 587, 1071, and1682 bp indicating *Brucella melitensis* biovar 3, and the fragments of 587 bp, and1682 bp sizes for *Brucella abortus* biovar 1, while *Brucella suis* reference strain has amplified the 272 bp, 587 bp, 1071 bp, and1682 bp sizes (Figure 4). These findings agreed with those of [24, 41, 42] using similar primers.

Results shown in Figure (4) revealed no vaccine strains among the Brucella isolates. The obtained results confirmed the identification of both *Brucella melitensis* and *Brucella abortus.*

For preventive purpose, it seems it is essential to break the side of the epidemic triangle connecting the environment and the susceptible host. This can be done by highlighting the hygienic measures essential to limit the bacterial load in the environment and diminish the possibility of contact of animals with viable Brucellae in contaminated environments. The

important issues include the aborted materials which should be properly disposed as early as possible, the manure and the contaminated bedding that should be properly removed with removing of 20 cm. of the ground and replacing by a new layer mixed with lime and removing of shade to expose the area to the sun light as explained by [51].

Conclusion

This study revealed the enzootic occurrence of brucellosis in Aswan governorate, Egypt. Several risk factors are responsible for the transmission of brucellosis among animals that include importation of animals from enzootic countries, mixed farming and movement of animals and lack of vaccination in Aswan governorate in spite the enzootic pattern of the disease. Possible survival of Brucella organisms in manure of infected animals as proved in this study suggests that manure could be a vehicle for indirect dissemination and could be an important potential risk factor.

Author contributions

All authors contributed in creating this article and approved the final manuscript.

Conflict of interest statement

Authors declare no conflict of interest

TABLE 1. Primers sequences, target genes, and amplicon sizes for conventional and Multiplex PCR.

Target gene	*Primers sequences*	*PCR product (bp)*
Immunodominant antigen, gene bp26	**BMEI0535f GCG-CAT-TCT-TCG-GTT-ATG-AA** **BMEI0535r CGC-AGG-CGA-AAA-CAG-CTA-TAA**	**450**
Erythritol catabolism, gene eryC	**BMEII0428f GCC-GCT-ATT-ATG-TGG-ACT-GG** **BMEII0428r AAT-GAC-TTC-ACG-GTC-GTT-CG**	**587**
Glycosyltransferase, gene wboA	**BMEI0998f ATC-CTA-TTG-CCC-CGA-TAA-GG** **BMEI0997r GCT-TCG-CAT-TTT-CAC-TGT-AGC**	**1682**
Outer membrane protein, gene omp31	**BMEII0843f TTT-ACA-CAG-GCA-ATC-CAG-CA** **BMEII0844r GCG-TCC-AGT-TGT-TGT-TGA-TG**	**1071**
Erythritol catabolism, gene eryC (D-erythrulose-1-phosphate dehydrogenase)	**BMEII0428f GCC-GCT-ATT-ATG-TGG-ACT-GG** **BMEII0428r AAT-GAC-TTC-ACG-GTC-GTT-CG**	**587**
ABC transporter binding protein	**BR0953f GGA-ACA-CTA-CGC-CAC-CTT-GT** **BR0953r GAT-GGA-GCA-AAC-GCT-GAA-G**	**272**
Ribosomal protein S12, gene rpsL	**BMEI0752f CAG-GCA-AAC-CCT-CAG-AAG-C** **BMEI0752r GAT-GTG-GTA-ACG-CAC-ACC-AA**	**218**

TABLE 2. Lyophilized reference Brucella strains

Species	Biovar	Strain	ATCC	NCTC
Brucella melitensis	1	16M Rev.1	23456	10094
	3	Ether	23458	10509
Brucella abortus	1	544	23448	10093
Brucella suis	1	1330	23444	10316

ATCC[1]: American Type Culture Collection, USA
NCTC[2]: National Collection of Type Cultures, UK.

TABLE 3. Seroprevalence of brucellosis at Aswan governorate

Animals	Number of tested cows	Number of sero-positive cows*	*Abortion*
Imported cows	145	2 (1.4%)	10 (6.9 %)
Local cows	787	20 (2.5 %)	9 (1.14 %)
Total cows	932	22 (2.4%)	19 (2 %)
Ewes	319	4 (1.2%)	4 (1.2 %)
Does	581	6 (1%)	6 (1 %)

*In case of abortion, serum samples were collected three weeks after abortion

TABLE 4. Brucella isolation from different clinical samples at Aswan governorate

Samples	No. of samples	Brucella isolation	*Brucella abortus* 1	*Brucella melitenisis* 3
Milk of native cows	10	4(40%)	2	2
Aborted materials of imported cows	5	4(80%)	-	4
Manure samples of native cows	5	2(40%)	-	2
Total		10	2(20%)	8(80 %)

Figure legends

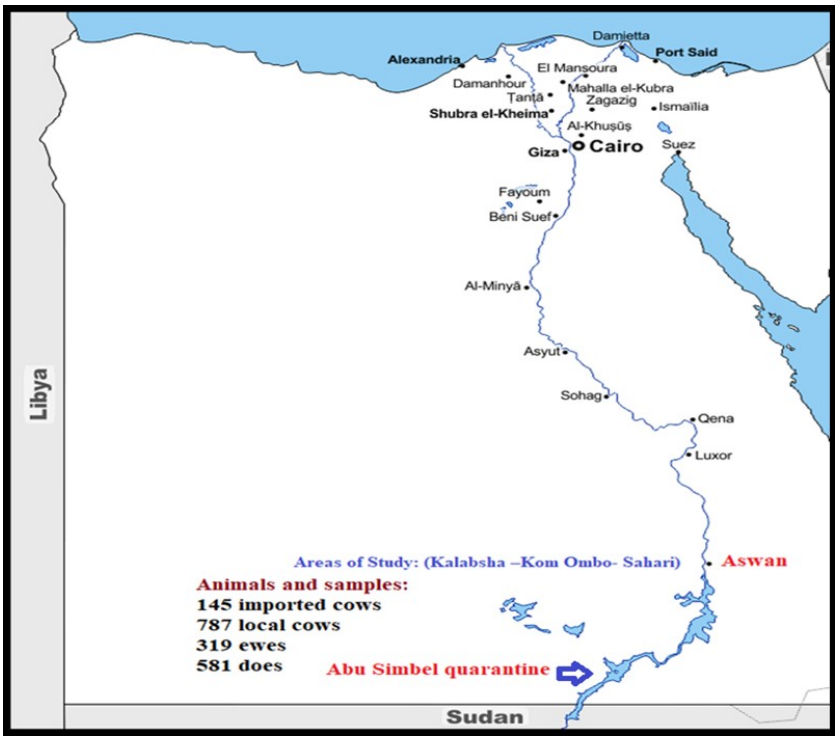

Fig. 1. Area of study, Animals and clinical samples

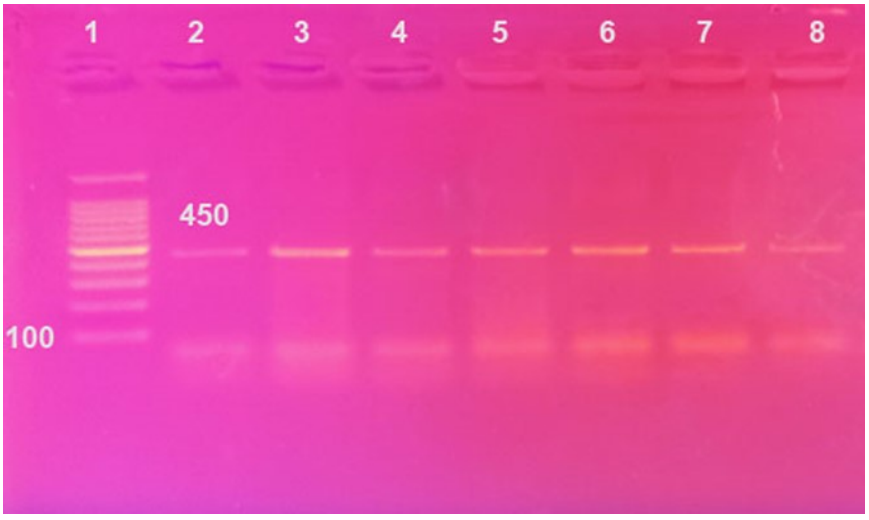

Fig. 2. Amplification of target gene immunodominant antigen, gene bp26 flanked the 450 bp. by conventional polymerase chain reaction (PCR).

Lane (1): 100bpDNA ladder.

Lane (2) B. melitensis reference strain

Lane (3:6) Brucella milk DNA extract

Lane (7, 8) Brucella manure DNA extract

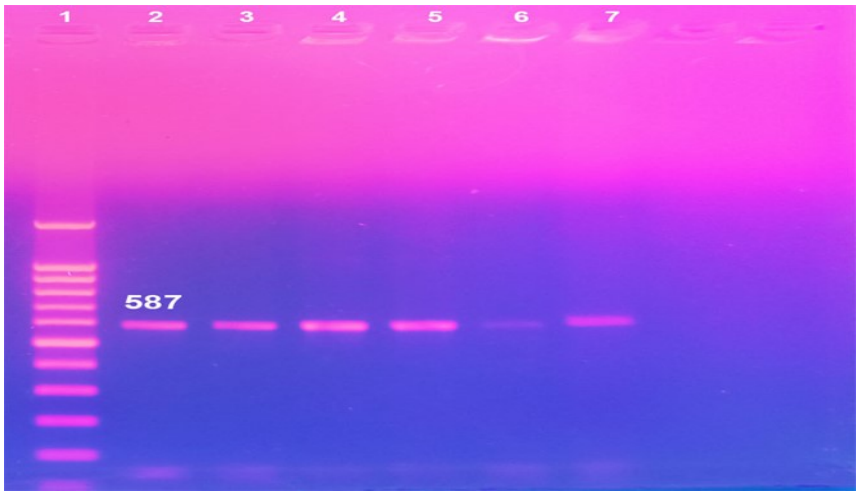

Fig. 3. Amplification of the set of primers specific for pathogenic Brucella strains flanked the 587 bp.

Lanes 1: 100bpDNA ladder

Lanes 2: 7, Brucella DNA extract**.**

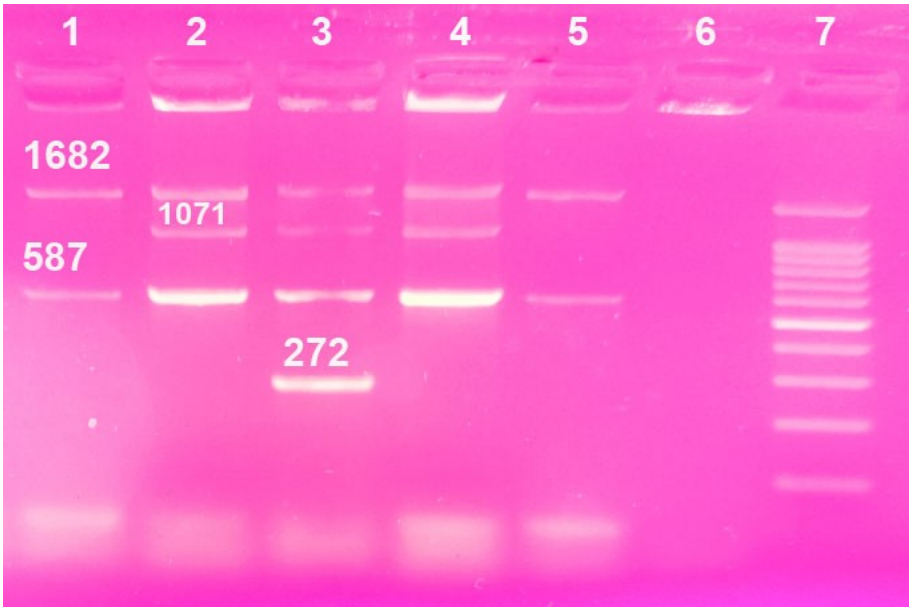

Fig. 4. Multiplex PCR of Brucella isolates

Lane (1) B. abortus 544 reference strain (587 - 1682 bp.)

Lane (2) B. melitensis reference strain Ether (587 - 1071 - 1682 bp.)

Lane (3) B. suis reference strain (272 - 587 - 1071 – 1682 bp.)

Lane (4) B. melitensis field strain (587 - 1071 -1682 bp.)

Lane (5) B. abortus field strain (587 - 1682 bp.)

Lane (6) Control negative,

Lane (L) 100 bp marker.

References

1. OIE. Infection with Brucella abortus, Brucella melitensis and Brucella suis, OIE (World Organisation for Animal Health) Rome (2018).
2. Quinn, P.J., Carter, M.E., Markey, B. & Carter, G.R. Clinical Veterinary Microbiology. London: Wolfe Publishing, 261–267 (1994).
3. Al-Majali, A.M. Seroepidemiology of caprine brucellosis in Jordan. *Small Rumin Research.,* **58**, 13–18 (2005).
4. Al-Majali, A.M., Majok, A., Amarin, N. & Al-Rawashdeh, O. Prevalence of, and risk factors for, brucellosis in Awassi sheep in Southern Jordan. *Small Rumin Research.,* **73**,300–303(2007).
5. Silva, I., Dangolla, A. and Kulachelvy, K. Seroepidemiology of Brucella abortus infection in bovids in Sri Lanka. *Preventive Veterinary Medicine*, **46**, 51–59 (2000).
6. Ko, J. and Splitter, G.A. Molecular host interaction in brucellosis: Current understanding and future approaches to vaccine development for mice and humans. *Clinical. Microbial. Research*, **16**, 65-78 (2003).
7. Godfroid, J., Scholz, H.C., Barbie, T., Nicolas, C., Wattiau, P., Fretin, D., Whatmore, A.M., Cloeckaert, A., Blasco, J.M., Moriyon, I., Saegerman, C., Muma, J.B., Al Dahouk, S., Neubauer, H. and Letesson, J.J. Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. *Preventive Veterinary Medicine,* **102** (2), 118–131 (2011).
8. Celli, J., de Chastellier, C., Franchini, D. M., Pizarro-Cerda, J., Moreno E. and Gorvel, J. P. Brucella evades macrophage killing via VirB-dependent sustained interactions with the endoplasmic reticulum. *J. Exp. Med.,* **198**, 545-556 (2003).
9. Celli, J., Salcedo, S.P. and Gorvel, J.P. Brucella coopts the small GTPase Sar1 for intracellular replication. *Proc. Natl. Acad. Sci. USA*, **102**, 1673-1678 (2005).
10. Starr, T., Ng, T.W., Wehrly, T.D., Knodler, L. A. and Celli, J. Brucella intracellular replication requires trafficking through the late endosomal/lysosomal compartment. *Traffic*, **9**, 678-694 (2008).
11. Constable, P.D., Hinchcliff, K.W., Done, S.H. and Gruenberg, W. A textbook of diseases of cattle, horses, sheep, pigs, and goats. Vol 1. 11th ed. Elsevier, 3251 Riverport lane, St. Louis, Missouri63043. (2017)
12. Trujillo, I.Z., Zavala, A,N., Caceres, J.G. and Miranda, C.Q. Brucellosis infection. *Infect. Dis. Clin. North Am.,* **8**, 225-241 (1994).
13. Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L. and Tsianos, E. V. The new global map of human brucellosis. *Lancet Infect. Dis.,* **6**, 91-94 (2006).
14. Crawford, R.P., Huber, J.D. and Adams, B.S. Epidemiology and surveillance. In: Nielsen K, Duncan JR,. Animal Brucellosis. CRC Press: Boca Raton., pp. 131–15. (1990).
15. Alexander, B., Schnurrenberger, P. R. and Brown, R. R. Number of Brucella abortus in the placenta, umbilicus and fetal fluid of two naturally infected cows. *Veterinary Record,* **108**, 500 (1981).
16. Corbel, M.J. Brucellosis: an overview. *Emerg. Infect. Dis.,* **3**, 213-221 (1997).
17. Wallach, J.C., Ferrero, M.C., Delpino, M.V., Fossati, C.A. and Baldi, P.C. Occupational infection due to Brucella abortus S19 among workers involved in vaccine production in Argentina. *Clin. Microbiol. Infect.,* **14**, 805-807 (2008).
18. Blasco, J. M. A review of the use of B. . melitensis Rev. 1 vaccine in adult sheep and goats. *Preventive Veterinary Medicine,* **31**, 275-283 (1997).
19. Shareef, J.M. A review of serological investigations of brucellosis among farm animals and humans in Northern provinces of Iraq (1974-2004). *J. Vet. Med. B Infect. Dis. Vet. Public Health,* **53** (1), 38–40 (2006).
20. WHO, FAO report. Brucellosis in humans and animals: World Health Organization in collaboration with the Food and Agriculture Organization of the United Nations and the World Organization for Animal Health (2006).
21. Sanogo, M., Abatih, E., Thys, E., Fretin, D., Berkvens, D. and Saegerman, C. Risk factors associated with brucellosis seropositivity among cattle in the central savannah-forest area of Ivory Coast. *Prev. Vet. Med.,* **107**, 51–6 (2012).
22. Dean, A.S., Fournié, G., Kulo, A.E., Boukaya, G.A., Schelling, E. and Bonfoh, B. Potential risk of regional disease spread in West Africa through cross-border cattle trade. *PLoS ONE,* **8**, e755 (2013).
23. Kanouté, Y.B., Gragnon, B.G., Schindler, C., Bonfoh, B. and Schelling, E. Epidemiology of brucellosis, Q fever and rift valley fever at the human and livestock interface in northern Côte d'Ivoire. *Acta Trop.,* **165**, 66–75 (2017).
24. Hosein, H,I., Zaki, H,M., Safwat, N.M., Menshawy, A.M.S., Rouby, S., Mahrous, A. and Madkour, B.E. Evaluation of the General Organization of Veterinary Services control program of animal brucellosis in Egypt: An outbreak investigation of brucellosis in buffalo. *Veterinary World,* **11**(6), 748-757 (2018).
25. Morales-Estradaa, A.I., Hernández-Castrob, R., López-Merinoa, A., Singh-Bedic , J. and Contreras-Rodríguez, A. Isolation, identification, and antimicrobial susceptibility of Brucella spp. cultured from cows and goats manure in Mexico. *Arch. Med. Vet.,* **48**, 231-235 (2016).
26. Alton, G.G., Jones, L.M., Angus, R.D. and Verger, J.M. Techniques for the brucellosis laboratory. Vol 1, 3rd Edition. Institut National de la recherche Agronomique (INRA) (1988).
27. Ewalt, D.R., Packer, R.A. and Harris, S.K. An improved selective medium for isolating Brucella sp. from bovine milk. In: Proceeding of the Process International Symposium Veterinary Laboratory Diagnosticians. 577-589 Alton, G.G., Jones, L.M., Angus, R.D. and Verger, J.M. Techniques for the

brucellosis laboratory. 1988: Institute National de la recherche Agronomique (INRA). 37. (1983)

28. Garcia-Yoldi, D., Marin, C.M., De Miguel, M.J., Munoz, P.M., Vizmanos, J.L. and Lopez-Goni, I. Multiplex PCR assay for the identification and differentiation of all Brucella species and the vaccine strains Brucella abortus S19 and RB51 and Brucella melitensis Rev1. *Clinical Chemistry,* 52, 779-781 (2006)

29. Lopez-Goni, I., Garcia-Yoldi, D., Marin, C.M., De Miguel, M.J.,Munoz, P.M., Blasco, J.M., Jacques, I., Grayon, M., Cloeckaert, A. and Ferreira, A.C. Evaluation of a multiplex PCR assay (Bruce-ladder) for molecular typing of all Brucella species, including the vaccine strains. *Journal of Clinical Microbiology*, **46**, 3484-3487(2008)

30. Wareth, G., Hikal, A., Refai, M., Melzer, F., Roesler, U. and Neubauer, H. Animal brucellosis in Egypt. *J. Infect. Dev. Ctries,* **8**(11), 1365-1373 (2014).

31. Lamyaa, M.B. Public health importance of brucellosis in Menofia Governorate. Ph. D. Thesis. Fac. Vet. Med. Moshtohor. Zagazig Uni. (2005).

32. Lobna, M.A.S. Epidemiological studies on Brucellosis in animal and man with special reference to prevention and control. Ph.D. Thesis. Fac. Vet. Med., Moshtohor. Benha Univ. (2006).

33. Rehab, A. Epidemiological characterization of Brucella strains in Egypt, PhD thesis, faculty of veterinary medicine, infectious diseases Beni- Suef University (2011)

34. El-Bauomy, E.M. Further studies on brucellosis in sheep and goats. Ph.D. Thesis. Fac. Vet. Med., Cairo Univ. (1993)

35. Zayed, E.A. A study on brucellosis in sheep and goats. M.V.Sc. *Thesis*, Fac. Vet. Med. Cairo Univ (1997).

36. Earhart, K., Vafakolov, S., Yarmohamedova, N., Michael, A., Tjaden, J., Soliman, A. Risk factors for brucellosis in Samarqand Oblast, Uzbekistan. *Int. J. Infect. Dis.,* **13**, 749 – 753 (2009).

37. Omer, M.K., Skjerve, E., Holstad, G., Woldehiwet, Z. and Macmillan, A.P. Prevalence of antibodies to Brucellaspp. in cattle, sheep, goats, horses and camels in the State of Eritrea, influence of husbandry systems. *Epidemiol. Infect.,* **125**, 447–453(2000)

38. Verger, J.M., Garin Bastuji, B., Grayon, M. and Mahe, A.M. Brucella melitensis infection in cattle in France. *Ann. Rech. Vet.,* **20**, 93-103 (1989)

39. Samaha, H., Al-Rowaily, M., Khoudair, R.M. and Ashour, H.M. Multicenter Study of Brucellosis in Egypt. *Emerg. Infect. Dis.,* **14**(12) 1916–1918 (2008).

40. Menshawy, A., Perez-Sancho, M., GarciaSeco, T., Hosein, H. I., Garcia, N., Martinez, I., Sayour, A.E., Goyache, J., Azzam, R. A. A. and Dominguez, L. Assessment of genetic diversity of zoonotic Brucella spp. Recovered from livestock in Egypt using multiple locus VNTR analysis. *BioMed. Research International,* **1**,7 (2014).

41. Hosein, H.I., Rouby S.R., Menshawy, A. and AbdAl-Ghany, A. E. Sensitivity and specificity of the commonly used diagnostic procedures of bovine brucellosis. *Veterinary Sciences: Research and Reviews,* **3**(3), 45-50 (2017)

42. Hosein, H.I., Hamdy, M., Zaitoun, A., Menshawy, A.M., Rouby, S.R., Madkour, B., Mazeed, A.M. and Abdel-Ra'ouf, A. Brucella Prevalent Strains Circulating in Egypt during 2020-2021: Bacteriological and Molecular Study. *Journal of Veterinary Medical Research,* **28** (1), 12–20 (2021).

43. Khan, A U, Shell, W., Melzer, F, Sayour, A, Ramadan, E., Elschner, M., Moawad, A., Roesler, U., Neubauer, H. and El-Adawy H. Identification, Genotyping and Antimicrobial Susceptibility Testing of Brucella spp. Isolated from Livestock in Egypt. *Microorganisms*, **7** (12), 60(2019).

44. Xavier, M.N., Paixão, T.A., Poester, F.P., Poester, A, Lage, P. and Santos R. L. Pathology, immunohistochemistry, and bacteriology of tissues and milk of cows and fetuses experimentally infected with Brucella abortus. *J. Comp. Pathol.,* **140**, 149-157 (2009).

45. Godfroid, J., Debolle, X., Roop, R.M. , O'Callaghan, D., Tsolis , R.M., Baldwin, C., Santos, R.L., Mcgiven, J., Olsen, S., Nymo, I.H., Larsen, A., Al Dahouk, S. and Letesson, J.J.. The quest for a true One Health perspective of brucellosis. *Rev. Sci. Tech. Off. Int. Epiz.,* **33** (2), 521-538 (2014).

46. Ficht, T.A. Intracellular survival of Brucella: Defining the link with persistence. *Vet. Microbiol.,* **2516**, 1-11 (2003).

47. Neta, C., Alcina, V., MoAl, J.P.S., Xavier, M.N., Paixão, T.A., Lage, A.P. and Santos, R.L.. Pathogenesis of bovine brucellosis. *Vet. J.,* **184**, 146-155 (2010)

48. Nicoletti, P. The epidemiology of bovine brucellosis. *Adv. Vet. Sci. Comp. Med.,* **24**, 69-98 (1980).

49. Mitscherlich, E. and Marth, E. H. Microbial Survival in the Environment. Springer-Verlag. Berlin. (1984)

50. Sammartino, L.E., Gil, A. and Elzer, P. Capacity building for surveillance and control of bo-vine and caprine brucellosis. In: FAO Animal Production and Health Proceedings -AO/WHO/OIE Expert and Technical Consultation. Rome., 7, 55 (2006).

51. Hosein, H.I. Infectious diseases of domestic animals, Text book, 5th ed., Laser center for publication, Beni Suef, Egypt (2022).

بعض عوامل الخطر المرتبطة بمرض البروسيلا في الحيوانات في محافظة أسوان، مصر

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أجريت هذه الدراسة خلال عام 2022 لتقييم الوضع الحالي لمرض البروسيلا في محافظة أسوان كمحافظة حدودية، حيث يتم استيراد الحيوانات الحية علي نطاق واسع من خلال محجر أبو سمبل. قامت الدراسة الحالية بتقدير معدل الانتشار المصلي لمرض البروسيلا بين الأبقار المحلية والمستوردة، وحددت سلالات البروسيلا المنتشرة حاليًا في محافظة أسوان، كما تم توصيف أهم عوامل الخطر المرتبطة بديناميكية انتشار مرض البروسيلا . تم اختبار 932 بقرة منها 145 بقرة مستوردة و787 بقرة محلية من مناطق مختلفة، و319 نعجة و581 نعجة في محافظة أسوان لمرض البروسيلا باستخدام اختبار روز البنغال (RBPT)، واختبار (BAAPT) تم تأكيد اختبارات الفحص والعينات الإيجابية عن طريق اختبار التثبيت التكميلي (CFT). تم تقدير معدل الانتشار المصلي لمرض البروسيلا (1.4%) في الأبقار المستوردة و20 (2.5%) في الأبقار المحلية، مع انتشار إجمالي قدره 22 (2.4%) في الأبقار و4 (1.2%) في النعاج و6 (1%) في النعاج. . تم جمع عينات مختلفة اشتملت على 10 عينات من حليب البقر و 5 عينات من المواد المجهضة من البقر و 5 عينات من روث البقر. وتمت معالجة العينات لعزل البكتيريا واختبار PCR. تم عزل ثمانية عزلات من البروسيلا melitensis biovar 3 وعزلتين من البروسيلا abortus biovar1 وتم تشخيصها على الأسس البكتريولوجية والجزيئية. تم عزل Brucella melitensis biovar 3 من عينتين من روث الأبقار المصابة، مما يشير إلى أن الروث يمكن أن يكون وسيلة للانتشار غير المباشر للعدوى في كل من الحيوانات والإنسان ويمكن أن يكون عامل خطر محتملًا مهمًا.

الكلمات المفتاحية: البروسيلات، السماد، تفاعل البوليميراز المتسلسل، عوامل الخطر، الانتشار المصلي.