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# Diagnosis, Treatment and Control of Livestock and Human Brucellosis

Brucellosis, caused by intra-cellular *Brucella* species is one of the most important emerging zoonotic chronic diseases distributed worldwide since the discovery of *Brucella melitensis* by Bruce in 1887. WHO classified this disease a major but neglected zoonotic disease as this disease hinders animal productivity and affects human health. Mainly *B. abortus* (cattle), *B. melitensis* (small ruminants) and *B. suis* (swine) cause animal and human brucellosis in under developedcountries of the world. Cross-species transmission of these *Brucella* spp. can occur and cattle may be affected with all these three species. This zoonotic disease is a major public and animal health problem in many regions of the world especially in countries where rural incomes come largely from livestock rearing and dairy production, which can considerably impact socio-economic development. In animals, brucellosis mainly affects reproduction and fertility, with abortion and reduced milk yield. In humans, the clinical picture resembles many other febrile diseases, but sacroilitis and hepato-splenomegaly are the most prominent. As an occupational zoonotic disease, infection of humans results from direct contact with infected animals and consumption of contaminated milk and milk products. Cattle ranchers/dairy farmers/milkmen, veterinarians, abattoir workers, meat inspectors, lab workers, hunters, travelers, etc. are at risk. Brucellosis is endemic in Bangladesh but still there is no vaccination program. Therefore, vaccination trials in animals with the commercial SRB51 vaccine and its comparison with locally prepared vaccine is essential. Preparatory to vaccine development molecular characterization of the causal agent of brucellosis and an epidemiological risk factor analysis is also very important. This KGF sponsored project dealt with molecular characterization of strains and epidemiological risk factor analysis of brucellosis in livestock and humans and development of a treatment strategy for human and animals infected with brucellosis.

## Methodology

A questionnaire was developed by the project team for collection of epidemiological data from different project areas. Project workers collected aborted cattle fetus and milk and sera from the Military Dairy Farm (MDF), Savar, and the Central Cattle Breeding and Dairy Farm (CCBDF), Savar, and human serum samles from the Mymensingh Medical College Hospital (MMCH), Mymensingh and relevant data of the suspected cases during this reporting period. Suspected dairy cattle were screened for Brucellosis by the Rose Bengal Test (RBT), rapid test, Milk Ring Test (MRT), impression smear from aborted material and then modified Zeihl Neelsen staining, classical biotyping, guineapig inoculation, histopathology, polymerase chain reaction (PCR) and sequencing, Multi Locus Variable Number of Tandem Repeat Analysis (MLVA), CFT, SAT, were performed. A total 1003 cattle sera (503 from the CCBDF and 500 from

MDF) were screened with RBT, MRT and rapid kit test and 715 human sera were screened with RBT and rapid kit test in MDF and MMCH. Positive cases were re-tested with ELISA, conventional PCR with sequencing and construction of phylogenetic tree. Real time PCR and MLVA, Bruce ladder PCR, Crystal Field Theory (CFT), subcutaneous adipose tissue (SAT) tests were performed. A total of 1003 cattle sera (503 from CCDBF and 500 from MDF) were screened with RBT, MRT, and rapid test kit in MDF and MMCH. Positive cases were re-tested with ELISA, conventional PCR with sequencing and construction of phylogenetic tree. Real time PCR and MLVA (Multi Locus Variable Number of Tandem Repeat Analysis), Bruce ladder PCR, CFT and SAT were also performed.

## Results and Outputs

Out of 1003 cattle serum samples, 43 reacted positive in RBT (Fig. 1) and out of 1003 milk samples, 14 reacted positive in MRT, 24 in ELISA and SAT (slow agglutination test). Among 715 human serum samples 16 reacted positive in RBT in MDF, Savar and MMCH. Numerous

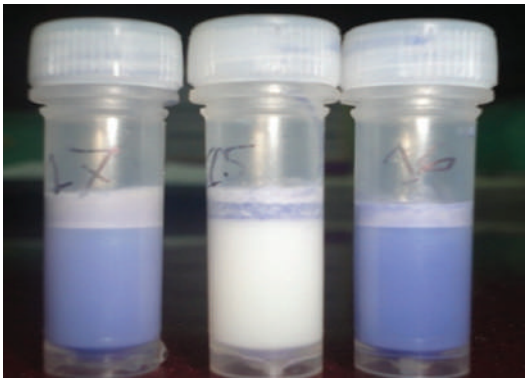

**Fig. 1. The milk ring test (MRT) for Brucellosis: Milk in the middle tube showing a ring of cream more intensely colored than the underlying milk indicates a positive test while the milk samples in the the two corner tubes showing reverse colorations tested negative**

changes in major organs after inoculation with aborted fetal contents. On the basis of the different characteristics ($CO_2$ requirement for growth, production of $H_2S$ in culture, biochemical test like oxidase, catalase and urease, growth in safranin, thionin and fuchsin and agglutination against anti A and Anti M antibody) and motility test, the *Brucella* species and biovar was determined as *Brucella abortus biovar 3*. The molecular technique (MLVA) indicated the strain to be similar as the Asian strain. The guineapig inoculation technique could be used as a good alternative to the culture method and for the confirmatory diagnosis of brucellosis from con taminated clinical samples like placenta.

pink colored *Brucella* like organisms (Fig. 2) with a blue background were observed under microscope and on the basis of different characteristics ($CO_2$ requirement for growth, production of $H_2S$ in culture, biochemical test like oxidase, catalase and urease, growth in safranin, thionin and fuchsin and agglutination against anti A and Anti M antibody), motility test. The *Brucella* species and biovars were determined as *Brucella abortus* biovar 3.

For a confirmatory diagnosis of brucellosis, isolation of Brucella bacteria is the best method which is also considered as a “gold standard” test.

The histopathological study for the first time in Bangladesh using guineapig inoculation techniques successfully detectd pathological

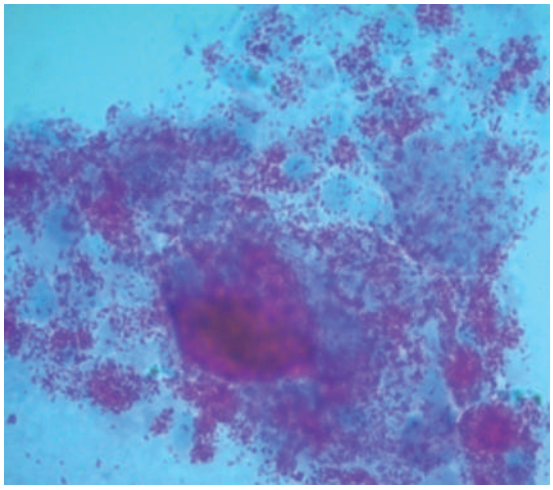

**Fig. 2. Numerous pink organisms in blue background stained by modified ZeihlNeelsen stain (1000x)**

Positive human and cattle samples revealed 602 base pair bands in conventional PCR and amplifications of *B. abortus* specific DNA were recorded through real time PCR. Histopathology of naturally infected aborted fetus indicated lesions in different organs such as fibrinous pleuritic in lungs, focal hepatitis in liver, capsulitis with thickening of capsule in kidney, fibrinous pericarditis in heart, congestion and haemorrage in spleen. Experimentally Brucella infected guineapigs also revealed characteristic lesions in different organs such as granulomas, hemorrhages and necrosis in lungs parenchyma, hemorrhagic endocarditis, monocytes in heart, congestion, hemorrhage and multifocal accumulation of mononuclear cells in kidney. Hemorrhage, congestion and fatty change in liver and caseaus necrosis of splenic foci were also recorded.

A heat killed vaccine was prepared from the isolated *Brucella* organism and inoculated into guineapig subcutaneously at dose ($4\times10^{10}$cfu/2ml). Serum samples were collected upto 9th week for observing the immune response. The antibody (Ab) level started to rise significantly ($p<0.01$) from the 2nd week, reached a peak level in the 4th week and then started to decline significantly ($p<0.01$) up to the 9th week. Heat killed vaccine was administered to indigenous cattle as an inoculum dose of $10 \times 10^{10}$ cfu/5 ml) through a single subcutaneously (SC) injection. For comparison, the live attenuated commercial Brucella abortus strain RB51 vaccine (CZ Veterinaria, SA, Spain) @ 2.0 ml ($10\text{-}34\times10^{9}$) SC as a single dose was given to cattle of another set.

Evaluation of Ab titers of the two groups were done by RBT and ELISA. The Ab titer of cows inoculated with locally prepared heat killed vaccine started to rise significantly and reached a peak level at day 28 and then started to decline significantly ($p < 0.05$) from day 40. On the contrary, the mean Ab titer from the cow inocluated with the commercial RB51 vaccine started to appear insignificantly and reached the peak level at day 60, the changes being very significant from day 0 ($p < 0.05$); after 60 days, the Ab level started to decrease and reach the lowest level at day 150, and the Ab level at day 180 was found to be similar as that at day zero.

Out of four *Brucella*-infected dairy cows treated with antibiotics (oxytetracycline and streptomycin) injections for 48 days showed all-animal negative to *Brucella*-infection by using sero-molecular methods. These four treated animals receiving dual antibiotics therapy showed significantly ($p < 0.05$) increased level of Hb ($10.38 \pm 1.02$) and PCV ($40.83 \pm 1.17$) in comparison with pre-treatment Hb ($8.75 \pm 0.87$) and PCV (39.33) values. The biochemical constituents especially glucose and AST values decreased in comparison with pre-treatment values.

The ELISA results showed that the mean OD value (antibody titer) of the serum of cows infected with Brucella was 2.28 at 0 day of therapy and 1.39, 0.98, 1.17 at day 30 and 90 and 180 days, respectively. The OD value started to decline significantly at day 30 ($p < 0.004$) than from day 0 and decrease up to 90 days ($p < 0.0001$) then started to rise at day 180 insignificantly ($p < 0.210$). After completing the therapy (180 days) change of OD value (antibody titer) is statistically significant from the start of the therapy ($p < 0.003$).

Univariate and binary logistic regressions were used to identify important risk factors of Brucellosis. Three variables (age, parity and abortion) were found to be significantly associated with B. abortus infection in lactating cows. *B. abortus* is the causal agent of bovine brucellosis which was identified for the first time as an etiological agent of human brucellosis in occupationally exposed dairy farm workers in Bangladesh.

## Expected Impact

*Brucella abortus* was detected as the causal agent of bovine brucellosis which was identified for the first time as an etiological agent of human brucellosis in occupationally exposed dairy

farm workers of Bangladesh. The findings of this project woukd indirectly contribute to an enhancement of nutrition security for the people through production of Brucella free livestock leading to an improvement in the quality of animal protein in milk and meat) to country's nutritional security and poverty elimination in future.

Mass vaccination of livestock against brucellosis would be cost effective and would result in a net economic benefit. Combined long acting oxytetracycline and streptomycin against clinical Brucella infection showed some encouraging results in crossbred dairy cows. Results of this study would be helpful to policy makers in planning prevention and control strategies to reduce health hazards and economic losses due to brucellosis in humans and animals.

## Recommendations

- Control of brucellosis in livestock should be a priority inituativ to curb human brucellosis
- Collaboration between the Department of Livestock Services and Department of Public Health needs to be strengthened in terms of research and extension for control of brucelloisis. Actvities of the One Health Hub in Bangladesh should be enhanced and brucellosis should be included in the priority list comprising campaigns for public education, awareness and prevention, and development of an infrastructure for disease surveillance and reporting with a viewb to eradicating brucellosis in livestock and wildlife species
- Further studies are required to explore the cross-species transmission of B. abortus and B. melitensis in animals and humans as well as to study the immune response of the inactivated Brucella vaccine with adjuvant in cattle, sheep, goat and buffaloes.

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This Technical Bulletin has been prepared on the basis of technical information available from a completed BKGET-KGF Funded CGP Project, the details of which are given below:

**Project Code and Title:** TF44-L/17. Livestock and Human Brucellosis: Molecular Diagnosis, Treatment and Control

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