Animal Health Prod and Hyg (2013) 2(2) : 188 - 192

Animal Health, Production and Hygiene

www.aduveterinaryjournal.com

Research Article

Forelimb Lameness in Merino and Akkaraman Sheep Associated with Naturally Occuring *Brucella melitensis* Infection

Kerem Ural[1], Şükrü Kırkan[2], İbrahim Akın[3], Uğur Parın[2], Mehmet Gültekin[1], Gökhan Duran[4], Bülent Ulutaş[1]

[1]Department of Internal Medicine, Faculty of Veterinary, Adnan Menderes University, Aydin-Turkey. [2]Department of Microbiology, Faculty of Veterinary, Adnan Menderes University, Aydin-Turkey. [3]Department of Surgery, Faculty of Veterinary, Adnan Menderes University, Aydin-Turkey. [4] Göksa Veterinary Clinic Ziraat mah., 655 sok., 26-C, Altindag, Ankara-Turkey.

A B S T R A C T

Background/Aim: Brucellosis has been still been recognized as an important infectious disease among ruminants worldwide. Disease reports in recent years indicated an elevation in the number of outbreaks in sheep. The objective of the present study was to describe forelimb lameness observed in a mixed Merino and Akkaraman sheep flock associated with *Brucella melitensis* infection. **Material and Method**: A descriptive case control study was conducted on 107 sheep (in a sheep flock consisting 450 mixed Merino and Akkaraman) during the period of April-September 2012. The brucellosis status of the present flock was established through serological testing of sera samples by use of Rose Bengal Plate (RBPT), serum agglutination (SAT) tests and polymerase chain reaction (PCR) applied in series. **Results and Conclusion:** Out of those 107 sera, RBPT revelaed 93 (86%) positivity, and out of the latter animals SAT recorded 60 (65%) positive samples with titers equal or above 1/40, whereas PCR recorded 83 (89%) positivity. Seventeen out of 107 cases tested showed forelimb lameness, interestingly all those animals were detected to have forelimb lameness with SAT titers 1/40 and > and PCR positivity. Clinical, epizootiological and microbiological aspects of Brucella infection were described in the present article.

Keywords: forelimb, lameness, Merino, Akkaraman, sheep, Brucella melitensis

Merinos ve Akkaraman Koyunlarında Doğal *Brucella melitensis* Enfeksiyonuna Bağlı Ön Bacak Topallığı

Ö Z E T

Özbilgi/Amaç: Brusellozis, halen geviş getirenlerin dünya çapında önemli bir enfeksiyöz hastalığı olarak kabul edilmektedir. Son yıllarda hastalığa ilişkin raporlar koyun salgınlarının sayısında bir artışı göstermektedir. Bu çalışmada karışık Merinos ve Akkaraman koyun sürüsünde *Brucella melitensis* enfeksiyonuna bağlı şekillenen ön bacak topallığının tanımlanması amaçlandı. **Materyal ve Metot**: Nisan-Eylül 2012 ayları arasında 450 başlık karışık Merinos ve Akkaraman koyun sürüsünde yapılan tanımlayıcı vaka kontrol çalışması 107 koyun üzerinde gerçekleştirildi. Sürüde brusellozis durumunun belirlenmesi amacıyla serum örneklerinde sırasıyla Rose Bengal Plate (RBPT), serum aglütinasyon (SAT) testleri ve polimeraz zincir reaksiyonu (PCR) uygulandı. **Bulgular ve Sonuç:** Yüzyedi serumdan 93'ü (%86) RBPT pozitif tespit edildi ve bu hayvanlardan yapılan testlerde, titresi 1/40 ve üzerinde olan 60 (%65) örnek SAT pozitif bulunurken, 83(%89) örnek ise PCR pozitif olarak kaydedildi. 107 koyundan 17'sinde ön bacak topallığı gözlendi ve ilginç olarak bu hayvanların tümünün 1/40 ve üzeri SAT titrelerine sahip olduğu ve PCR pozitif oldukları tespit edildi. Bu makalede Brucella enfeksiyonu klinik, epizootiyolojik ve mikrobiyolojik yönden tanımlandı.

Anahtar kelimeler: ön bacak, topallık, Merinos, Akkaraman, koyun, Brucella melitensis

---

Correspondence to: Kerem Ural, Department of Internal Medicine, Faculty of Veterinary, Adnan Menderes University, Aydın, Turkey. E-mail: uralkerem@gmail.com

Introduction

Brucellosis has still been recognized as one of the most important and widespread zoonoses in all over the world according to Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the Office International des Epizooties (OIE) data. Infection therefore disease conditions are caused by several bacteria involvig the genus Brucella, that has the ability to infect a specific animal species (Alton, 1990, Blood and Radostits, 1990).

Given the zoonotic feature and importance of this bacterial disease of domestic animals and humans, it causes economic losses, and potentially threatens human health. *Brucella sp.* may be transmitted to humanbeing from infected animals, especially within consumption of contaminated milk or milk products, besides more or less contact with their carcasses (Renukaradhya et al., 2002). Regarding *Brucella* genus, six species of are recognized currently, *B. abortus* and *B.melitensis* are responsible for diseases characterized by placentitis, abortion and infertility in cattle and sheep, respectively (Alton, 1990). Human infections are often due to *B. melitensis,* with only a few instances of *B. abortus* infection (Manthur et al., 1994). Human brucellosis, speicifically resulting from *B. melitensis* infection is an important public health problem in rural areas (Wallach et al., 1998).

Brucella melitensis is endemic in the Mediterranean region, but infection is widespread world-wide. North America (except Mexico) is believed to be free from the agent, as are Northern and Central Europe, South-East Asia, Australia and New Zealand (OIE 2009). *B. melitensis* is the major cause of abortion in sheep and goats in Turkey (Öngör et al., 2001; Güler et al., 2003). Therefore, it is difficult to diagnose *Brucella* species, classical detection methods such as serological, cultural and biochemical tests (Bricker, 2002). However, these methods are not completely satisfactoring. Bacteriological isolation is a time consuming procedure, besides handling the microorganism is hazardous. Serological methods are not conclusive, because not all infected animals produce significant levels of antibodies and because cross-reaction with other bacteria can give false negative results (Gupta et al., 2006). Recently, detection studies based on PCR (Polymerase Chain Reaction) have become widespread (Gupta et al., 2006). PCR is mostly preferred due to its sensitivity and rapidity (Baily, 1997; Gupta et al., 2006).

Brucellosis in humanbeing is a systemic infectious disease with a variety of clinical signs, in that osteoarthricular involvement results in a single swollen and painful joint (Zaks et al., 1995; Bosilkovski et al., 2004; Pourbagher et al., 2006; Wernaers and Handelberg, 2007). Arthritis associated with *B. melitensis* has well been described in the literature and is seen occasionally in both ram and ewes, indeed most of the non-pregnant sheep may remain asymptomatic (DelVecchio et al., 2002; Lopes et al., 2010). Lameness due to *B. melitensis* has also been reported in swine (Glynn and Lynn, 2008).

Lameness related with Brucellosis has rarely been reported in the veterinary litearture, in which vaccination against *Brucella ovis* resulted in arthricular involvement in 2 case series (Kater and Hartley, 1963; West et al., 1978). In the present article the aim was to define forelimb lameness observed in a mixed Merino and Akkaraman sheep flock associated with naturally occuring *Brucella ovis* infection.

Material and Methods

Interpretation of study design

The present study was based on data obtained from a commercial sheep flock reared under semi-extensive system of management in Denizli province of Turkey from April to September 2012. The flock was composed of animals collected from a private enterprise located in Ankara previously. One of the present authors (G. D.) randomly performed a visit to the flock prior to disposal in the latter city, coinciding with the onset of the clinical signs. The focus of the present survey was on sheep residing in randomly selected sheltering units, following their handover. Currently there is a relatively strong market for sheep culled for production issues in Ankara. This complex condition depends on the number of sheep slaughtered. In the present survey the owner handover the diseased sheep for culling purpose.

The investigation involved interaction with sheep owner, history taking, physical examination (by blinded authors K.U., M.G., G.D and B.U.) for signs and causes of lameness. The body and limb conformations were observed by another blinded author (I.A.) during the sheep was at resting and locomotion, as reported previously (Bokko and Chaudhari, 2001). Among the flock sheep presenting signs of lameness were approached very gently, properly restrained and were forwarded to necessary physical locomotor examination.

The limb conditions that tended to exhibit lameness among sheep such as interdigital pouch inflammation, owergrown hooves, traumatic injury, comformational deformity, fracture that may manifest as lameness were exluded among sheep examined.

Sampling

A total of 107 blood samples susceptible for Brucellosis were withdrawn out of 450 sheep. The collected samples were forwarded immediately to the microbiology laboratory in cold chain. All blood samples were treated with Rose Bengal Plate (RBPT), serum agglutination (SAT) tests and polymerase chain reaction (PCR) analysis by two of the authors (S.K., U.P.) (Koneman, 2006).

Serological Tests

Blood samples were collected from affected animals (more than 1 year old) through venipuncture, by use of a single use vacutaineer system. Following withdrawal of samples were transported to the laboratory. All blood samples were enrolled in to two separate portions. One group was used for PCR examinations and the rest for serological tests. Regarding serological tests, all blood samples were separated into sera with centrifugation. Serological testing of the serum samples (n=107 samples) was accomplished by use of RBPT and SAT. RBPT positive samples were further investigated to SAT for the determination of sera antibody titer.

PCR methodology

Finally serologically positive and doubtful samples were examined within PCR for the detection of Brucella DNA.

Reference strain

B. melitensis Rev. 1 vaccine and *B. abortus* S19 vaccines were used as positive control.

Oligonucleotide primers

B. abortus and *B. melitensis* primer sequences were used as previously described by Bricker and Halling (1994).

DNA extraction from blood samples

A volume of 200 µl blood samples was extracted by DNA extraction kit (Fermentas®) as recommended by manufacturer.

PCR assay

In the present study, previously reported oligonucleotide primers specific for IS711 (Güler et al., 2003), *B. melitensis* and *B. abortus* were used. Assay was performed in a final volume of 25 µl mixture containing PCR buffer [60 mM Tris-HCl (pH 9.0), 15 mM $(NH_4)_2SO_4$, 1.5 mM MgCl] (MBI Fermentas), 250 µm (each) of the four deoxynucleotide triphosphate (MBI Fermentas), a primer cocktail consisting of three primers (1 µm IS711-specific primer, 0.1 µm each of the *B. melitensis* and *B. abortus*-specific primer), 1.25 U Taq polymerase and 2.5 µl template DNA. Amplification reactions were performed in Mastercycler Personal Thermal Cycler (Eppendorf AG, Germany) within the following steps: initial denaturation at 95°C for 3.0 minutes, cycling at 95°C for 2.0 minutes for denaturation, at 55.5°C for 2.0 minutes for annealing, at 72°C for 2.0 minutes for 35 cycles with the a final extension at 72°C for 4 minutes (Leal-Klevezas et al., 1995) .

Following completion of PCR, the products were analyzed via electrophoresis through a 1.5% agarose gel after which the gel was stained with ethidium bromide and photographed after visualized on an UV transilluminator.

Results

Serological results

In this research, 93 (87%) sera samples were RBPT positive and 14 (13%) samples negative. SAT were applied for 93 RBPT positive sera samples for the detection of antibody titer.

Regarding SAT, 6 (6%) sera samples showed antibody titers of 1/5-1/10, 27 (29 %) as 1/20 and 60 (65%) sera samples possessed antibody titer of 1/40 or higher. According to these results, 60 sera samples were found to as Brucella infection serologically positive. Comparison of RBPT, SAT and PCR among 107 sheep was shown in table 1.

Table 1. Comparison of RBPT, SAT and PCR for detection of Brucellosis among 107 sheep.
Tablo 1. 107 koyunda Brucellozis açısından RBPT, SAT ve PCR'ın karşılaştırılması.

	RBPT		SAT			PCR	
Antibody titers (n=107)	Positive	Negative	1/5-1/10	1/20	1/40 and >	Positive	Negative
	93	14	6	27	60	83	10

Only RBPT positive cases were subjected to SAT and PCR. 17 out of those 107 sheep were detected to have forelimb lameness with SAT titers 1/40 and > and PCR positivity.

Yalnızca RBPT pozitif vakalara SAT ve PCR uygulandı. 107 koyundan SAT titresi 1/40 ve üzeri olan ve PCR pozitif bulunan 17 koyunda ön bacak topallığı gözlendi.

PCR results

In our study, 83 (89%) out of 93 RBPT Brucella positive samples of Merino and Akkaraman sheep produced a 731 bp PCR band specific to *B. melitensis*, approximately.

Determination of the detection sensitivity of PCR

Detection limit of the PCR assay was eveluated for sheep blood samples. To determine colony forming unit (cfu), a concentrated culture of the *B. melitensis* was prepared in sterile saline and $5x10^5$ cfu/ml dilution was made. $5x10^5$ cfu/ml dilution was serially diluted 2-fold to 5 cfu/ml. From the all dilutions, 0.1 ml suspensions onto two tryptose soy agar plates and incubated at 37ºC for 48 hours. Then colonies on plates were counted. Twenty microliters of each dilution was boiled for 10 min and added directly to the PCR mixture. The bacterial concentration of the positive PCR results were obtained with different aliquots containing at least 10^3 cfu of *B. melitensis* organisms were detected per milliliter of sheep blood. However, some aliquots containing at least 100 cfu of *B. melitensis* organisms were detected per milliliter of sheep blood.

Interestingly 17 out of 107 sheep with SAT titers at 1/40 or higher and PCR positivity were showing the clinical signs of lameness with probable osteoarthricular involvement (Figures 1 and 2).

Discussion

The sheep is predisposed to lameness by several of factors. Unsuitable environmental conditions, wetness of housing floor, overgrown/cracked/fissured hoof (Devendra and McLorey, 1982), fracture and trauma (Adams, 1974), systemic/local bacterial infections (inflammation of anatomical structures and glands (Blood and Radostits, 1990) may all be involved and responsible for lameness. The role of predisposing factors varies depending on the age, herd size and management systems adopted by farmers (Harris et al., 1988). Arthritis causing lameness may be detected in both ewe and rams (Lopes et al., 2010).

Brucella melitensis (*B. melitensis*) is the principle cause of ovine brucellosis and the disease may be characterized with infertility, retained fetal membrane, abortion, orchitis, epididymitis and finally arthritis (Corbel, 1997; Boschiroli et al., 2001; Renukaradhya et al., 2002), although arthritis assocaited with Brucellosis has not widely been documented in sheep liteature. Interestingly in paralel to the purpose of the study reported herein, the present authors would like to describe a case series of sheep showing forelimb lameness associated with Brucellosis.

Human brucellosis is a life-threatening disease that may have variable clinical presentations (Colmenero et al., 2002). A subset of patients develops chronic brucellosis with more severe form of the disease resulting in osteo-articular signs involving arthritis or genitourinary changes, as orchitis, epididymitis and glomerulonephritis (Hartigan, 1997; Colmenero et al., 2002), similar to sheep. Brucellar arthritis has been well documented in human being (Gotuzzo et al., 1987; Wernaers and Handelberg, 2007). Surprisingly it has also been stated that patients presenting with arthritis due to brucellosis

Figure 1. Forelimb lameness a) lateral and b) backwards view in a Merino sheep. Osteoarthricular involvement was evident in Brucella melitensis infected sheep.

Şekil 1. Merinos ırkı bir koyunda ön bacak topalllığı a) yandan ve b) arkadan görünüm. Brucella melitensis enfekte koyunda görülen osteoartrikuler tutulum.

Figure 2. Forelimb lameness in lateral and front view in a White Karaman sheep.

Şekil 2. Akkaraman koyununda görülen ön bacak topallığının yandan ve önden görünümü.

entails a close direct/indirect contact with sheep, goat and cattle (Chadda et al., 2004). In a previous study relevant to epidemiological and clinical features of Brucella arthritis in children, the most common affected joints were the knee, hip and ankle, however arthritis of the upper extremities (wrist and elbow) was rare (Zamani et al., 2011).

There was sex predisposition as females showed signs of lameness in contrast to the males. This arises because many sheep owners prefer ewes for the purpose of multiplication. In the present study there was lameness in the forelimbs compared to the hindlimbs. This is in particular similar to the earlier reports (Egwu et al., 1994). More body weight was loaded on the forelimbs (59 %) in contrast to the hind limbs (41 %), of body weight, in propelling the body (Kim and Breur, 2008). This may be presenting that the forelimbs are subjected to more injuries from trauma and concussions than the hindlimbs. This is in agreement with the observations by Adams (1974).

Lameness leads to reduced performance in association with diminished food intake, and reproductive efficiency, lowered milk production, body weight loss, and libido disorders (Harris et al., 1988). The economic implication of this situation is tremendous in terms of reduced market value, and overall reduced productivity of sheep (Bokko and Chaudhari, 2001).

Thirty cows with persistent serological reactions to *Brucella abortus (B. abortus)* had lameness in association with chronic granulomatous arthropathy in a previous study. Synovial tissue revealed organisms of Brucella morphology in fluorescent antibody-stained cryostat sections and the synovial fluids contained high titres of antibodies against *B. abortus* and *Yersinia enterocolitica* (Corbel et al., 1989). In chronic stage of Brucellosis, hygromas and joint inflammation may be observed in male goats (Megid et al., 2010).

Lameness after exposure to the combined use of *B. abortus* strain 19 and *Br. ovis* vaccine was previously detected in 40 out of 300 Perendale rams. *B. abortus* strain 19 was the etiological agent resulting in epiphysitis as determined by radiological examination of affected limbs (West et al., 1978). In the present study the vaccination status of the flock was unknown as the mixed flock was composed of animals collected from Ankara region of the market. However it should bear in mind that prior vaccination with Brucella strains may be related to side effects involving lameness.

The present authors believe that the severe ankle lesions on the forelimbs impeded the sheep's movement and contributed to emaciated condition. It is not known whether *B. melitensis* directly or indirectly caused lesions in other tissues nor whether concurrent disease of other relevant etiology was present. The articular lesions in this study was suggested to be in association within Brucellosis. Further studies may be warranted with larger sheep populations naturally infected with Brucellosis.

References

Adams OR (1974). In: Lameness in Horses, 3rd, Lea and Febiger (Eds): Philadelphia, pp. 4–32.

Alton GG (1990). Brucella melitensis. In: Animal Brucellosis, Nielsen.K and Duncan JR (Eds): CRC Pres, Boston, pp. 205-215.

Baily GG, Krahn JB, Drasar BS and Stocker NG (1997). Detection of Brucella abortus and Brucella melitensis by DNA amplification. The American Journal of Tropical Medicine and Hygiene, 95, 271-275.

Blood DC and Radostits OM (1990). In: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. 10th Ed., Mackay's Kent, Britain. pp: 449–487.

Bokko BP and Chaudhari SUR (2001). Prevalence of lameness in sheep in the north east region of Nigeria. International Journal of Agriculture and Biology, 3 (4): 519 – 521.

Boschiroli ML, Foulongne V and O'Callaghan D (2001). Brucellosis: a worldwide zoonosis. Current Opinion in Microbiology, 4, 58-64.

Bosilkovski M, Krteva L, Caparoska S and Dimzova M (2004). Osteoarticular involvement in brucellosis: study of 196 cases in the Republic of Macedonia. Croatian Medical Journal, 45, 727-733.

Bricker BJ (2002). PCR as a diagnostic tool for brucellosis. Veterinary Microbiology, 90, 435-446.

Bricker BJ and Halling SM (1994). Differentiation of Brucella abortus bv. 1, 2, and 4 Brucella melitensis, Brucella ovis, and Brucella suis bv. 1 by PCR. Journal of Clinical Microbiology, 32, 2660-2666, 1994.

Chadda VS., Soni PK., Gupta A., Gupta BK., Chadda S., Nayak KC. (2004). Incidence of brucellosis in arthritis and chronic low back pain in high risk group. Journal of Association of Physicians of India; 52: 338.

Colmenero Jde D, Queipo-Ortuño MI, Maria Reguera J, Angel Sua-

rez-Muñoz M, Martín-Carballino S and Morata P (2002). Chronic hepatosplenic abscesses in Brucellosis. Clinico-therapeutic features and molecular diagnostic approach. Diagnostic Microbiology and Infectious Disease, 42, 159-67.

Corbel MJ, Stuart FA, Brewer RA, Jeffrey M and Bradley R (1989). Arthropathy associated with Brucella abortus strain 19 vaccination in cattle. I. Examination of field cases. British Veterinary Journal, 145(4): 337-346.

Corbel MJ (1997). Brucellosis: an overview, Emerging Infectious Diseases, 3, 213–221.

DelVecchio VG, Kapatral V, Redkar RJ, Patra G, Mujer C, Los T, Ivanova N, Anderson I, Bhattacharyya A, Lykidis A, Reznik G, Jablonski L, Larsen N, D'Souza M, Bernal A, Mazur M, Goltsman E, Selkov E, Elzer PH, Hagius S, O'Callaghan D, Letesson JJ, Haselkorn R, Kyrpides N and Overbeek R (2002). The genome sequence of the facultative intracellular pathogen Brucella melitensis. Proceedings of the National Academy of Sciences of the United States of America, 99, 443-448.

Devendra C and McLorey GB (1982). In: A Textbook of sheep and Goat Production in the Tropics. International Tropical Agriculture Series. Longman, London. Pp. 352–353.

Egwu GO, Adamu SS, Ameh JA, Onyeyili PA, Abana PS, Chaudhari SUR and Rabo JS (1994) Retrospective, clinic pathological and microbiological studies of interdigital pouch lameness in sheep in an arid zone of Nigeria. Bulletin of Animal Health and Production in Africa, 42, 5–11.

Glynn MK and Lynn T V (2008). Brucellosis. Journal of the American Veterinary Medical Association, 233, 900–908.

Gotuzzo E, Seas C, Guerra JG, Carrillo C, Bocanegra TS, Calvo A, Castañeda O and Alarcón GS (1987). Brucellar arthritis: a study of 39 Peruvian families, Annals of the Rheumatic Diseases, 46, 506-509.

Gupta VK, Deepak K, Verma P K, Singh SV and Vihan VS (2006). Polymerase Chain Reaction (PCR) for Detection of Brucella melitensis in goat milk. Small Ruminant Research, 65, 79-84.

Güler L, Gündüz K and Ok Ü (2003). Comparison of polymerase chain reaction and bacteriological culture for the diagnosis of sheep brucellosis using aborted fetus samples. Veterinary Microbiology, 93, 53-61.

Harris DJ, Hiburt CD and Anderson GA (1988). Incidence, cost and factors associated with foot lameness in dairy cattle in South Victoria. Australian Veterinary Journal, 65, 171.

Hartigan P (1997). Human brucellosis: epidemiology and clinical manifestations. Irish Veterinary Journal, 50, 179-80.

Kater JC and Hartley WJ (1963). Lameness in rams following vaccination against Brucella ovis infection. New Zealand Veterinary Journal, 11, 3, 65-67.

Kim J and Breur GJ (2008). Temporospatial and kinetic characteristics of sheep walking on a pressure sensing walkway. Canadian Journal of Veterinary Research, 72, 1, 50-55.

Koneman E (2006) Chapter 9: Brucella species. In: Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th Edit., Winn W, Allen S, Janda W, Koneman E, Procop G, Schreckenberger P and Wood G (Eds.), Lippincott Williams and Wilkins Publication, United States of America, pp. 482-491.

Leal-Klevezas DS, Martinez-Vazquez IO, Lopezmerino A and Martnez-Sorano JP (1995) Singlestep PCR for detection of Brucella spp. from blood and milk of infected animals. Journal of Clinical Microbiology, 33, 3087-3090.

Lopes LB, Nicolino R, Haddad JPA (2010) Brucellosis - Risk Factors and Prevalence: A Review. The Open Veterinary Science Journal, 4, 72-84.

Manthur BG, Peerapur BV, Shetty SKV, Purohit SN, Shrikant B and Sarayu L (1994) Brucellosis among the patients attending B.L.D.E.A's medical college hospital, Bijapur- a serological and cultural study. Indian Journal of Medical Microbiology,12, 1, 35-38.

Megid J, Mathias LA and Robles CA (2010) Clinical Manifestations of Brucellosis in Domestic Animals and Humans. The Open Veterinary Science Journal, 4, 119-126

Öngör H, Muz A and Çetinkaya B (2001) Atık yapmış koyunlarda brusellosis teşhisinde ELISA ile diğer serolojik testlerin karşılaştırılması. Turkish Journal of Veterinary and Animal Science, 25, 21-26.

Pourbagher A, Pourbagher MA, Savas L, Turunc T, Demiroglu YZ, Erol I and Yalcintas D (2006) Epidemiologic, clinical and imaging findigns in brucellosis patients with osteoarticular involvement. American Journal of Roentgenology, 187, 873-880.

Renukaradhya GJ, Isloor S and Rajasekhar M (2002) Epidemiology, zoonotic aspects, vaccination and control/eradication of brucellosis in India. Veterinary Microbiology. 90, 183–195.

Wallach JC, Samartino LE, Efron A and Baldi PC (1998) Human infection by Brucella melitensis: An outbreak attributed to contact with infected goats. FEMS Immunology and Medical Microbiology, 19, 315-321.

Wernaers P and Handelberg F (2007) Brucellar arthritis of the knee: A case report with delayed diagnosis. Acta Orthopaedica Belgica, 73, 795-798.

West DM, Johnstone AC, Bruere AN and Chapman HM (1978) Epiphysitis in rams following vaccination against Brucella ovis infection. New Zealand Veterinary Journal, 26, 5, 133-134.

Zaks N, Sukenik S, Alkan M, Flusser D, Neumann L and Buskila D (1995) Musculoskeletal manifestations of brucellosis :A study of 90 cases in Israel. Seminars in Arthritis and Rheumatism, 25, 97-102.

Zamani A, Kooraki S, Mohazab RA, Zamani N, Matloob R, Hayatbakhsh MR and Raeeskarami SR (2011) Epidemiological and clinical features of Brucella arthritis in 24 children. Annals of Saudi Medicine, 31(3): 270-273.