

## Correspondence

### Presumptive diagnosis of brucella epididymoorchitis by modified cold ZN staining of testicular pus sample

Sir,

Brucellosis is a well known zoonotic disease. Consumption of unpasteurised milk and milk products is a common mode of transmission of brucella to humans. Rare modes of transmission include transplacental transfer, breast feeding, respiratory and even sexual intercourse<sup>1</sup>.

Brucellosis known for its varied manifestations is common in north Karnataka<sup>2,3</sup>. Therefore, a very high index of suspicion is necessary for early diagnosis. Deep-seated chronic infections leading to various complications and a lot of morbidity are strongly associated with untreated brucellosis<sup>4,5</sup>. Epididymoorchitis is a frequent genitourinary complication and up to 20 per cent of men with brucellosis suffer from this complication<sup>6,7</sup>.

Protean manifestations of brucellosis, lack of awareness about the disease and slow growth of the causative agent make the laboratory diagnosis difficult. Culture is the gold standard for laboratory diagnosis of brucellosis. But, culture being less sensitive and often delayed, serology is the mainstay of diagnosis<sup>4,8-10</sup>. Therefore, importance of rapid diagnostic techniques cannot be overemphasized. Use of modified cold ZN stain on blood culture broths for presumptive and early identification of brucella has been documented<sup>11</sup>. We present here a case of *Brucella* epididymoorchitis, where modified cold ZN staining of pus smears was used for rapid presumptive diagnosis of brucella infection.

A 53 year old diabetic male, came to Surgery OPD at SDM Medical College Hospital, Dharwad (Karnataka, India) in the last week of September 2007 with complaints of fever for one week and pain and swelling in both the testes since 5 days. On examination, the patient had mild fever (99°F), moderate hepatosplenomegaly and bilaterally inflamed testes. A presumptive diagnosis of bilateral epididymoorchitis

was made at this stage, the patient was admitted and was put on amoxicillin-clavulanic acid (1.2 g b.d., i.v.), metronidazole (500 mg t.i.d., i.v.) and diclofenac sodium (25 mg i.m.)

The laboratory investigations revealed following findings; fasting blood sugar - 252 mg/dl and postprandial blood sugar - 445 mg/dl. HIV by Tridot (BioMed Industries, Himachal Pradesh) and HBsAg by Hepacard (BioMed Industries, Himachal Pradesh) were negative. Ultrasonography (USG) abdomen showed hepatomegaly with grade II fatty infiltration and non specific moderate splenomegaly. USG scrotum showed bilateral epididymoorchitis with right testicular abscess and left sided hydrocele.

Widal test for enteric fever (Span Diagnostics Ltd., Surat, India) and quantitative buffy coat (QBC) for malarial parasite (QBC Diagnostics Inc., Philipsburg, PA, USA) were negative. In our laboratory every sample received for Widal test is also tested with Rose Bengal Plate (agglutination) Test for Brucellosis (RBPT) [Indian Veterinary Research Institute [IVRI], Izatnagar, India]. The RBPT for this sample was strongly positive. The serum was therefore, subjected to standard tube agglutination test (SAT – suspension of pure smooth culture of *Brucella abortus* strain 99 in phenol saline, IVRI, Izatnagar) to know the titre of brucella agglutinins. The patient showed alarmingly high titres of 10,240 IU/ml with SAT.

The patient was posted for right sided orchidectomy. The right testicle showed pus pockets, which yielded at least 15 ml of frank pus. The pus was collected in sterile plain bulb and was transported to the microbiology laboratory immediately. Blood for culture was also collected and processed.

The pus was plated on chocolate agar and MacConkey's agar. Two tubes each of thioglycollate

broth and brain heart infusion broth were inoculated simultaneously. Pus smears were stained by Gram stain and modified cold ZN stain<sup>12</sup>. Gram stain performed on several smears showed only pus cells and no organisms. However, the modified cold ZN stained smears showed acid-fast coccobacilli arranged in groups of 5 to 10 along with pus cells (Fig.).

Because of the initial suspicion of brucella infection all the procedures were carried out in the bio-safety cabinet (Kartos International, Class II, Type A). The culture plates revealed growth after 48 h incubation. The growth on chocolate agar showed tiny grey colonies and MacConkey's agar showed non-lactose fermenting tiny colonies. Growth from both the media was provisionally and separately identified as brucella species with the help of following reactions. Gram stain - Gram negative coccobacilli, modified cold ZN stain - acid fast coccobacilli, oxidase - positive, catalase - positive, motility - non-motile, urease test - positive.

Slide agglutination of growth from chocolate agar showed strong agglutination with high titre brucella antiserum stored in our laboratory. The blood culture, after 5 days of incubation, also grew a similar organism with the entire set of characteristics identical with the growth from pus. The isolates were confirmed as *Brucella* species by PCR at The Maratha Mandal Dental College Molecular Biology Laboratory, Belgaum, Karnataka. The isolates were also sent to Indian Veterinary Research Institute (IVRI), Izatnagar for identification, and were identified as *Brucella melitensis* biotype 1. The patient was treated with

rifampicin (450 mg b.d.) and doxycycline (100 mg b.d.) for a period of 6 wk after orchidectomy. His fever subsided, left sided hydrocele resolved completely, and his blood culture was negative for brucella after completion of treatment.

Early diagnosis of brucellosis will be vital not only for early initiation of specific antibiotic therapy to the patient but also for safety of the laboratory personnel handling the samples. Direct microscopy of blood and bone marrow is found to be less useful in diagnosis of brucellosis<sup>13</sup>. Though molecular diagnostic techniques like real time PCR is very useful for rapid diagnosis of this condition, it is not yet available for routine use in countries like India<sup>14</sup>. Culture, therefore, is the gold standard for unequivocal diagnosis of brucellosis<sup>8</sup>.

In the present case, a presumptive identification of brucella epididymoorchitis was done in about 15 min after receiving the pus sample. To conclude, rapid presumptive diagnosis of brucellosis by modified cold ZN stain of exudates is possible especially when there is a strong serological evidence of brucella infection. The staining technique does not need great expertise or sophisticated laboratory equipment and hence can be easily used at peripheral laboratories. However, further confirmation of the test result by culture is necessary. As brucellosis is one of the most commonly encountered laboratory acquired infections, presumptive diagnosis of brucellosis would be of great help in the safety of laboratory workers as well<sup>15,16</sup>.

#### Acknowledgment

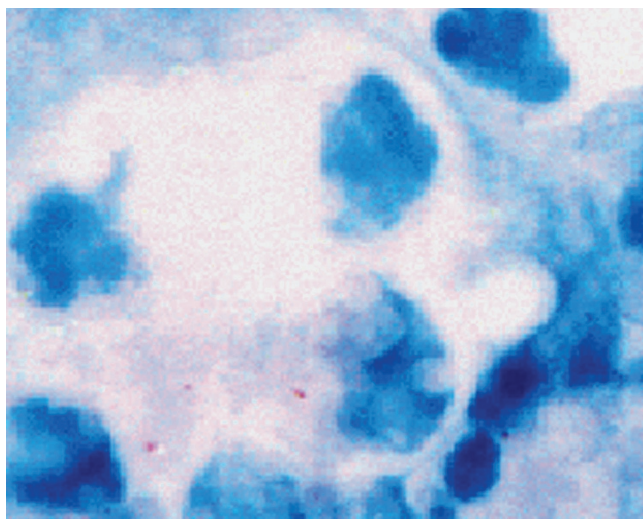
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**R.D. Kulkarni\***, **Sneha K. Chunchanur**  
**Ajantha G.S., Shubhada C. & Pavitra Jain**  
Department of Microbiology  
SDM College of Medical Sciences & Hospital  
Dharwad 580 004, Karnataka, India

\*For correspondence:  
atul410@yahoo.com

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**Fig.** Modified cold ZN stained smears showed acid-fast coccobacilli arranged in groups of 5 to 10 along with pus cells.

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