



RESEARCH ARTICLE

Ultrasonographic and Histopathological Findings in Rams with Epididymo-Orchitis Caused by *Brucella melitensis*

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ABSTRACT

Epididymo-orchitis is one of the most important reproductive diseases in rams caused by various pathogens. The purpose of this study was to describe the clinical, ultrasonographic and histopathological characteristics of epididymo-orchitis caused by *Brucella melitensis* in rams. Nine rams with a large unilateral scrotal swelling were admitted to the surgery clinic. Full case history, thorough clinical examination, Rose Bengal test (RBT), Microagglutination test, ultrasonographic and histopathological examinations were carried out for all rams. The mean age of the diseased rams was 4 ± 1.7 years. The diseased rams showed positive RBT (++) and the mean antibrucella titer was $1/140 \pm 7.8$. For rams admitted between 1 to 2 months from the onset of clinical signs, the main ultrasonographic findings included thick hyperechoic scrotum, thick hyperechoic testicular tunics, anechoic fluid into vaginal cavity, enlarged testis, absence of testicular echogenic pattern, wide appearance of the mediastinum testis and multiple hypoechoic testicular and epididymal abscesses. For rams admitted between 2 to 3 months from the onset of clinical signs, hyperechoic multiple testicular abscesses, hyperechoic fibrous foci and thick hyperechoic epididymes were seen. The pathognomonic lesions were multiple focal testicular and epididymal abscesses, microgranulomas and microcalcification, necrosis of germinative epithelium, atrophy of seminiferous tubule with absence of spermatogenesis and interstitial edema associated with inflammatory cells infiltration. Scrotal lymph node showed focal caseous lymphadenitis with capsular edema. In conclusion, the ultrasonographic and histopathological findings of epididymo-orchitis caused by *B. melitensis* in rams are characteristics and vary depending on the chronological stage of the disease.

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INTRODUCTION

Brucellosis is considered one of the most common global zoonosis caused by the genus *Brucella* especially *B. abortus*, *B. melitensis*, and *B. suis* (McDermott *et al.*, 2013; Cvetnić *et al.*, 2014). Epidemiological investigations showed that rams kept under intensive systems were subjected to large-scale invasion of their genitalia by bacteria which led to infection of the accessory glands and epididymo-orchitis (Büyükcangaz *et al.*, 2013). A variety of bacteria were associated with this affection in rams including, *Escherichia coli* (Marthur *et al.*, 2011), *Histophilus ovis* (Philbey *et al.*, 1991), *Brucella ovis* (Dénes and Glávits, 1994), *Actinobacillus seminis* (Puente-Redondo *et al.*, 2000), *Brucella melitensis* (Chand *et al.*,

2002), *Arcanobacterium pyogenes* (Gouletsou *et al.*, 2004) and *Salmonella enterica* subsp. *diarizonae* (Ferreras *et al.*, 2007).

Brucella melitensis is a significant problem in small ruminants, particularly in developing nations where infections can be widespread (Ducrototy *et al.*, 2014). The predominant symptoms in naturally infected sheep and goats are abortions, stillbirths, the birth of weak offspring and decreased milk yield in females, acute orchitis and epididymitis in males and occasionally, arthritis in both sexes (Culter *et al.*, 2005).

Brucella is a Gram-negative, facultative intracellular bacterium that causes zoonotic brucellosis in both animals and humans. Out of 10 classified *Brucella* species, *B. melitensis*, *B. abortus*, *B. suis* and *B. canis* are of

zoonotic importance (He, 2012). *Brucella ovis* causes chronic epididymitis associated with the formation of spermatocele, sperm granuloma or abscess in rams. It is emphasized that chronic inflammatory processes were observed in both epididymides even if the clinically palpable epididymo-orchitis occurred unilaterally (Dénes and Glávits, 1994).

In last decade, ultrasonography became a routine tool for diagnosis and prognosis of various surgical affections in large animal practice (Abu-Seida *et al.*, 2012; Kotb *et al.*, 2014). It appeared to be useful for the diagnosis of intra-scrotal abnormalities, especially during investigation of the long-standing stage of the disease, after clinical findings have subsided (Abu-Seida, 2012). In addition, serological tests, gross lesions and microscopic findings of epididymo-orchitis had an additional diagnostic importance in rams (Gouletsou *et al.*, 2004). To the best of our knowledge, there are little researches dealt with epididymo-orchitis in breeding rams naturally infected with *Brucella melitensis*. Therefore, the purpose of this study was to describe the clinical, ultrasonographic and histopathological characteristics of a confirmed epididymo-orchitis caused by *Brucella melitensis* in nine breeding rams.

MATERIALS AND METHODS

Animals and clinical examination: Nine breeding rams with unilateral scrotal swelling were admitted to the surgery clinic at Faculty of Veterinary Medicine, Cairo University during the period between 2011-2014. Full case history was obtained for each ram. Pulse and respiration rates, ruminal motility and body temperature were measured. Scrotal, testicular and epididymal palpation was applied. In addition, the scrotal lymph nodes and regional vasculatures were examined.

Serological tests: A 5 ml blood sample obtained from jugular vein was collected in a tube without anticoagulant, preserved in a refrigerator for two hours and centrifuged at 3000 xg for 15 minutes. The supernatant was collected as serum. In addition, Rose Bengal antigen (Brucella unit, Abbasia, Cairo), white Brucella antigen (Brucella unit, Abbasia, Cairo) and control positive serum were used for the serological tests.

Rose Bengal plate test (RBT): It was carried out according to the method specified by (WHO, 2006). The test was performed on flat cards by mixing equal volumes (0.03 ml) of serum and antigen (Rose Bengal antigen, Brucella unit, Abbasia, Cairo, Egypt) on a rocker at a rate of 12-14 rocks/minute. The test was read after 4 minutes at ambient temperature for agglutination. The appearance of a large-grained (++) or small-grained (+) agglutinate indicated a positive result.

Microagglutination test: Positive serum samples were confirmed by microagglutination test. The test was performed in standard 96-well U-bottom micro-titre plates by placing 80 µl of phenol saline (0.5% [w/v] phenol in 0.15 M sodium chloride) into the first well and 50 µl volumes of phenol saline in the remaining wells of the same row. A volume of 20 µl serum was added and mixed in the first well. Then 50 µl was transferred to the next well.

Further volumes of 50 µl were transferred to subsequent wells to give a series of doubling dilutions. An equal volume of standard *B. melitensis* agglutination suspension, diluted to working strength in phenol saline, was then added to each well. The plate was incubated at 37°C for 24 hours. All results were visually recorded in comparison with standard.

Ultrasonography and castration: Scrotal ultrasonographic examination was done for all rams using Toshiba ultrasound device (Toshiba just vision, Japan) connecting with 3.5-5.0 MHZ convex transducer. The examined area was shaved and ultrasound coupling gel was applied. The scrotum and its contents were scanned in both sagittal and transverse planes (Abu-Seida, 2012).

Open castration was carried out as usual for treatment of all affected rams under lumbo-sacral epidural analgesia using 3-5 ml Lidocaine hydrochloride 1% solution (Lidocaine Hydrochloride Injection BP 1.0% w/v, Mercury Pharma International Ltd, Co Dublin, Ireland).

Histopathology: Multiple specimens were collected from the excised testes, epididymes and scrotal lymph nodes. These specimens were fixed in 10% neutral buffered formalin solution, processed by conventional methods, sectioned at 4-6 microns and stained with hematoxyline and eosin for histopathological examination (Bancroft and Gamble, 2013).

Statistical analysis: All data are presented as mean±SD. All tests were performed using statistical analysis software SPSS (Statistical Packages for the Social Sciences 19.0; IBM, Armonk, NY, USA).

RESULTS

Clinical findings: All of the affected rams were used for breeding and had not vaccinated against brucellosis. The mean age of the diseased rams was 4±1.7 years. The mean admission time from the onset of clinical signs was 2.5±0.7 months. The vital parameters including temperature, ruminal motility, pulse and respiratory rates weren't affected. The main respiratory rate/minute, pulse rate/minute, rectal temperature (°C) and ruminal motility/ 2 minutes were 15.5±1.3, 87.9±2.4, 38.4±0.4 and 2.6±0.5, respectively. All rams had unilateral hard and/or doughy large scrotal swelling. Both testis and epididymis of the affected side were thickened and slightly painful. The second testis appeared apparently normal. The right and left testes were affected in five and four rams, respectively. The scrotal lymph nodes (superficial inguinal lymph node) were also enlarged (Fig. 1a). The external abdominal vein of the affected side was congested.

Serological and Ultrasonographic findings: All rams demonstrated high positive Rose Bengal tests (++) and the mean antibrucella titer was 1/140±7.8. For rams admitted between 1 to 2 months from the onset of clinical signs (3 rams), the main ultrasonographic findings were enlarged testis with thick hyperechoic scrotum, thick hyperechoic tunica vaginalis, anechoic fluid into vaginal cavity, wide appearance of the mediastinum testis, loss of normal testicular echogenicity and hypoechoic testicular abscesses

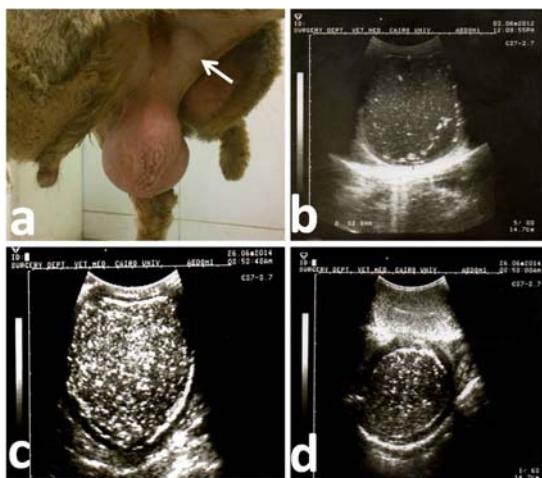


Fig. 1: a) A 3-year-old breeding ram showing unilateral epididymo-orchitis with enlarged scrotal lymph node (superficial inguinal lymph node) (arrow). b) Testicular ultrasonogram of a 4-year-old breeding ram showing large hypoechoic abscess with small hyperechoic flakes and thick hyperechoic scrotum. c) Testicular ultrasonogram of a 5-year-old breeding ram showing large hyperechoic abscess with thick hyperechoic tunica vaginalis scrotum. d) Testicular ultrasonogram of a 3-year-old breeding ram showing large hypoechoic abscess with thick hyperechoic tunica vaginalis scrotum and anechoic fluid filled vaginal cavity. Notice the apparently normal testis with normal rete testis and echogenicity.



Fig. 2: a) Testicular ultrasonogram of a 6-year-old breeding ram with epididymo-orchitis showing multiple hypoechoic abscesses. b) Testicular ultrasonogram of a 7-year-old breeding ram with epididymo-orchitis showing large hyperechoic fibrosis and multiple anechoic areas separated by hyperechoic fibrous bands. c) Testicular ultrasonogram of a 3-year-old breeding ram with epididymo-orchitis showing large hyperechoic testicular abscess and thick hyperechoic epididymal tail. d) Ultrasonogram of the spermatic cord of a 5-year-old breeding ram with epididymo-orchitis showing hyperechoic thick cord and hyperechoic epididymis containing hypoechoic fluid and hyperechoic flakes.

with small hyperechoic flakes (Fig. 1b, 1c & 1d). The apparently normal testis had small size, normal echogenicity and rete testis (Fig. 1d).

For rams admitted between 2 to 3 months from the onset of clinical signs (6 rams), enlarged testis with thick hyperechoic scrotum and tunics, hyperechoic testicular abscesses (Fig. 1c), multiple hypoechoic abscesses (Fig. 2a), large areas of hyperechoic fibrosis and multiple anechoic areas separated by hyperechoic fibrous bands

(Fig. 2b) were the main ultrasonographic features. All affected rams showed thick hyperechoic epididymis (Fig. 2c) containing hypoechoic fluid with hyperechoic flakes (Fig. 2d).

Histopathological findings: The mean weight of excised testes 1.6 ± 0.7 kg. The gross lesions included severe enlargement of the scrotal contents, thick indurated scrotum, fibrous adhesions between testicular tunics, multiple testicular and epididymal abscesses with creamy pus and foci of fibrosis and calcification. The affected testes showed necrotizing orchitis. Necrosis of germinative epithelium, atrophy of seminiferous tubule with absence of spermatogenesis and interstitial edema associated with inflammatory cells infiltration were seen (Fig. 3).

Multifocal micro-abscesses formation was also seen in some areas of the affected testes. These abscesses appeared as a central core of necrosis containing cell debris and bacterial colonies enclosed with fibrous capsule and inflammatory cells (Fig. 4a & 4b). Multiple pyogranulomas with central core of neutrophils surrounded by chronic mononuclear inflammatory cells, giant cells and enclosed by fibrous capsule were also seen (Fig. 4c). In addition, microcalcification with accumulation of chronic inflammatory cells and surrounded by fibrous capsule was observed (Fig. 4d). The affected epididymis showed focal abscesses, microcalcification, accumulation of chronic inflammatory cells and fibrous capsule (Fig. 5a). The scrotal lymph node of the affected rams showing capsular edema and inflammatory cells infiltration (Fig. 5b). In addition, chronic lymphadenitis with proliferating fibrous connective tissue encircled the lymphoid follicles (Fig. 5c) and focal caseous necrosis (Fig. 5d) was also seen.

DISCUSSION

Egypt is a highly populous country, has a large proportion of poor livestock keepers, and is a hotspot for neglected zoonosis. Sheep constitute an important part of livestock in Egypt providing milk, meat, hide and wool (Abu-Seida, 2014). Epididymo-orchitis caused by *B. melitensis* is an important pathological affection of rams often leads to lowered fertility or sterility (Chand *et al.*, 2002). But most of the available studies on *B. melitensis* dealt with seroprevalence and prevention (Spicic *et al.*, 2010; Ducrototy *et al.*, 2014 and Hamidi *et al.*, 2015), only one study was carried out on its clinical, serological and pathological findings (Chand *et al.*, 2002) and no study was performed on its ultrasonographic diagnosis in naturally infected rams. Therefore, this study was conducted to describe the clinical, serological, ultrasonographic and histopathologic findings of epididymo-orchitis caused by *Brucella melitensis* in naturally infected breeding rams.

In the present study, all of the affected animals were breeding rams. This finding agrees with that reported by (Chand *et al.*, 2002). This might be due to venereal transmission. In this respect, Cutler *et al.* (2005) mentioned that the transmission of *B. melitensis* during breeding is possible, but seems to be uncommon during natural mating. However, the possibility of infection through oral ingestion, mucous membrane, or cutaneous wounds could not be ruled out (Chand *et al.*, 2002).

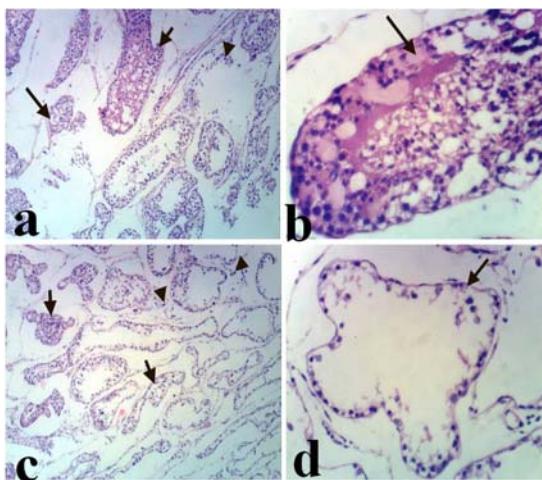


Fig. 3: a) Photomicrograph of a testis of breeding ram showing necrotizing orchitis. Notice necrosis of germinative epithelium (small arrow), atrophy of seminiferous tubule (large arrow) and interstitial edema associated with inflammatory cells infiltration (arrow head). b) Photomicrograph of a testis of breeding ram showing marked necrosis and desquamation of germinative epithelium (arrow). c) Photomicrograph of a testis of breeding ram showing necrosis and atrophy of seminiferous tubules with incomplete spermatogenesis (arrows) and interstitial mononuclear inflammatory cells infiltration (arrow head). d) Photomicrograph of a testis of breeding ram showing marked necrosis of germinative epithelium lining seminiferous tubules with absence of spermatogenesis (arrow). H&E Stain. a,c: X 100; b,d: X 400.

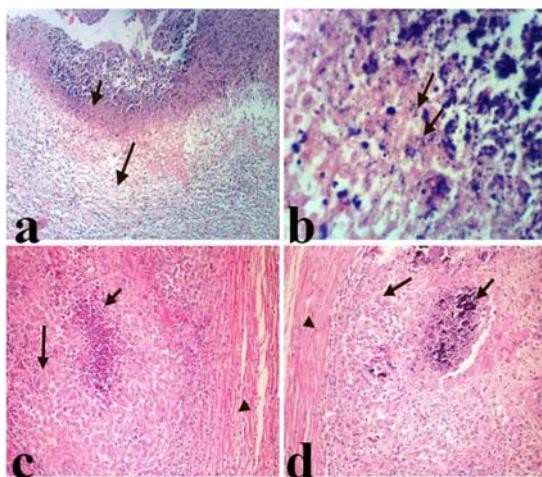


Fig. 4: (a) Photomicrograph of a testis of breeding ram with epididymo-orchitis showing abscess formation. Notice central core of necrosis containing cell debris, bacterial colonies (small arrow) and encased with fibrous capsule and inflammatory cells (large arrow). (b) Higher magnification of previous photo demonstrating the bacterial colonies (coccobacilli of *Brucella*). (c) Photomicrograph of a testis of breeding ram with epididymo-orchitis showing pyogranuloma with central core of neutrophils (small arrow), surrounded by chronic mononuclear inflammatory cells and giant cells (large arrow) and encased by fibrous capsule (arrow head). (d) Photomicrograph of a testis of breeding ram with epididymo-orchitis showing microcalcification (small arrow), accumulation of chronic inflammatory cells (large arrow) and fibrous capsule (arrow head). H&E Stain. a: X100; b: X1000; c and d: 200X.

All the vital parameters were within the normal range due to the subsiding of the clinical signs in the long-standing stage of the disease. This agrees with the findings of Chand *et al.* (2002). The scrotal lymph node was enlarged and the external abdominal vein of the affected side was congested. These could be attributed to the chronic inflammation of the affected testis and epididymis.

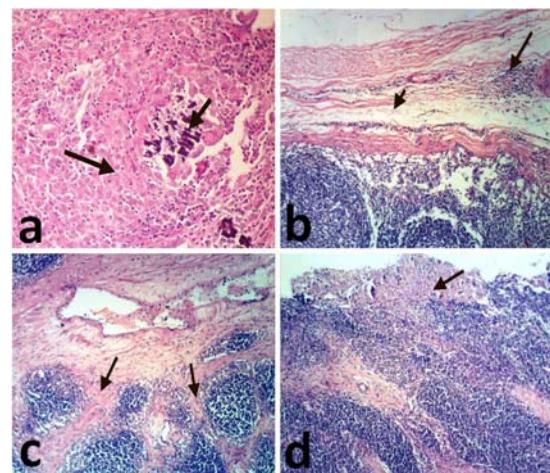


Fig. 5: a) Photomicrograph of epididymis of breeding ram with epididymo-orchitis showing microcalcification (small arrow) and accumulation of chronic inflammatory cells (large arrow). b) Photomicrograph of the scrotal lymph node of ram with epididymo-orchitis showing edema (small arrow) and capsular inflammatory cells infiltration (large arrow). c) Photomicrograph of the scrotal lymph node of breeding ram with epididymo-orchitis showing chronic lymphadenitis. Notice proliferating fibrous connective tissue encircled the lymphoid follicles (arrows). d) Photomicrograph of the scrotal lymph node of breeding ram with epididymo-orchitis showing focal caseous necrosis (arrow). H&E Stain. a: X200; b-d: X100.

All of the affected rams had unilateral epididymo-orchitis. This is in agreement with Chand *et al.* (2002) who recorded both unilateral and bilateral *B. melitensis* induced epididymo-orchitis.

There are several serological tests available for brucellosis diagnosis and surveillance. Among these tests, the Rose Bengal plate test (RBT) is the recommended method. The OIE considers this test "prescribed test for trade" (Godfroid *et al.*, 2010). Therefore the present study depended upon this test because it is a quick, cheap and effective test for the diagnosis of brucellosis. But positive RBT samples should be confirmed by a quantitative test. Therefore the present study used microagglutination test for measurement of the antibrucella titer. In this respect, Da Costa *et al.* (2010) found that the attenuated *Brucella melitensis* Rev 1 vaccine, used against brucellosis, interferes with serological diagnostic tests. This finding was excluded in our study because all of the examined rams were not vaccinated against brucellosis.

The ultrasonographic features of epididymo-orchitis varied depending on the chronological stage of the disease. Similar finding was recorded in experimentally induced orchitis associated with *Arcanobacterium pyogenes* in rams (Gouletsou *et al.*, 2004). The echogenicity of the testicular abscess depended mainly upon the consistency of its pus content. Therefore the abscess contained watery pus appeared hypoechoic with hyperechoic necrotic tissue flakes, while the abscess contained caseated pus appeared hyperechoic. In late stage of the disease, the affected testis lost its normal echo pattern and large hyperechoic areas of fibrosis and calcification were seen. The enlarged testis and thick hyperechoic scrotum, testicular tunics and epididymis were attributed to the chronic inflammation. In addition, the appearance of anechoic fluid into the vaginal cavity could be explained by the accumulation of the inflammatory exudates.

Although surgical castration was a successful treatment for the diseased rams, all rams were disposed of to prevent any spread of the disease. The veterinary surgeons should follow restricted aseptic precautions to avoid the zoonotic infection. Animals are the only significant source of human brucellosis, specially veterinarians, abattoir workers, and livestock keepers. Transmission is via direct contact and through consumption of unpasteurized dairy products. Human brucellosis is a grave and debilitating disease that may lead to permanent sequelae, requires prolonged and combined antibiotic therapy, and is fatal in 1-5% of untreated cases (Zinsstag *et al.*, 2011).

The histopathological findings also varied according to the chronological stage of the disease. The prominent early histopathological changes were necrotizing orchitis, necrosis of germinative epithelium, atrophy of seminiferous tubule with absence of spermatogenesis and interstitial edema associated with inflammatory cells infiltration. Multifocal abscesses, granulomas with concurrent fibrosis and mineralization were observed later. These findings were in agreement with those reported in epididymo-orchitis caused by *B. melitensis* in naturally infected rams (Chand *et al.*, 2002) and in experimentally induced orchitis associated with *Arcanobacterium pyogenes* in rams (Gouletsou *et al.*, 2004).

Brucellae are coccobacilli or short rods, usually arranged singly but sometimes in pairs or small groups (Spicić *et al.*, 2010). Interestingly in the present study, single, pairs and small colonies of coccobacilli were seen during histopathological examination which suggested *B. melitensis* as a causative agent for epididymo-orchitis in the examined rams. Similarly, bacterial colonies were previously identified in thrombosed spermatic cord vessels, scrotal lymph nodes, lung and liver of a ram with unilateral orchitis and epididymitis caused by *Salmonella enterica subspecies diarizonae* infection (Ferreras *et al.*, 2007).

Conclusion: The main characteristics of epididymo-orchitis caused by *B. melitensis* in rams are slightly painful enlarged testis and epididymis, enlarged scrotal lymph nodes, congested external abdominal vein and high antibrucella titer. Ultrasonographic findings include; thick hyperechoic scrotum and tunica vaginalis, anechoic fluid into vaginal cavity, wide appearance of the mediastinum testis, loss of normal testicular echogenicity, hypoechoic testicular abscesses with small hyperechoic flakes, areas of hyperechoic fibrosis and anechoic fluid separated by hyperechoic fibrous bands. Grossly, thick indurated scrotum, fibrous adhesions between testicular tunics, multiple testicular and epididymal abscesses with creamy pus and foci of fibrosis and calcification are seen. Necrotizing orchitis, atrophy of seminiferous tubule with absence of spermatogenesis, multifocal abscesses, granulomas, fibrosis and mineralization are the main histopathological findings.

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