Short Communication

Prevalence of Brucellosis among Camels in District Muzaffargarh, Pakistan

Sami Ullah[1*], Tariq Jamil[2], Muhammad Hassan Mushtaq[1], Muhammad Hassan Saleem[3]

[1]Department of Epidemiology and Public Health; [2]University Diagnostic Laboratory; [3]Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, Outfall Road (54600), Lahore, Pakistan.

Abstract | Brucellosis is an important zoonotic disease of animals and humans. Camel has often been neglected for brucellosis study in Pakistan due to many reasons. A study was conducted to investigate prevalence among camels in district Muzaffargarh. A total of 387 serum samples (300 females; 87 males) were collected and subjected to Rose Bengal plate test (RBPT) and polymerase chain reaction (PCR). Out of 387 samples, 2 (0.51%) were found seropositive by RBPT however, none of them was found positive by PCR. All the seropositive samples came from adult female camels (≥6 years of age) only. Common watering points (e.g. ponds) showed significant association ($P<0.05$), however, age and sex had non-significant association ($P>0.05$). The study concluded seroconversion and potential risk factor involved in the spread of brucellosis in camel herds. Routine screening together with public awareness is required to control brucellosis in the area.

Keywords | Seroprevalence, PCR, Brucellosis, Camels, Muzaffargarh

Brucellosis is an important zoonotic disease of domestic and wild animals including humans (Hamdy and Amin, 2002; Radostits et al., 2007). Causative agents of Brucellosis are gram negative bacteria, belonging to genus *Brucella*, five out of nine known species have potential zoonotic significance (Godfroid et al., 2005; Seleem et al., 2010). Camel is known to be susceptible for both *B. abortus* and *B. malitensis*, but it is not considered as a primary host (Gwida et al., 2011; Wernery and Kaaden, 2002). Epidemiological reviews on camel brucellosis have shown a cosmopolitan prevalence (Abbas and Agab, 2002; Gwida et al., 2012; Seleem et al., 2010; Sprague et al., 2012). Camels get subsequent infection from other animals sharing their habitat and husbandry practices (Abbas et al., 1992). Clinical manifestation of this disease is in the form of reproductive loss leading to abortion, still birth or infertility (Olsen and Tatum, 2010). Camel plays important socio-economic role among nomadic pastorals of arid and semi-arid lands in the country (Ahmad et al., 2010). The pastoral households are at great public risk due to their close physical contact and assistance in calf delivery, cleaning new-borns, suckling and carrying the young calf from field to nomadic house (Abbas and Agab, 2002). Ingestion of unpasteurized milk and its products is a source of brucellosis transmission in humans (Shimol et al., 2012; Warsame et al., 2012). The disease could pose serious socio-economic threats to livestock owners in pastoral and rural community (Megersa et al., 2011). Previous studies have indicated brucellosis as endemic in livestock farms of Pakistan with increasing prevalence rates (Munir et al., 2011).

Despite its demographic and socioeconomic importance, data regarding camel brucellosis remains scanty and restricted to other food animals in the country (Abubakar et al., 2012; Gul and Khan, 2007). For accurate diagnosis serological tests like Rose Bengal plate agglutination test (RBPT) are cheap and easy for herd based screening of animals with high sensitivity and low specificity (Ali et al., 2013; Ghanem et al., 2009; OIE, 2012). Confirmation of seropositive animals by more specific antigen based tests, like indirect-ELISA and PCR are helpful in interpreting
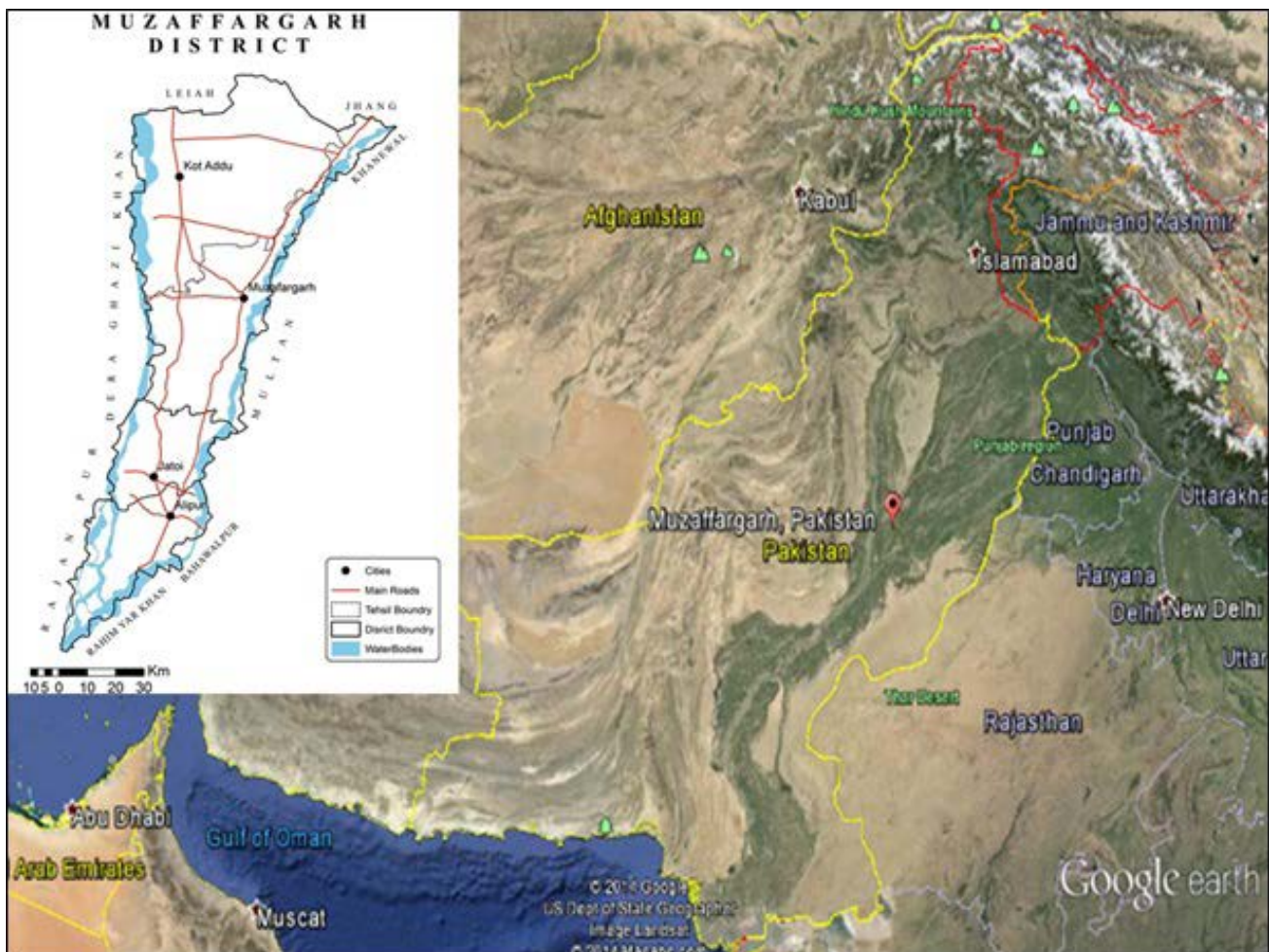
Figure 1: Location map of district Muzaffargarh

diagnosis more accurately (Gall and Nielsen, 2004; Schelling et al., 2003).

Muzaffargarh is located between 30.0703° N-71.1933° E in south-west of Punjab province, at the bank of river Chenab of Pakistan. The district comprises of four administrative divisions i.e. Kot Addu, Muzaffargarh, Jatoi and Alipur. The area is rich in camel and cattle population having arid ecology with 102110 acres of forested area. This district was severely affected by Indus and Chenab river floods in 2010 (Figure 1). The present study estimated the prevalence and associated factors of brucellosis among camels of district Muzaffargarh by Rose Bengal plate agglutination test (RBPT) and polymerase chain reaction (PCR) for seroprevalence and molecular diagnosis respectively. This was the first time to study camel brucellosis through molecular identification in the district.

A total of 387 blood samples were collected randomly from different camel herds (> 6 months of age) among four divisions of district Muzaffargarh along with various study factors. These samples were initially screened by RBPT. Serologically positive samples were then processed for genome extraction and PCR. Sample size was determined by

assuming population unknown, prevalence 50% and confidence interval 95% in the formula (Thrusfield, 2007).

Serum drawn from blood samples were initially screened for RBPT as described previously (Alton et al., 1975). Standardized antigens were collected from Biologicals Supply Section, Veterinary Research Institute, Zarar Shaheed Road, Lahore (54810).

Table 1: Primers used for PCR targeting *BCSP31* gene

Type of Primer	Primer sequence	Product size
Forward Primer	5-TGGCTCGGTTGC-CAATATCAA-3'	
Reverse Primer	5-CGCGCTTGCCT-TTCAGGTCTG-3'	223 bp

Brucella genome was extracted and purified by using QIAmp DNA Mini Kit (Qiagen, Germany) according to manufacturer's instructions. Genome quantity and quality was measured by NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Scientific, USA). The extracted genome was amplified for polymerase chain reaction by using *brucella* genus specific 223 primers targeting outer membrane protein encoding gene (bcsp 31). Polymerase chain reaction

Table 2: Association between seroprevalence of brucellosis in camel herds and studied parameters using Chi-square analysis

Parameters		Seropositive	Seronegative	P-value
Age	≤ 6 years	00	87	0.445
	>6 years	2	298	
Sex	Male	00	133	0.305
	Female	2	252	
Herd composition	Only camels	2	355	0.681
	Mixed herd (other animals)	00	30	
Water Source	Rivers	00	87	0.046
	Ponds	2	93	
	Traditional wells	00	205	

technique was used for molecular identification of brucellosis in the samples. PCR was performed in 25 µL reaction mixture as described previously (Gemechu et al., 2011) having concentrations of 20 pmol of primers (Table 1), 0.2 mM dNTPs (10mM), 1× PCR buffer, 1.5 mM MgCl$_2$, 0.5 U Taq DNA polymerase, and 10 µl of template DNA. Cycle conditions consisted of initial denaturation for 5 min at 93°C, template denaturation at 90°C for 1 min, 30 sec of primer annealing at 58°C and final extension at 72°C for 7 min 40 cycles.

PCR products were visualized on 1.5% agarose gel stained with ethidium bromide (10 mg/ml) under UV-light using gel documentation system (Bio-Rad, USA).

Pearson's Chi-square analysis was used to determine association of the factors with the positivity of the infection with 95% confidence interval.

Out of 387 camel blood samples, 2 (0.51%) camels were found positive by RBPT. Followed by PCR and Gel Electrophoresis, the samples were found negative. Out of 387 camel serum samples, 300 (77.51%) samples came from adult (≥6 years) animals whereas, remaining 87 (22.49%) samples came from younger (<6 years) animals. The two seropositive samples had come from adult animals whereas younger animals were found all negative by RBPT. Based upon sex of animals, 133 (34.4%) of the total samples came from male camels whereas 254 (65.63%) came from female camels. The two seropositive samples were found both form female camels (0.78% prevalence rate among female camels). Among risk factors studied, watering points showed a significant association for brucellosis transmission (*P*<0.05) (Table 2).

A total of 387 camel blood samples were processed by RBPT by which two samples were found positive showing overall 0.51% seroprevalence. Previous studies in Pakistan have reported 0.0-17.2% seroprevalence among camels (Ahmad and Munir, 1995; Ajmal et al., 1989; Gul and Khan, 2007; Gul et al., 2014; Nasrin et al., 1998). Higher

seroprevalence of brucellosis among livestock of intensive/semi-intensive system may be indicative of transmission through close contact (Abbas and Agab, 2002). Historical isolation and nomadic raising system may have prevented camel herds from brucellosis in the area. Although brucellosis is less prevalent in camels than cattle and buffaloes, consuming raw camel milk may threat human health for infection transmission. Other studies have revealed a seroprevalence ranging between 0.3-3.9% in different areas of Africa (Ghanem et al., 2009; Megersa, 2004; Omer et al., 2000). Herd composition, especially mixing of small ruminants (sheep and goats) is known for prevalence of brucellosis in camels (Abbas and Agab, 2002; Omer et al., 2000). The camel herds, in the present study, were not mixed with other livestock species neither had prior vaccination history. However, lack of vaccination against brucellosis may be reason for the existing infection among herds. Vaccination can be suggested for minimizing the infection level among camel herds, however the control remains questionable (Dawood, 2008; Treanor et al., 2010).

Molecular identification of seropositive samples by PCR revealed 0 (0%) detection for brucellosis. Higher seroprevalence by RBPT reveals its high sensitivity but low specificity. This may be true due to fluctuating antibody titers and/or presence of antibodies in healthy animals where disease is endemic (Gul and Khan, 2007; Gwida et al., 2011). RBPT antigen is also reported to cross-react with *Salmonella urbana, Yersinia enterocolitica* and *E. coli* that further questions its specificity (Abubakar et al., 2012; Gall and Nielsen, 2004). PCR is more reliable than serological tests but its efficacy remains highly variable (Khamesipour et al., 2014; Hassanain et al., 2012).

Seroprevalence of brucellosis was found higher in females 2/254 (0.87%) than males 0/133 (0%). Similar results were found in a study at Karachi where females and males were found 1.8% and 0% seroprevalent respectively (Siddiqui, 2009). Our results were also supported by (Abbas and Agab, 2002; Bekele, 2004; Omer et al., 2010; Teshome et al., 2003; Warsame et al., 2012), however contradicted by

(Gul et al., 2014; Tassew and Kassahun, 2014) who found slightly higher prevalence rate in male camels. This higher prevalence have been attributed to pregnancy, lactation, physiological differences of females than males and the presence of erythritol (Gyles et al., 2008; Radostits et al., 2007; Warsame et al., 2012). However, the association of sex with the seroprevalence of brucellosis was found insignificant (*P*>0.05).

Regarding age, adult animals were found more seroprevalent i.e. 0.7% (≥ 6 years) as compared to 0% (≤ 6years). Adult camels have also shown higher seroprevalence rates elsewhere (Abbas and Agab, 2002; Omer et al., 2010). Age, in our study, didn't show significant association with the seroprevalence of brucellosis (*P*>0.05) as supported by (Amin et al., 2005; Ghanem et al., 2009; Teshome et al., 2003; Tilahun et al., 2013).

Watering sources, in the study area, were composed of some fixed points like ponds and traditional wells shared by humans and all kind of livestock prevailing in that area. These watering sources showed statistically significant (*P*<0.05) association with the seropositivity of the infection. This seropositivity cloud have been associated in a variety of ways e.g. (i) drinking of contaminated water by discharges of infected animals (ii) physical contact with sub-clinical infected animals while taking rest at these common places.

The study can conclude that, among camels, both sexes are susceptible for brucellosis irrespective of age. Herds mixed with other animals and sharing common watering points may show higher prevalence rates than isolated and independent water source herds.

Public awareness is needed as preventive regimen especially in nomads. Routine screening and culling of seropositive animals is recommended among herds at least on annual basis. Vaccination of animals can be recommended in disease endemic areas, provided that its cost-effectiveness and profitability studied earlier (Roth et al., 2003). For human prevention, public awareness is recommended not to consume raw/unpasteurized milk. In the study it was found that 94% of the herd owners consumed raw milk, 4% boiled and 2% sour milk. Further studies may include population based prevalence study, antigen based identification of the etiology through ELISA/PCR and calculation of Odd's ratio for associated rick factors among camel herds of Punjab.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST

The authors declare no conflict of interest to disclose.

REFERENCES

• Abbas B, Agab H (2002). A review of camel brucellosis. Prev. Vet. Med. 55(1): 47-56. http://dx.doi.org/10.1016/S0167-5877(02)00055-7

• Abbas B, Chabeuf N, Saint-Martin G, Bonnet P, Maillaird A, Beshir H, Musa B (1992). Camel pastoralism in the Butana and north eastern Sudan: an interdisciplinary study. Nomad. Peopl. 31: 64-84. http://www.jstor.org/stable/43123374

• Abubakar M, Mansoor M, Arshed MJ (2012). Bovine brucellosis: old and new concepts with Pakistan perspective. Pak. Vet. J. 32: 147-155.

• Ahmad R, Munir M (1995). Epidemiological investigation of brucellosis in Pakistan. Pak. Vet. J. 15: 169-172.

• Ahmad S, Yaqoob M, Hashmi N, Ahmad S, Zaman M, Tariq M (2010). Economic importance of camel: unique alternative under crisis. Pak. Vet. J. 30(4): 191-197.

• Ajmal M, Ahmed MD, Arshad M (1989). Serosurveillance of brucellosis. Pak. Vet. J. 9: 115-117.

• Ali S, Ali Q, Nji Iabatih E, Ullah N, Muhammad A, Khan I, Akhter S (2013). Sero-prevalence of *brucella* abortus among dairy cattle and buffaloes in Pothohar Plateau, Pakistan. Pak. J. Zool. 45(4): 1041-1046.

• Alton GG, Jones LM, Pietz DE (1975). Laboratory techniques in brucellosis. Monogr. Ser. World Health Organ. (55): 1-163.

• Amin K, Rahman MB, Rahman MS, Han JC, Park JH, Chae JS (2005). Prevalence of *brucella* antibodies in sera of cows in Bangladesh. J. Vet. Sci. 6(3): 223-226.

• Bekele M (2004). Sero-epidemiological study of brucellosis in camels (*Camelus dromedarius*) in Borena lowland pastoral areas, Southern Ethiopia, M.Sc. Thesis, Adis Ababa, Ethiopia.

• Dawood HA (2008). Brucellosis in camels (*Camelus dromedorius*) in the south province of Jordan. Am. J. Agri. Biol. Sci. 3(3): 623-626. http://dx.doi.org/10.3844/ajabssp.2008.623.626

• Gall D, Nielsen K (2004). Serological diagnosis of bovine brucellosis: a review of test performance and cost comparison. Rev. Sci. Tech. 23(3): 989-1002.

• Gemechu MY, Gill JPS, Arora AK, Ghatak S, Singh DK (2011). Polymerase chain reaction (PCR) assay for rapid diagnosis and its role in prevention of human brucellosis in Punjab, India. Int. J. Prev. Med. 2(3): 170.

• Ghanem YM, El-Khodery A, Saad AA, Abdelkader AH, Heybe A, Musse YA (2009). Seroprevalence of camel brucellosis (*Camelus dromedarius*) in Somaliland. Trop. Anim. Health Prod`. 41(8): 1779-1786. http://dx.doi.org/10.1007/s11250-009-9377-9

• Godfroid J, Cloeckaert A, Liautard JP, Kohler S, Fretin D, Walravens K, Garin-Bastuji B, Letesson JJ (2005). From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. Vet. Res. 36(3): 313-26. http://dx.doi.org/10.1051/vetres:2005003

• Gul S, Khan A (2007). Epidemiology and epizootology of

brucellosis: a review. Pak. Vet. J. 27(3): 145-151.

- Gul ST, Khan A, Rizvi F, Hussain I (2014). Sero-prevalence of brucellosis in food animals in the Punjab, Pakistan. Pak. Vet. J. 34(4): 454-458.

- Gwida M, El-Gohary A, Melzer F, Khan I, Rosler U, Neubauer H (2012). Brucellosis in camels. Res. Vet. Sci. 92(3): 351-355. http://dx.doi.org/10.1016/j.rvsc.2011.05.002

- Gwida MM, El-Gohary AH, Melzer F, Tomaso H, Rosler U, Wernery U, Wernery R, Elschner MC, Khan I, Eickhoff M, Schoner D, Neubauer H (2011). Comparison of diagnostic tests for the detection of *brucella* spp. in camel sera. BMC Res. Notes. 4: 525. http://dx.doi.org/10.1186/1756-0500-4-525

- Gyles CL, Prescott JF, Songer G, Thoen CO (2008). Pathogenesis of Bacterial Infections in Animals. Wiley.

- Hamdy ME, Amin AS (2002). Detection of *Brucella* species in the milk of infected cattle, sheep, goats and camels by PCR. Vet. J. 163(3): 299-305. http://dx.doi.org/10.1053/tvjl.2001.0681

- Hassanain NA, Ahmed WM (2012). Efficacy of serological tests in comparison with PCR for diagnosis of Brucellosis. World J. Med. Sci. 7(4): 243-247. http://dx.doi.org/10.5829/idosi.wjms.2012.7.4.65190

- Khamesipour F, Rahimi E, Shakerian A, Doosti A, Momtaz H (2014). 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. 64(2): 245-256. http://dx.doi.org/10.2478/acve-2014-0023

- Megersa B (2004). Sero-epidemiological study of brucellosis in camels (Camelus dromedarius) in Borena lowland pastoral areas, Southern Ethiopia. M.Sc. Thesis, Adis Ababa, Ethiopia.

- Megersa B, Biffa D, Abunna F, Regassa A, Godfroid J, Skjerve E (2011). Seroprevalence of brucellosis and its contribution to abortion in cattle, camel, and goat kept under pastoral management in Borana, Ethiopia. Trop. Anim. Health Prod. 43(3): 651-656. http://dx.doi.org/10.1007/s11250-010-9748-2

- Munir R, Farooq U, Fatima Z, Afzal M, Anwar Z, Jahangir M (2011). Sero-prevalence of brucellosis in bovines at farms under different management conditions. Br. J. Dairy Sci. 2: 35-39.

- Nasrin M, Afzal H, Siddique M (1998). Sero-prevalence of *Brucella abortus* and *Brucella melitensis* in camels. J. Anim. Plant Sci. 8: 61-62.

- OIE (2012). Bovine Brucellosis. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Paris, Vol-I: 01-35.

- Olsen S, Tatum F (2010). Bovine brucellosis. Vet. Clin. North Am. Food Anim. Pract. 26(1): 15-27. http://dx.doi.org/10.1016/j.cvfa.2009.10.006

- Omer MK, Skjerve E, Holstad G, Woldehiwet Z, Macmillan AP (2000). Prevalence of antibodies to *Brucella* spp. in cattle, sheep, goats, horses and camels in the State of Eritrea; influence of husbandry systems. Epidemiol. Infect. 125(2): 447-453.

- Omer MM, Musa MT, Bakhiet MR, Perrett L (2010). Brucellosis in camels, cattle and humans: associations and evaluation of serological tests used for diagnosis of the disease in certain nomadic localities in Sudan. Rev. Sci. Tech. 29(3): 663-669.

- Radostits OM, Done SH, Gay CC, Hinchcliff KW, Constable PD (2007). Veterinary medicine: a textbook of the diseases of cattle, sheep, pigs, goats, and horses. Elsevier Science Health Science Division. Pp. 389-390.

- Roth F, Zinsstag J, Orkhon D, Chimed-Ochir G, Hutton G, Cosivi O, Carrin G, Otte J (2003). Human health benefits from livestock vaccination for brucellosis: case study. Bull. World Health Org. 81: 867-876.

- Schelling E, Diguimbaye C, Daoud S, Nicolet J, Boerlin P, Tanner M, Zinsstag J (2003). Brucellosis and Q-fever seroprevalences of nomadic pastoralists and their livestock in Chad. Prev. Vet. Med. 61(4): 279-293. http://dx.doi.org/10.1016/j.prevetmed.2003.08.004

- Seleem MN, Boyle SM, Sriranganathan N (2010). Brucellosis: a re-emerging zoonosis. Vet. Microbiol. 140(3-4): 392-398. http://dx.doi.org/10.1016/j.vetmic.2009.06.021

- Siddiqui A (2009). Incidence of camel brucellosis in Pakistan. (Copyright 2009). https://www.researchgate.net/profile/Muhammad_Siddiqui/publication/233991733_paper_on_brucellosis/links/02bfe50ddaac44951d000000.pdf

- Shimol SB, Dukhan L, Belmaker I, Bardenstein S, Sibirsky D, Barrett C, Greenberg D (2012). Human brucellosis outbreak acquired through camel milk ingestion in southern Israel. Isr. Med. Assoc. J. 14(8): 475-478.

- Sprague LD, Al-Dahouk S, Neubauer H (2012). A review on camel brucellosis: a zoonosis sustained by ignorance and indifference. Path. Global. Health. 106(3): 144-149.

- Tassew H, Kassahun F (2014). Sero-epidemiological study of camel brucellosis in Mehoni District, South Eastern Tigray, Ethiopia.

- Teshome H, Molla B, Tibbo M (2003). A seroprevalence study of camel brucellosis in three camel-rearing regions of Ethiopia. Trop. Anim. Health Prod. 35(5): 381-390. http://dx.doi.org/10.1179/2047773212Y.0000000020

- Thrusfield M (2007). Veterinary Epidemiology. 3rd Edition, Blackwell Sci. Ltd. 232-4.

- Tilahun B, Bekana M, Belihu K, Zewdu E (2013). Camel brucellosis and management practices in Jijiga and Babile districts, Eastern Ethiopia. J. Vet. Med. Anim. Health. 5(3): 81-86. http://dx.doi.org/10.5897/JVMAH2013.0206

- Treanor JJ, Johnson JS, Wallen RL, Cilles S, Crowley PH, Cox JJ, Maehr DS, White P, Plumb GE (2010). Vaccination strategies for managing brucellosis in Yellowstone bison. Vaccine. 28: 64-72. http://dx.doi.org/10.1016/j.vaccine.2010.03.055

- Warsame I, Alemu S, Temesgen W, Molla W (2012). Seroprevalence and Associated Risk Factors of Camel (Camelus dromedaries) Brucellosis in and Around Dire Dawa, Ethiopia. Glob. Vet. 8(5): 480-483.

- Wernery U, Kaaden O (2002). Infectious Diseases of Camelids. Blackwell Wissenschafts Verlag, Berlin. 109-15.