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Reliable and highly specific techniques for the detection of *Brucella* spp. antibodies in camel milk

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Abstract

Brucellosis is one of the most serious zoonotic diseases in human and farm animals caused by *Brucella* species. This study aims to: (i) estimate the current prevalence of *Brucella* among camels in Erbil Governorate; (ii) evaluate the milk ring test as a diagnostic tool for screening of brucellosis in camels; (iii) study the association between months and percentage of positive samples to *Brucella*. During the period, January - June 2021, a total of 250 raw camel milk samples (130 samples from farms and 120 from sale points) were randomly collected. The brucellosis is diagnosed using the Milk Ring Test (MRT), indirect ELISA, and bacteriological isolation of *Brucella* species. The prevalence of *Brucella* antibodies in camel milk samples is 11.6% and 10.4% according to MRT and ELISA, respectively. The overall isolation percentage of *Brucella* species is 8.4%. The detection rate of isolates in sale points is higher 10.0% than the isolation rate from farm 6.9%. The results also reveal that 4.6% and 5.8% of isolates are *Brucella abortus*; while, 5.8% and 4.2% are *Brucella melitensis* from the milk of farm and sale points, respectively. The highest rate of brucellosis according to MRT is observed in February 18.6%, while the lowest rate is documented in May 7.5%. We recommend using MRT for the diagnosis of *Brucella* spp. in routine screening of brucellosis in milk collection centers, dairy factories, and farm. Customers are also recommended to heat the milk adequately to eliminate this milk-borne pathogen before drinking milk or manufacturing processes.

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Introduction

Brucella species are facultative intracellular, non-motile, non-sporing, gram-negative coccobacilli. They are aerobic, but some strains require 5-10% CO_2 for primary isolation. Growth *in vitro* is slow and primary isolation may require up to 4 weeks of incubation at 37° C. In vitro growth is slow but enhanced if serum was added to the growth medium (1). Brucellosis is a global bacterial zoonotic disease that is transmissible to humans and a wide range of domestic and wild animals, particularly food-producing animals comprising camels, cattle, buffaloes, sheep, goats, pigs, and reindeer (1,2). Camels in all rearing countries, except Australia, are infected by the two predominant species of the genus: *B. abortus* and *B. melitensis*. Brucellosis in camels is an insidious disease, since it hardly provokes any clinical signs, and may furthermore be faced with difficulties in laboratory diagnosis due to the lack of sufficiently validated tests (3). The prevalence of infection ranges widely between regions (4). For instance, the documented prevalence in East African countries range between <1% to 40%, while sporadic small-sized studies in the Middle East showed a prevalence of < 30% (5,6). Despite the massive rearing and exploitation of camels in many populations' daily life in the Middle East, little is known about the true prevalence of brucellosis in the countries of this region. The presence of *Brucella* species in milk or dairy products can occur from either a direct passage from udders, contamination by animal

excreta, or unsanitary handling of the milking utensils (4,7). The transmission to human occurs through the consumption of contaminated food of animal origin (milk or meat), and from mothers to breastfed babies (8,9). A meta-analysis study of *Brucella* spp. in raw milk has found the prevalence in the Middle East to be 29.0% (95% CI: 23-35%), which poses a serious threat to public health (6). In fact, human brucellosis is a multisystem disease with a broad spectrum of clinical manifestations and life-threatening complications, such as meningitis, osteomyelitis, sacroiliitis, spondylitis, hepatic abscesses, peripheral arthritis, bronchopneumonia, epididymitis, prostatitis, orchitis, encephalitis, and cardiovascular complications (10,11). The diagnosis of brucellosis is rather complicated, and it must be obligatorily confirmed by laboratory testing. Bacteriological culture is still the definitive test for *Brucella* infections; however, various serological and molecular diagnostic tests (Rose Bengal test, ELISA, complement fixation, and slide agglutination test) are available with different accuracies and performance requirements (12-14). The choice of the diagnostic test depends on the overall epidemiological situation in the region, objectives of the study, validation of the diagnosis, monitoring, cross-sectional studies or confirmation of brucellosis free status of the region (15,16). Different studies had found the sensitivity and specificity of MRT to range from 80-88% and 97-99% in the detection of brucellosis in milk samples from cows, sheep, and goats (17-19). However, another recent study found a lower sensitivity and specificity (73.3% and 84.6%, respectively) for the detection of brucellosis in milk samples from goats and sheep (20). This variation is mostly attributed to differences in disease prevalence and the accuracy of the reference test used for the evaluation of the diagnostic test (21). Nonetheless, and practically speaking, the adoption of MRT is supported by the observed high specificity of results as a straightforward and cheap screening/diagnostic method for excluding brucellosis rather than confirming the infection in the suspected animal. Recently, brucellosis in ewes, nanny goats, cows, and buffaloes in Erbil Governorate has been screened (17,18,22). However, to the best of the author's knowledge, *Brucella* infection in the camel's population has not been thoroughly addressed.

The objectives of this study are to: (i) estimate the current prevalence of *Brucella* among camels in Erbil Governorate; (ii) evaluate the milk ring test as a diagnostic tool for screening of brucellosis in camels; (iii) study the association between months (sample collection time) and frequency of *Brucella*.

Materials and methods

Ethical approve

This study obtained approval from the scientific board, College of Science, Knowledge University, Erbil, Iraq, the approval issue 001, dated 10/11/2020.

Samples collection

During the period, January - June 2021, a total of 250 raw camel milk samples (130 from farms and 120 samples from sale points) were randomly collected from suburban farms at the outskirts of Erbil city and retail milk shops in Erbil Governorate. For each sample, about 100 ml of milk sample were collected into labelled sterile plastic containers with screw lid, under hygienic conditions. On the same day of collection, all the samples were transported under cool conditions (inside an icebox ~ 5°C) to Department of Medical Laboratory Sciences at College of Science, Knowledge University, Erbil, Iraq. The samples were stored in a deep freezer at −18°C and were analyzed within 48 hours of collection (23).

Milk Ring Test (MRT)

Detection of *Brucella* antibodies in raw milk was done by the Milk Ring Test, MRT. The test was carried out by adding one drop (~ 0.05 ml) of MRT antigen solution (JOVAC Jordan) to 1 ml of whole milk in a narrow test tube (11*100 mm). The antigen milk mixture was incubated at 37°C for 1-3 hours. If the anti-*Brucella* antibodies are present in the milk, they bind to the antigen and rise with the cream layer to form a blue ring above the white milk column. If antibodies are absent, the mixture remains homogeneously bluish white throughout the tube (19). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the MRT were calculated according to standard equations below (21), using the bacterial isolation diagnostic method as the gold standard. Sensitivity = [TP / (TP + FN)] ×100. Specificity = [TN / (FP + TN)] ×100. Positive predictive value = [TP / (TP + FP)] ×100. Negative predictive value = [TN / (FN + TN)] ×100. Accuracy = [TP + TN / (TP + FP + TN + FN)] ×100. Where TP is the number of true positive, FP is the number of false positive, TN is the number of true negative, and FN is the number of false negative.

ELISA test

The indirect ELISA test to detect IgG were performed according to a published protocol (24). Briefly, all reagents were allowed to come to room temperature and homogenized by vortex before use. Samples were centrifuged to separate the creamy portion from the lactoserum containing the antibodies. Lactoserum was processed and tested according to the instructions provided by the kit manufacturer (Euroimmun AG, Germany).

Isolation and identification of *Brucella*

Isolation of *Brucella* species from the raw milk samples was performed under aseptic conditions (25). Inoculated tubes and plates (*Brucella* broth and *Brucella* agar, HiMedia, India) were incubated aerobically and in the presence of 5%-10% carbon dioxide at 37°C. The plates were observed for up to 7 days for the presence of suspected colonies of

Brucella. Biochemical tests were performed for identification purposes of the suspected isolates (17). The identification of *B. abortus* and *B. melitensis* were confirmed by its definitive biochemical tests (1).

Statistical analysis

Data were analyzed using SPSS software version 25, and confidence intervals were estimated using normal distribution approximation at an alpha level of 0.05. Chi-square test was applied to test the odd between the groups. P value less than 0.05 was considered significant. Association of seroprevalence with months was evaluated using correlation coefficient test.

Results

Seroprevalence of *Brucella*

According to the MRT, the overall prevalence of *Brucella* antibodies in raw milk samples was 11.6% (29/250). Similarly, 10.4% (26/250) of milk samples were positive for the presence of anti-*Brucella* antibodies, detected by ELISA (Table 1). Based on both tests, there is no significant difference between raw milk collection sites in terms of positivity rate (P=0.416). Statistically, it is estimated that 7.91-16.23% (95% CI) of camels would be seropositive for *Brucella* in Erbil Governorate if screened by MRT assay. No significant differences were found between MRT and ELISA in terms of brucellosis detection (χ^2 = 0.183, P=0.668).

Prevalence of *Brucella* species

The overall isolation percentage of *Brucella* species from camel raw milk samples was 8.4% (21/250). It is obviously clear that the detection rate in sale points was higher 10.0% than the isolation rate from a farm 6.9%. However, such an increase is not statistically different (p=0.378). Regarding the identified species of *Brucella* from camel raw milk samples, *B. abortus* comprised about two thirds 61.9% of total isolates (13/21 isolates), while the remaining isolates were of *B. melitensis* (Table 2).

Table 1: Prevalence of *Brucella* antibodies in camel raw milk

Collection Site		Samples	Positive Samples n (%)	95% CI	*p* Value
MRT:	Farm	130	13 (10.0)	5.43 - 16.49	0.416
	Sale points	120	16 (13.3)	7.82 - 20.75	
	Total	250	29 (11.6)	7.91-16.23	
ELISA:	Farm	130	11 (8.5)	4.30 - 14.64	0.302
	Sale points	120	15 (12.5)	7.17 - 19.78	
	Total	250	26 (10.4)	6.91 - 14.87	

Table 2: Isolation of *Brucella* species from camel raw milk

Collection site	No.	*B. abortus* n (%)	*B. melitensis* n (%)	Total n (%)
Farms	130	6 (4.6)	3 (2.3)	9 (6.9)
Sale points	120	7 (5.8)	5 (4.2)	12 (10.0)
Total	250	13 (5.2)	8 (3.2)	21 (8.4)

Comparison of MRT and ELISA to culture approach

The MRT technique detected more cases of brucellosis 11.6% than the traditional culture method 8.4% in both groups of milk samples. Sensitivity, specificity, positive predictive value, and negative predictive value of MRT and ELISA are given in (Table 3).

Temporal distribution of seropositive samples

Variations of *Brucella* antibodies occurrence in camel raw milk samples during six months have been investigated (Figure 1). The highest rate of prevalence of *Brucella* antibodies detected by MRT was found in February 18.6%, while the lowest rate was documented in May 7.5%. According to the statistical calculations, there is a weak positive correlation (r^2 = 0.16) between the progress of winter-spring months and the prevalence of brucellosis.

Table 3: Evaluation of MRT and ELISA in detecting camel brucellosis

	MRT	ELISA
Sensitivity	80.77% (60.65-93.45)	80.77% (60.65-93.45)
Specificity	96.62% (93.46-98.53)	99.13% (96.91-99.89)
PPV *	72.41% (56.43-84.18)	91.30% (72.29-97.69)
NPV *	97.86% (95.42-99.02)	97.86% (95.42-99.02)
Accuracy	95.06% (91.70-97.34)	97.28% (94.47-98.90)

* PPV; positive predictive value, NPV; negative predictive value.

Discussion

Brucellosis is an infectious disease of livestock and wild animals and the commonest human zoonosis. Transmission to humans occurs in several ways, commonly through consumption of contaminated food, particularly raw milk or meat and their products (26-28). Our findings are inconsistent with a previous country-wide survey of the camel brucellosis in Iraq that was carried out in 2005, in cooperation with FAO, in which no antibodies were detected against *Brucella* in 540 serum samples screened using the Rose Bengal and ELISA tests (29). Indeed, prevalence of

camel brucellosis was reported in nearby countries such as Saudi Arabia, where the seroprevalence of brucellosis in camels appeared to follow two distinct patterns: a low (2-5%) prevalence in nomadic or extensively kept camels and a high 8-15% prevalence in camels kept intensively or semi-intensively (5). Moreover, 12.1% of camels were found infected with *Brucella* in Jordan (30). These findings indicate a current increasing trend of brucellosis in camel population in Erbil.

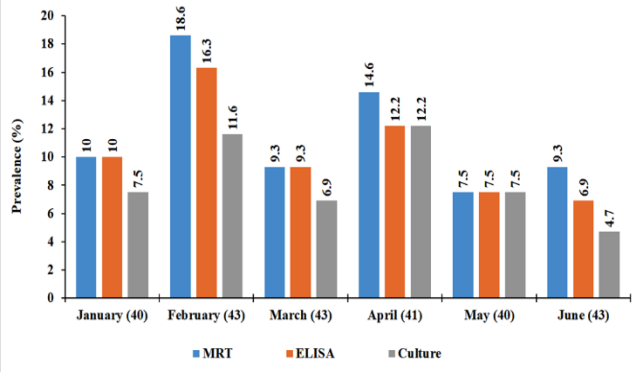

Figure 1: Monthly variations of *Brucella* spp. antibodies in camels during study period. Numbers in parentheses denote the number of samples collected in the corresponding month.

Similar prevalences were also reported in Ethiopia 11.7-15.5% (31), Iran 11.38% (32) and Egypt 11-13% (33). In contrast, lower rates were reported in Libya 5.7% (34), Mongolia 2.3% (35) and Oman 1.3-3.7% (36). Such variations are influenced mostly by the epidemiology in the study area and the testing method (12,16,28). The efficiency (accuracy) of MRT in detecting camel brucellosis is markedly similar to the ELISA (95.06% vs 97.28%) in comparison to culture method, which candidates the MRT a good alternative screening/diagnostic method. Based on the findings of the study, the 2% difference in accuracy can be sacrificed for the sake of simplicity and rapidity of RMT in comparison to the ELISA (5,12).

Regarding the detected species, *B. abortus* was more prevalent than *B. melitensis*. This observation is anticipated since cattle and other livestock are the major host species for *B. abortus* (1,27,37). These findings are also in good agreement with the previously published literature (3,5,31). In fact, isolation of Brucella is a difficult, tedious, time-consuming, and potentially risky laboratory work (1,12). Therefore, most recent studies employ culture-independent diagnostic assays such as Rose Bengal Test, Slide Agglutination, and ELISA. The overall isolation rate of *Brucella* spp. in this study 7.1% was similar to a Nigerian study that detected brucellosis in livestock by the bacteriological approach (38). The sampling of different areas with different *Brucella* epidemiology or during the dry season may account for such variations in isolation rates.

The isolation of *Brucella* from milk samples may be improved if more than one culture medium is used (12,13). On the other hand, higher isolation rates were also reported from different countries. In Syria, a recent study isolated *Brucella melitensis* from bovine raw milk samples at a rate of 25% (39). Furthermore, in San Paulo, 30% of bovine screened milk samples yielded *Brucella abortus* during a study of four years (40).

MRT primarily detects IgA and IgM antibodies against *Brucella* spp. in raw milk. The sensitivity and specificity of MRT is reported to being 85% and 95%, respectively (17,41). The sensitivity and specificity of MRT reported in this work clearly prove its good value as a straightforward, inexpensive screening test to detect brucellosis in raw milk of cattle and buffaloes. However, a higher sensitivity of 100% and lower specificity of 75-73.5% have been reported for the MRT testing of cow and buffalo milk samples (42). It should be noted that the slight drawback of MRT specificity in comparison to molecular diagnostic techniques is compensated by the fact that the MRT is cheap and easy to perform. Meanwhile, ELISA and PCR approaches are expensive and unavailable in many developing countries. Albeit, a recent Syrian study has found that PCR and culture approach yielded the same results, while the MRT showed lower rates of positive results (39).

The temporal distribution of seropositive raw milk samples of camel shows a poor correlation between months and the prevalence of brucellosis. The seasonality of brucellosis in camel is still largely unknown. The wet season has been reported to be a risk factor for the infection (43,44). It is believed that *Brucella* spp. do not survive in the dry and warm weather (43). It is worthwhile to say that camels appear to be infected by spill-over of *Brucella* from other ruminants and cattle (5,43). In such cases, camels in cohabitation with other mammals are exposed continuously to *Brucella* regardless of the season and may show such irregular seasonality. Nonetheless, the final genotypic solid evidence supporting this observation is still missing.

Conclusion

The rate of brucellosis in camel milk at Erbil Governorate is alarming to the risk for humans. MRT can be used for efficient and everyday monitoring due to its simple, rapid, sensitive, and cheap technique for routine screening of brucellosis in raw milk. The epidemiology and seasonal variations in brucellosis rates in camel at Erbil are not completely clear. Further research addressing this subject are greatly recommended.

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Conflict of interest

There is no conflict of interest.

References

1. Scholz HC, Banai M, Cloeckaert A, Kämpfer P, Whatmore AM. *Brucella*. In: Rujillo ME, Dedysh S, DeVos P, Hedlund B, Kämpfer P, Rainey FA, Whitman WB, editors. Bergey's manual of systematics of archaea and bacteria. London: John Wiley & Sons Ltd; 2018.
2. Noomi BS, Ahmed SS, Khalaf HY, Jafar NA. Immune response strategies of *Brucella melitensis* and their antigens in rats. Iraqi J Vet Sci. 2022;36:27-30. DOI: 10.33899/ijvs.2022.134990.2431
3. Musa MT, Eisa MM, El Sanousi EM, Abdel Wahab MB, Perrett L. Brucellosis in camels (*Camelus dromedarius*) in Darfur, western Sudan. J Comp Pathol. 2008;138:151-5. DOI: 10.1016/j.jcpa.2007.10.005
4. Gutema F, Tesfaye J. Review on camel brucellosis: Public health importance and status in Ethiopia. Acad Res J Agric Sci Res. 2019;7:513-29. [available at]
5. Abbas B, Agab H. A review of camel brucellosis. Prev Vet Med. 2002;55:47-56. DOI: 10.1016/S0167-5877(02)00055-7
6. Abedi AS, Hashempour-Baltork F, Alizadeh AM, Beikzadeh S, Hosseini H, Bashiry M, Taslikh M, Javanmardi F, Sheidaee Z, Sarlak Z, Mofid V, Fakhri Y, Mousavi Khaneghah A. The prevalence of *Brucella spp*. in dairy products in the Middle East region: A systematic review and meta-analysis. Acta Trop. 2020;105241. DOI: 10.1016/j.actatropica.2019.105241
7. Adetunji SA, Ramirez G, Ficht AR, Perez L, Foster MJ, Arenas-Gamboa AM. Building the evidence base for the prevention of raw milk-acquired brucellosis: A systematic review. Front Public Health. 2020;8:76. DOI: 10.3389/fpubh.2020.00076
8. Enkelmann J, Stark K, Faber M. Epidemiological trends of notified human brucellosis in Germany, 2006-2018. Int J Infect Dis. 2020;93:353-8. DOI: 10.1016/j.ijid.2020.02.019
9. Tuon FF, Gondolfo RB, Cerchiari N. Human-to-human transmission of Brucella - a systematic review. Trop Med Int Health. 2017;22:539-46. DOI: 10.1111/tmi.12856
10. Liu X, Yang M, Song S, Liu G, Zhao S, Liu G, Hornok S, Wang Y, Jiang H. *Brucella melitensis* in Asian badgers, northwestern China. Emerg Infect Dis. 2020;26:804-6. DOI: 10.3201/eid2604.190833
11. Wiethoelter AK, Mor SM, Massey PD, Robson J, Wilks K, Hutchinson P. Pigs, pooches and pasteurisation: The changing face of brucellosis in Australia. Aust J Gen Pract. 2018;47:99-103. DOI: 10.31128/AFP-08-17-4289
12. Poester FP, Nielsen K, Ernesto Samartino L, Ling Yu W. Diagnosis of brucellosis. Open Vet Sci J. 2014;4:46-60. DOI: 10.2174/1874318801004010046
13. Hassan WS, Hassan SD, Abdulrazzaq KM, Al-Obaidi QT. Serological detection of the latent infection of brucellosis in calves in Mosul city, Iraq. Iraqi J Vet Sci. 2022;36:7-10. DOI: 10.33899/ijvs.2022.134936.2421
14. Godfroid J, Nielsen K, Saegerman C. Diagnosis of brucellosis in livestock and wildlife. Croat Med J. 2010;51:296-305. DOI: 10.3325/cmj.2010.51.296
15. Almashhadany DA. Prevalence of brucellosis in human and camels in Thamar province/Yemen. J Saudi Soc Agric Sci. 2014;13:132-7. [available at]
16. Smirnova E, Vasin A, Sandybayev N, Klotchenko S, Plotnikova M, Chervyakova O, Sansyzbay A, Kiselev O. Current methods of human and animal brucellosis diagnostics. Adv Infect Dis. 2013;3:177-84. DOI: 10.4236/aid.2013.33026
17. Almashhadany DA. The significance of milk ring test for identifying Brucella antibodies in cows and buffaloes' raw milk at Erbil governorate, Kurdistan region, Iraq. Iraqi J Vet Sci. 2019;33:395-400. DOI: 10.33899/ijvs.2019.163085
18. Al-mashhadany DA. The utility of MRT to screen brucellosis among ewe and nanny goats milk in Erbil governorate/Kurdistan region/Iraq. Int J Biol Pharm Allied Sci. 2018;7:1786-802. DOI: 10.31032/ijbpas/2018/7.9.4551
19. Almashhadany DA. Serological and bacteriological evaluation of brucellosis in milk of small ruminants. Food Res. 2021;5:216-22. DOI: 10.26656/fr.2017.5(4).700
20. Almashhadany DA. Diagnosis of brucellosis in sheep and goats raw milk by fast and reliable techniques. Iraqi J Vet Sci. 2021;35:663-8. DOI: 10.33899/ijvs.2021.127697.1523
21. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's diagnostic microbiology. 12th ed. Philadelphia: Elsevier Health Sciences; 2007. 922 p.
22. Al-mashhadany DA. The role of milk ring test in monitoring brucellosis among cow milk in Erbil governorate/Kurdistan region/Iraq. Int J Biol Pharm Allied Sci. 2018;5:802-19. DOI: 10.31032/ijbpas/2018/7.5.4439
23. Almashhadany DA, Osman AA. Isolation, serotyping, and antibiogram of Salmonella isolates from raw milk sold at retail vending in Erbil city, Iraq. Bull Univ Agric Sci Vet Med Cluj-Napoca Anim Sci Biotechnol. 2019;76:116-22. DOI: 10.15835/buasvmcn-asb:0020.19
24. Funk N, Tabatabai L, Elzer P, Hagius S, Martin B, Hoffman L. Indirect enzyme-linked immunosorbent assay for detection of *Brucella melitensis*-specific antibodies in goat milk. J Clin Microbiol. 2005;43:721-5. DOI: 10.1128/jcm.43.2.721-725.2005
25. Corbel M, Food and Agriculture Organization of the United Nations, World Health Organization & World Organization for Animal Health. Brucellosis in humans and animals. Geneva: World Health Organization; 2006.
26. El-Sayed A, Awad W. Brucellosis: Evolution and expected comeback. Int J Vet Sci Med. 2018;6:S31-5. DOI: 10.1016/j.ijvsm.2018.01.008
27. Seleem MN, Boyle SM, Sriranganathan N. Brucellosis: A re-emerging zoonosis. Vet Microbiol. 2010;140:392-8. DOI: 10.1016/j.vetmic.2009.06.021
28. Shoukat S, Wani H, Ali U, Parveez A, Ara S, Ganguly S. Brucellosis: A current review update on zoonosis. J Immunol Immunopathol. 2017;19:61-9. DOI: 10.5958/0973-9149.2017.00009.0
29. Sharief D, Al-Adhad B, Mahmood T, Al-Kubaisi A, Saleem H, Saeed E, Mahdi A. T2-P47 - Survey of the seroprevalence of brucellosis in ruminants in Iraq. Proceedings of the 11th International Symposium on Veterinary Epidemiology and Economics; 2006. 1 p. [available at]
30. Al-Majali AM, Al-Qudah KM, Al-Tarazi YH, Al-Rawashdeh OF. Risk factors associated with camel brucellosis in Jordan. Trop Anim Heal Prod. 2007;40(3):193-200. DOI: 10.1007/S11250-007-9080-7
31. Bekele WA, Tessema TS, Melaku SK. *Camelus dromedarius* brucellosis and its public health associated risks in the Afar national regional state in northeastern Ethiopia. Acta Vet Scand. 2013;55:89. DOI: 10.1186/1751-0147-55-89
32. Khamesipour F, Rahimi E, Shakerian A, Doosti A, Momtaz H. Molecular study of the prevalence of *Brucella abortus* and *Brucella melitensis* in the blood and lymph node samples of slaughtered camels by polymerase chain reaction (PCR) in Iran. Acta Vet Brno. 2014;64:245-56. DOI: 10.2478/acve-2014-0023
33. Ahmed MS, Mohamed ZA, Mohamed MD, Mohamed AB, Sherif MS, Emad EY. Seroprevalence of camel brucellosis (*Camelus dromedarius*) and phenotypic characteristics of *Brucella melitensis* biovar 3 in Shalateen city, Red sea governorate, Egypt. Afr J Microbiol Res. 2017;11:1259-66. DOI: 10.5897/ajmr2017.8629
34. Al-Griw HH, Kraim ES, Farhat ME, Perrett LL, Whatmore AM. Evidence of ongoing brucellosis in livestock animals in north west Libya. J Epidemiol Glob Health. 2017;7:285-8. DOI: 10.1016/j.jegh.2017.09.001
35. Bayasgalan C, Chultemdorj T, Roth F, Zinsstag J, Hattendorf J, Badmaa B, Argamjav B, Schelling E. Risk factors of brucellosis seropositivity in Bactrian camels of Mongolia. BMC Vet Res. 2018;14:342. DOI: 10.1186/s12917-018-1664-0
36. Alrawahi AH, Robertson I, Hussain MH, Saqib M. A cross-sectional seroepidemiological study of camel (*Camelus dromedarius*) brucellosis and associated risk factors in the sultanate of Oman. Open Vet J. 2019;9:133-9. DOI: 10.4314/ovj.v9i2.7

37. Al-mashhadany DA. Prevalence of bovine brucellosis in Thamar province/Yemen. Yemeni J Agric Res Stud. 2009;20:17-26.
38. Ocholi RA, Kwaga JP, Ajogi I, Bale JO. Phenotypic characterization of Brucella strains isolated from livestock in Nigeria. Vet Microbiol. 2004;103:47-53. DOI: 10.1016/j.vetmic.2004.06.012
39. Al-Mariri A. Isolation of *Brucella melitensis* strains from Syrian bovine milk samples. Bulg J Vet Med. 2015;18:40-8. DOI: 10.15547/bjvm.842
40. Langoni H, Ichihara SM, Silva AV, Pardo RB, Tonin FB, Mendonça LP, Machado JD. Isolation of *Brucella spp*. from milk of brucellosis positive cows in São Paulo and Minas Gerais states. Braz J Vet Res Anim Sci. 2000;37:0-0. DOI: 10.1590/s1413-95962000000600004
41. Al-Shemmari IM. Comparative study between conventional and molecular tests to detect the incidence of brucellosis in cattle and buffaloes in Babylon and Karbala provinces. Sci J Med Res. 2018;2:7-13. DOI: 10.37623/sjmr.2018.2502
42. Ibrahim AK, AbdelAll AA, Amin AS. Long-term diagnostic studies for detection of *Brucella spp*. in milk samples. Glob Vet. 2012;8:54-61. [available at]
43. Fatima S, Khan I, Nasir A, Younus M, Saqib M, Melzer F, Neubauer H, El-Adawy H. Serological, molecular detection and potential risk factors associated with camel brucellosis in Pakistan. Trop Anim Health Prod. 2016;48:1711-8. DOI: 10.1007/s11250-016-1148-9
44. Megersa B, Biffa D, Abunna F, Regassa A, Godfroid J, Skjerve E. Seroepidemiological study of livestock brucellosis in a pastoral region. Epidemiol Infect. 2012;140:887-96. DOI: 10.1017/S0950268811001178

تقنيات موثوقة ودقيقة للكشف عن الأجسام المضادة لبكتيريا البروسيلا في حليب الجمال

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الخلاصة

يُعد داء البروسيلا واحداً من اهم الأمراض البكتيرية المنقولة من حيوانات المزارع إلى الإنسان. وتهدف هذه الدراسة الى تقدير الانتشار الحالي لمرض البروسيلا بين الجمال في محافظة إربيل وتقييم اختبار الحلقة للحليب كفحص تشخيصي للكشف عن داء البروسيلا في الجمال. كما يتَطَرق البحث إلى الانتشار الموسمي للبروسيلا خلال فترة الدراسة. جمعت ٢٥٠ عينة حليب خام عشوائياً، منها ١٣٠ عينة من جمال المزارع، بينما ١٢٠ عينة من نقاط بيع حليب مختلفة خلال الفترة الزمنية ما بين كانون الثاني وحزيران من عام ٢٠٢٢. شُخص المرض باختبار الحلقة للحليب وبالمقايسة الامتصاصيَّة المناعيَّة للإنزيمات المرتبطة وكذلك بالعزل المباشر للبكتيريا من عينات الحليب. تظهر النتائج وجود الأجسام المضادة في ١١,٦ و ١٠,٤% من العينات اعتمادا على اختبار الحلقة والمقايسة الامتصاصيَّة المناعيَّة للإنزيمات المرتبطة، على التوالي، بينما عند عزل البكتيريا من ٨,٤% من العينات، كانت نسبة العزل من عينات الحليب المأخوذة من نقاط البيع ١٠,٠%، أي، اكثر من تلك المجموعة من المزارع ٦,٩%. أما بالنسبة للأنواع البكتيرية، شكل النوع بروسيلا ابورتوس نسبة ٤,٦ و ٥,٨% من عينات المزارع وعينات نقاط البيع على التوالي، بينما شكل النوع بروسيلا مليتينسيس نسبة ٢,٣ و ٤,٢% من عينات المزارع وعينات نقاط البيع، على التوالي. وفيما يخص الانتشار الموسمي، فإن اعلى نسبة سُجِلَت في شهر شباط ١٨,٦%، بينما اقل نسبة كانت في أيار ٧,٥%. وبناءً على هذه النتائج، فإنه يمكن الاعتماد على اختبار الحلقة للحليب في الكشف السريع عن داء البروسيلا في المزارع والمصانع ونقاط البيع. كما ننصح المستهلكين بتسخين الحليب بشكل كافي قبل تناوله أو استخدامه في تصنيع منتجات الحليب وذلك لتفادي الإصابة بالبروسيلا.