J. Vet. Med. OH Res. (2023). 5(1-2): 97-111 p-2664-2352 : ISSN: e-2664-2360
Website: www.lepvmbj.org DOI: 10.36111/jvmohr.2023.5(1-2).0037

THERAPEUTIC EFFICACY OF COMBINED OXYTETRACYCLINE AND STREPTOMYCIN WITH BENZYLPENICILLIN IN NATURALLY BRUCELLA-INFECTED DAIRY CROSS-BRED COWS IN BANGLADESH

S. M. A. K. Hussaini, M. S. Alam, M. M. Hasan, S. T. Sharmy, R. R. Sarker, F. Yeasmin, C. S. Chouhan, M. J. S. Bhuiyan, A. A. Maruf, F. Yasmin[1], A. K. M. A. Rahman, M. M. Rahman[2], M. R. Alam[2], H. Neubauer[3] and M. S. Rahman*

Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2200, Bangladesh; [1]Department of Training, Planning and Technology Testing Division, Bangladesh Livestock Research Institute, Savar; [2]Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2200, Bangladesh; [3]OIE Reference Laboratory for Brucellosis, Institute of Bacterial Infections and Zoonoses, Friedrich-Loeffler-Institute, Germany *Corresponding author e-mail: prithul02@yahoo.co.uk

ABSTRACT

Background: Brucellosis is an important infectious zoonotic disease caused by bacteria of the genus *Brucella*. It has global significance due to its adverse effects on public health, economics, and trade of animals and animal products. The causative agents of brucellosis, which have no plasmids or toxins and show distinctive virulence, are most significantly represented by intracellular survival. The commonly used antimicrobial drugs are not capable of entering the *Brucella*-infected cells that are safe from antibiotic treatment, but such treatments are only effective in the bacteremia phase of infection. Reports on the therapeutic management and cure of bovine brucellosis are limited in the literature. Therefore, an attempt was made to evaluate the combined oxytetracycline and streptomycin with benzylpenicillin injections in naturally *Brucella*-infected high-yielding dairy cross-breed cows.

Objectives: This study was conducted to determine the sero-molecular prevalence and therapeutic responses of combined oxytetracycline and streptomycin with benzylpenicillin in naturally Brucella-infected dairy cross-bred cows.

Materials and Methods: Serum samples of 460 (290 from Central Cattle Breeding and Dairy Farm, 170 from Military Dairy Farm, Savar, Dhaka) lactating cross-bred cows along with their milk samples were collected randomly. Serum samples were screened for brucellosis with the Rapid Antigen Kit Test, Rose Bengal Test (RBT), and Milk ring test (MRT), and positive samples were tested further with PCR for confirmatory diagnosis. Out of 11 all tests positive cows, of which three had a history of abortion were selected for therapeutic trials with combined long-acting oxytetracycline @ 25 mg/kg BW 3 doses at 24-hour intervals via intrauterine injection and streptomycin @ 20 mg/kg BW with benzylpenicillin @ 40,000 IU/kg 5 doses at 24 hours interval via intramuscular injections. Blood samples of all the *Brucella*-negative control and pre- and post-treatment stages of all the *Brucella*-infected cows were tested for *Brucella* by using PCR.

Results: Out of 460 randomly collected serum samples, 18 serum samples 3.9% (95% CI 2.4-6.2) were found positive using RBT and Rapid Antigen Kit Test and 13 of the samples 2.8% (CI 1.5-4.9) were positive respectively. The overall seroprevalence was found to be 3.9% (95% CI 2.4-6.2) using RBT and Rapid Antigen Kit Test and 2.8% (CI 1.5-4.9) using Milk Ring Test, respectively. The odds of brucellosis were 7.4 times (95% CI: 2.5-21.5) higher in cows with repeat breeding than those without repeat breeding. Moreover, the seroprevalence of brucellosis was significantly higher (Odds ratio: 15.7; 95% CI: 5.2-47.4) in cows with retention of fetal membranes than without retention of fetal membranes. Base pair PCR 602. However, of the three treated cows, three became pregnant on artificial insemination with a normal reproductive cycle which needs to explore its status in further research.

Conclusions: Combined antibiotic with oxytetracycline (I/U) and streptomycin with benzylpenicillin (I/M) against clinical *Brucella* infection showed some encouraging results and can be implemented at the field level.

Keywords: Brucellosis, Cross-bred cows, Rapid Kit test, Rose Bengal test, PCR, Intrauterine, therapeutic response

Article Info: Article Code No. © LEP: JVMOHR/0037/2023
Received: 15 October 2023 Revised: 30 November 2023 Accepted: 10 December 2023 Published: 30 December 20223

Cite this article: Hussaini SMAK, Alam MS, Hasan MM, Sharmy ST, Sarker RR, Yeasmin F, Chouhan CS, Bhuiyan MJS, Maruf AA, Yasmin F, Rahman AKMA, Rahman MM, Alam MR, Neubauer N and Rahman MS (2023). Therapeutic efficacy of combined oxytetracycline and streptomycin with benzylpenicillin in naturally Brucella-infected dairy cross-bred cows in Bangladesh. J. *Vet. Med. OH Res.* 5 (1-2): 97-111 [doi: 10.36111/jvmohr.2023.5(1-2).0037]

INTRODUCTION

Brucellosis is one of the most significant developing zoonotic chronic diseases of domestic and wild animals throughout the world which is caused by intracellular *Brucella* species since Bruce discovered *Brucella melitensis* in 1887.[1] However, many nations that were once thought to be endemic, such as Canada, Japan, Australia, New Zealand, Israel, western and northern Europe, as well as the majority of Latin America, have attained control.[2,3] According to reports, Syria has the greatest incidence of human brucellosis per million people, with 1,604.4 cases, followed by 391.0 cases in Mongolia, 268.8 cases in Iraq, 211.9 cases in Tajikistan, 149.5 cases in Saudi Arabia, and 141.6 cases in Iran.[4-7] Additionally, outbreaks of *B. abortus*-related brucellosis in cattle have been documented in Canada, Spain, Brazil, and even India. *B. melitensis-related* brucellosis outbreaks in cattle have also been documented there, but Bangladesh has not yet been reported.[8-13] The WHO has classified this disease as one of the top "neglected zoonotic diseases" in the world because of its specific negative effects on animal productivity and human health in underdeveloped nations.[14,15] Brucella infection is transmitted from infected animals to humans by direct contact with infected blood, placentas, fetuses or uterine secretions or through the consumption of infected and raw animal products, especially milk and milk products. The three main brucellosis-causing organisms in developing nations are *B. abortus* (cattle*), B. melitensis* (small ruminants), and *B. suis* (swine).[16] Cattle may become infected with any of these three species of *Brucella*, with *B. abortus* serving as the primary causative agent and *B. suis*, *B. melitensis*, and *B. suis* acting less commonly and sometimes, respectively.[3] This disease is endemic to the developing globe and may appear as acute outbreaks, chronic illnesses, or subclinical or carrier infections that last for the whole of life with recurrences possibly linked to abortion in late pregnancy in cattle.[17] Infected pregnant cattle develop placentitis, which is followed by late-term abortion, temporary or permanent infertility, weak newborn calves, and reduced milk production. Infected male cattle develop orchitis, and both male and female cattle develop infertility and hygromas. However, the disease is asymptomatic in non-gestational cattle, and the majority of infected animals only have one abortion during their lifetime. These animals may also develop into carriers and continue to shed the bacteria for a long time, particularly after parturition.[6,18] Infertility in cattle has been linked to brucellosis for the first time since reports from Bangladesh in 1967.[19] According to recently reviewed statistics, Bangladesh has a seroprevalence of brucellosis of 3.7% in cattle, 4.0% in buffaloes, 3.6% in goats, 7.3% in sheep, 4.8% in pigs, and 4.0% in dogs.[20,21] Bangladesh has not yet reported any clinical instances of brucellosis, but they may be related to the 435 (0.70%) documented aborted cases.[22] However, dairy cattle in Bangladesh have been isolated from the *B. abortus* biovar 3 strain.[23] Due to false-positive results from traditional serological tests, both sero-molecular methods and bacterial culture are needed to isolate and identify the bacteria.[24,25] Because it is resistant to standard medicines and antibodies, the intracellular pathogen *Brucella* typically lies dormant inside the phagosome vacuoles of the afflicted macrophages. As a result, the antibiotics work against the stage of active bacteremia but not against the stage of intracellular carrier. As a result, numerous researchers have attempted to cure the intracellular stage of brucellosis without success.[26-28] Because of this, effective antibiotic therapy for both animals and humans is still debatable and would be prohibitively expensive for most animal species.[29] Antibiotic therapy has

been used successfully in treating clinical brucellosis in humans, but it has not been utilized frequently to treat subclinical (intracellular bacteria) and clinical brucellosis in animals. This is mostly because these treatments are expensive and have a poor rate of antibiotic efficacy.[30] In 67 to 71.4% of infected cows, a combination of long-acting oxytetracycline (OTC) and streptomycin (ST) eradicated *Brucella abortus.*[31] While two rams who had clinical epididymitis before treatment remained affected, the combination treatment removed *Brucella ovis* from 11 of 12 (91.6%) experimentally infected treated rams.[32] OTC and ST are thought to be the most efficient treatments for brucellosis because they can penetrate the bacterial cell wall, block protein production, and provide a long-lasting concentration in plasma.[33] Long-acting oxytetracycline @ 25 mg/kg BW 16 doses at 72-hour intervals and streptomycin @ 25 mg/kg BW 10 doses at 24-hour interval injections was applied with the combination of anti-inflammatory and antihistaminic drugs in the treatment of brucellosis which was successful after 90 days of observation.[34] But, The limitation of this treatment was excessive cost and time-consuming.[34] Thus, the objective of the study was to develop a cost-effective treatment protocol, farmer-friendly, effective, and time-efficient.

MATERIALS AND METHODS

The study was conducted in Central Cattle Breeding and Dairy Farm (CCBDF) and Military Dairy Farm (MDF), Savar, Dhaka. By utilizing the jugular vein, 5 to 10 ml of blood from 460 randomly chosen lactating cows were drawn. Of these, 290 samples were taken from the CCBDF and 170 samples were taken from the MDF. To separate serum samples, the blood-filled tubes were centrifuged at 3,000 rpm for 10 minutes after being held vertically at room temperature for an hour. Clear sera were produced after centrifugation, and they were transferred to sterile, labelled Eppendorf tubes using sanitized Pasteur pipettes. The tubes were then stored at -20 °C until they were used for the Rose Bengal and fast kit tests. Animals that tested positive for the Rose Bengal and Rapid test kit tests and molecular testing (PCR).[13]

Laboratory Tests

The collected sera were subjected to two serological assays (a) the Rapid *Brucella* Antibody Test (RBT) and (b) the Rapid Kit Test. For confirmation, one molecular test polymerase chain reaction (PCR) was performed.

Rose Bengal Test (RBT)

According to the procedure that was described, the RBT using cattle sera was carried out.[35] Sufficient antigen (Instituto de Salud Tropical University's Edificio, CIMA, Avda, Pio XII, 55 E-31008, Pamplona, Spain), positive and negative controls, as well as test sera. Sera for a day's worth of testing was taken out of the refrigerator and warmed up. Equal quantities (30 µl) of serum were combined and rotated for 4 minutes on a glass plate. Any sign of agglutination is taken into consideration as a positive case of brucellosis in the tested serum, and the results are graded as positive in the case of clear agglutination and negative in the absence of such indications (Fig. 1).

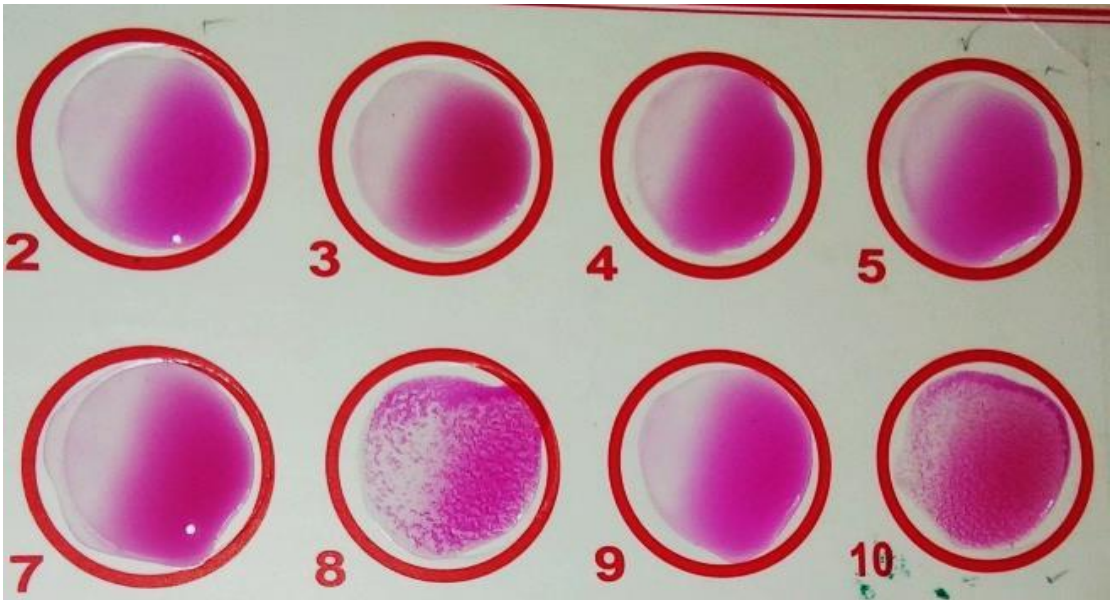

Fig. 1. Agglutination reaction (positive for brucellosis) with Rose Bengal antigen in samples No. 8 and No. 10.

Rapid Brucella Antibody Test Kit

Cattle sera were tested using an antigen Rapid test kit, or *Brucella* antibody test kit (Senspert® *Brucella* Ab Test Kit, Korea), to find *B. abortus* antibodies. The kit was maintained at a temperature between 2-30 °C. After opening the kit, it was used within ten minutes of the wrap. The test was carried out as per the instructions provided by the manufacturer. The specimen had well received a single drop (10 µl). Two drops (80 µl) of buffer were added after the fluid had completely drained into the specimen well. Results were read in 5-10 minutes. Despite the test's outcome, the purple band must show up on the control line. The outcome is determined by the existence of another band on the test line (Fig. 2).

Milk Ring Test (MRT)

The antigen was kept at room temperature (18 to 23^0C) for 1 hour before starting the test. After proper mixing, 1.0 ml of milk sample and 50 µl of MRT antigen reagent were added to each tube. The milk and MRT reagent were mixed with vortex and incubated for 1 hour at +37 ^{0}C and then between +2 to +8 ^{0}C for 18 to 20 hours. Milk in the middle tube indicates positive results showing a ring of cream more colored than the underlying milk (Fig. 3). Milk in the two corner tubes indicates negative results showing a ring of cream less colored than underlining milk.

DNA extraction

According to the instructions of the Purelink DNA extraction small kit (USA), DNA was extracted from the serum of cows that tested positive in a serological test.

Fig. 2. *Brucella* rapid test-the presence of two purple color bands within the result window indicating positive (right).

Fig. 3. Milk Ring Test (MRT) with cow milk

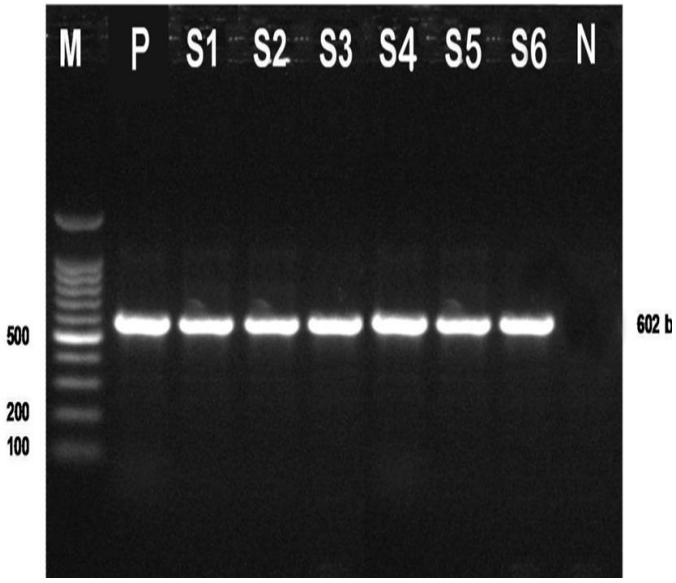

Fig. 4. Gel electrophoresis image of amplicons produced from *IS711* conventional PCR assay (602 bp) from milk and serum. Lane M = molecular marker, lane P = positive control, lane S1-S3 = milk, lane S4-S6 = serum from MDF, lane N = negative control

Primer design for this study

Using the online design application Primer 3 Plus, primer was created. To create the primer, the species *B. abortus* and the IS711 gene option were also searched. The IS711 gene's nucleotide sequences were copied from NCBI in FASTA format. The sequence has been put into the software company's website under the heading "source sequence below." Primers were chosen based on the source sequence's target area. The software chose the forward and reverse primers, as well as the amplicon size (602 BP).[36]

Polymerase chain reaction (PCR)

For each sample, PCR was used to amplify the IS711 repetitive genetic element, which is unique to the *B. abortus* species and measures 602 bp. In the current study, the forward and reverse primers were 5‟- GCTTGCCTTGATCTTTTTGG-3‟ and 5‟- AATGCAGACAGGCCCTAATG-3‟ respectively. Two µl of genomic DNA, 25 µl of PCR master mix (Promega Corporation, Madison, WI, USA), two µl of the forward and reverse primers (10-20 pmol each), and 19 µl of nuclease-free water were all incorporated into each 50 µl reaction mixture. Pre-heating at 95°C for 2 minutes was followed by 32 cycles of denaturation at 95 °C for 30 seconds, annealing at 48 °C for 30 seconds, and extension at 72 °C for 45 seconds. The overnight holding temperature was 4 °C and the final extension period was 5 minutes at 72 °C. The PCR results were examined using 1% agarose gel electrophoresis (Alpha Imager, Wiesbaden Germany), with ethidium bromide staining, and a gel documentation system (Alpha Imager) was used for photography.

Blood was collected from three cows who tested positive for serological and molecular markers, for PCR testing. The same procedure was used to collect blood for the serological test; 10 ml of blood was drawn from the jugular vein using a disposable sterile syringe (12.0 ml), and then 5 ml of blood was immediately transferred to a vacutainer tube and 5 ml to an EDTA vial (Becton Dickson), with the vacutainer tubes being labeled and kept in the refrigerator at 4 °C until processing. Sera were divided by centrifugation at 3000 rpm for 10 minutes and kept in 1.5 ml Eppendorf tubes at -20°C until tested.

Therapeutic trial

Selected cattle for treatment trials were those that tested positive in both serological and molecular tests. Six dairy cows were divided into two groups: a control group (*Brucella* negative) with three (n = 3) cows and a treated group (*Brucella* positive) with three (n = 3) cows. The three Holstein-Friesian cross-bred cows that tested positive for *Brucella* and had a history of abortion and stillbirth were chosen for this treatment study. Each of the three *Brucella*-positive cows received 25 ml of 3 intrauterine injections of oxytetracycline (Renamycin 100,® Renata Ltd., Bangladesh) @ 25 mg/kg BW every 24 hours after heat for 3 days, along with 5 days of intrauterine injections of Streptomycin @ 20 mg/kg and Benzylpenicillin @ 40,000 iu/kg (2.5 gm vial, ACME Laboratories Ltd., Bangladesh) every 24 hours.

Statistical analysis

The paired sample t-test was employed for statistical analyses using the SPSS program version 22 to determine the significant differences between *Brucella* negative and *Brucella* positive, pre-

and post-treatment groups. The assumption of statistical significance was a P value > 0.05.

RESULTS

Out of 460 randomly collected serum samples, 18 serum samples 3.9% (95% CI 2.4-6.2) were found positive using RBT and Rapid Antigen Kit Test and 13 of the samples, 2.8% (CI 1.5-4.9), and milk ring test were positive, respectively. The results of the univariable association between brucellosis sero status (RBT) and explanatory variables are presented in Table 1.

Table 1. Univariable association of Rose Bengal test results with explanatory variables

S/N	Variables	Categories	Results +ve	-ve	Chi-square test p-value
1.	Age (years)	≤ 7	05 (02.0)	244	0.04
		> 7	13 (06.2)	198	
2.	Parity	1-2	11 (04.5)	236	0.68
		3-4	07 (03.3)	206	
3.	Abortion	Yes	11 (09.3)	107	0.001
		No	07 (02.0)	335	
4.	Repeat breeding	Yes	12 (11.4)	093	<0.001
		No	06 (01.7)	349	
5.	Retention of placenta	Yes	13 (17.3)	349	<0.001
		No	05 (01.3)	380	
6.	Area	Central Cattle Breeding and Dairy Farm, Savar	11 (03.8)	279	1
		Military Farm, Savar	07 (04.1)	163	

Table 2 shows the results of the univariable association between brucellosis sero-status (RBT) and explanatory variables. Only age, repeat breeding and retention of fetal membranes were significantly associated with brucellosis.

Table 2. Risk factors retained in the final multivariable logistic regression model

Variable	Categories	Coefficients	SE	Odds ratio (95% Confidence Interval)	p-value
Repeat Breeding	Yes	1.99	0.57	7.4 (2.5-21.5)	< 0.001
	No	-	-	Reference	
Retention of Placenta	Yes	2.76	0.56	15.7 (5.2-47.4)	< 0.001
	No	-	-	Reference	

Finally, repeat breeding and retention of fetal membranes were associated with brucellosis. The odds of brucellosis were 7.4 times (95% CI: 2.5-21.5) higher in cows with repeat

breeding than without repeat breeding. Moreover, the seroprevalence of brucellosis was significantly higher (Odds ratio: 15.7; 95% CI: 5.2-47.4) in cows with retention of fetal membranes than without retention of fetal membranes.

Therapeutic response

Three *Brucella*-infected dairy cows treated for 5 days with antibiotic injections (oxytetracycline and streptomycin with Benzylpenicillin) were found to be negative for *Brucella* infection using sero-molecular techniques. These three treated animals that received dual antibiotic therapy showed significant results. Successfully those cows were recovered, and then the next heat artificial insemination was performed. The animal was observed until the next heat. In the next heat, artificial insemination was performed with liquid semen. The animal became pregnant and parturition was normal. The dam and calf were also *Brucella* negative. The calf was healthy. It can be implemented at the field level.

DISCUSSION

One of the most economically significant chronic zoonotic illnesses, brucellosis affects numerous species of food and companion animals, including humans, globally.[37] In some developed nations, this illness has been eradicated from their cattle herds, but it is still a common endemic disease in most developing nations, including Bangladesh.[38,39] Because of the widespread practice of slaughtering cattle due to reasons related to religion, society, and animal husbandry methods, bovine brucellosis has become a severe issue among Indian dairy cattle.[40] Economic losses from brucellosis were estimated to be BDT 60 million in 1983,[41] and more recently, they were assessed to be BDT 48.4364 million (US $ 0.61 million) yearly, exclusively from the Mymensingh area of Bangladesh.[42] Several sero-incidence investigations on brucellosis in humans and animals have shown Bangladesh as having a high prevalence of the disease.[38,39] Due to Bangladesh's large population, unsanitary circumstances, poor management methods, unrestricted cattle imports through borders, lack of focus on serosurvey, and monitoring and control of chronic and zoonotic brucellosis, the disease is endemic in both animals and humans.[41] Clinical brucellosis may only occur in late-gestation abortions and stillbirths, and these aborted cases are typically not reported to veterinary services and go untreated. However, because there are no clinical symptoms and because intracellular pathogens are involved, treatment for these aborted cases is rarely used. Treatment failure and relapse rates are also high, dependent on the drug combinations used, and treatment is expensive.[40] Small ruminants and cattle are frequently housed together, and *B. melitensis* can also infect cattle. Due to the lack of effective preventative and control methods, bovine brucellosis, which is zoonotic, continues to pose a severe threat to public health, social and economic advancement, food security, and food safety in developing nations.[40] *Brucella* antigens have been reported with infection and treatment methods, with the effect of antibiotic treatment on the murine antibody response.[43,44] In mice, the antibody response to Brucella cytoplasmic proteins is found to be affected by early antibiotic therapy. Since standard antibacterial medications can't penetrate, aggregate in, or remain in mammalian cells, infections with intracellular bacterial pathogens are challenging to treat.[45]

The bacterial lipopolysaccharide (LPS) interacts with the microphage membrane to produce a

phagosome, which is a membrane-bound protein (vacuole), which is how *Brucella* organisms enter macrophages. The bacteria release cyclic beta-1,2 glucans proteins and embed themselves on the outer side of the phagosome membrane, which prevents the phagosome from fusing with lysosomes. These actions allow the bacteria to resist respiratory burst phagosome metabolites and acidic pH. The lack of phagosome-lysosomal fusion, which normally kills and digests infections, offers phagosomes a haven for bacterial proliferation.[46] The bacteria spread throughout the body of the host after being released from the macrophages (bacteremia) and localize in the placenta and fetus of pregnant cattle that are predisposed to miscarriage and calf stillbirth. Dairy cow abortion is typically defined as the death of a fetus between the ages of 42 and 260 days; pregnancies lost before 42 days are typically referred to as early embryonic death; and a calf born dead between the ages of 260 days and its full term is classified as a stillbirth.[47] Mammalian macrophages have a lifespan of more than 90 days, and after that lifespan, the afflicted macrophages routinely undergo programmed death, which may release bacteria and result in bacteremia.[48]

The development of efficient antibacterial drugs against intracellular infections, particularly those that block inside the phagocytic macrophages, such as *Mycobacterium, Salmonella, Brucella*, *Listeria*, *Shigella*, and methicillin-resistant *Staphylococcus aureus* (MRSA), is currently a major issue. The bacteria multiply and build up a repository in these intracellular safe havens, frequently leading to chronic infections. Patients who contract the infection go on to carry the pathogens for the rest of their lives and either experience chronic infection or pass away from invasive forms of the infection. The therapeutic benefits of the majority of antibiotics, such as aminoglycosides, glycopeptides, and macrolides, are only partially effective against these intracellular bacteria.[49] A special class of cell-penetrating peptides with inherent antimicrobial activity known as cationic amphiphilic polyproline helices (CAPHs) has been proven to successfully target intracellular bacteria as a broad-spectrum antibiotic.[50]

The mononuclear phagocytic cells of the lymph nodes, liver, spleen, and bone marrow are where *Brucella* organisms, which are facultative intracellular infections, are found. Numerous antimicrobial medications show anti-*Brucella* action, although in vitro susceptibility test results are not always indicative of clinical success. This could be a result of the organism's intracellular position, which offers some security from human defenses and the effects of antimicrobial medications. Numerous studies on treatment trials against *Brucella* infections in humans and animals have been published, but none of them have been successful.[51]

Since the *Brucella* infection continues to be active inside phagosome vacuoles inside macrophages (phagocytic cells) of the reticulo-endothelium system, it is resistant to both antibiotics and antibodies. To treat brucellosis, it is therefore necessary to use antibiotics with permeability into the phagocytic cells where this bacterium is protected and multiplies in infected hosts.[52] Cows were considered to have received a successful treatment when *Brucella* organisms could no longer be found in udder secretions or any of the chosen tissue specimens taken from the treated animals. The persistence or resumption of *Brucella* organism shedding in udder secretions or the isolation of *Brucella* organisms from any tissue material collected are both considered to be irrefutable proof that a treatment has failed. Abortions in sick cows have decreased because of the use of broad-spectrum antibiotics such as aureomycin, terramycin,

tetracyclines, and streptomycin (ST), either alone or in combination. However, the consensus was that such treatment may not be suitable for the control of udder infections due to the high cost of therapy, the presence of antibiotic residue in milk, and the inability to cure udder infections in many cases.[40]

Streptomycin and oxytetracycline are considered to be the most efficient treatments for brucellosis because they can penetrate bacterial cell walls, limit protein synthesis, and provide long-lasting quantities in plasma.[53] Oxytetracycline and streptomycin together have been shown to have synergistic effects in vitro.[54] Oxytetracycline and streptomycin injections were combined, and the results were better than either treatment alone.[55] Both the antibiotics when given intramuscularly, showed some side effects like swelling and irritation.[34] Anti-inflammatory and anti-histaminic drugs were given to eradicate that problem. This intra-muscular treatment of OTD and ST was observed for up to 90 days and the treatment cost was 22,500 BDT.[34] Considering these facts, the OTC (Injection Renamycine® 100) was given three intrauterine infusions after heat. As OTC was given as an intrauterine infusion and didn't cause any irritation or swelling to the skin, an anti-inflammatory and anti-histaminic drug was unnecessary. The treatment was unnecessary to follow up to 90 days as the animal got pregnant after a short treatment. The streptomycin with Benzylpenicillin was given intramuscularly which has a synergistic effect with OTC and is non-irritant to skin. The treatment is cost-effective and requires less time than the previous study.[34] Therefore, this combination therapy of OTC intrauterine infusion and intramuscular ST with Benzylpenicillin protocol can be followed at the field level to treat brucellosis. Further research is needed to understand the mechanism of this developed treatment protocol.

CONCLUSIONS

Bangladesh is a brucellosis-endemic country, thus conducting periodic seroprevalence tests on animals that are sensitive to the disease can aid in the early detection of *Brucella* infection, which is necessary for the control and eradication of brucellosis. The consequences of brucellosis on animal health can be identified by serological tests and assessment of molecular detection primers. The effectiveness of long acting oxytetracycline and streptomycin coupled against sub-clinical *Brucella* infection in crossbred dairy cows exhibited some positive results. However, the antibody titer increased after 180 days of medication withdrawal which implies a return. The antibacterial effectiveness of frequently used antibiotics against intracellular pathogens is low because they have poor cellular absorption and frequently have restricted access to intracellular targets. The cell-penetrating peptides (CPPs) demonstrated excellent promise as carriers for antibacterial drugs and may help to develop novel therapeutic tools to treat infectious disorders brought on by intracellular pathogens. In this study, three treated animals received combined antibiotics (oxytetracycline and streptomycin with Benzylpenicillin) therapy oxytetracycline at intrauterine and streptomycin with Benzylpenicillin at intramuscularly showed significant effectiveness of the treatment. Successfully those cows were recovered, and then in the next heat, artificial insemination was performed. The animal became pregnant and parturition was normal. The dam and calf were also *Brucella* negative. The calf was healthy. It can be implemented field

level. Therefore, the interdisciplinary "One Health" character of brucellosis' consequences suggests that veterinary, medical, public health and other relevant experts should work together for brucellosis control and eradication in both animals and humans.

ETHICAL APPROVAL

All animal-related procedures and methods were carried out following the guidelines by the Animal Welfare and Experimentation Ethical Committee of the Bangladesh Agricultural University, Mymensingh (Ethical approval number - AWEEC/BAU/2023(55)

ACKNOWLEDGEMENTS

This research work was supported by LDDP Research and Innovation Sub-project, Livestock and Dairy Development Project, Department of Livestock Services, Farmgate, Dhaka-Transmission dynamics of brucellosis and abortion risk factors in large dairy herds in Bangladesh (Project No. 2022/07/LDDP).

REFERENCES

01. Corbel MJ (1997). Brucellosis: an overview. *Emerging Infectious Diseases* 3: 213-221 [doi: 10.3201/eid0302.970219]
02. Pappas GD, Papadimitriou P, Akritidis N, Christou L and Tsianos EV (2006). The new global map of human brucellosis. *The Lancet Infectious Diseases* 6: 91-99 [doi: 10.1016/s1473-3099(06)70382-6]

03. OIE (2016). Brucellosis. https://www.oie.int/en/animal-health-in-the-world/animal-diseases/Brucellosis/

04. Ebrighgt JR, Altantsetseg T, Oyungerel R (2003). Emerging infectious diseases in Mongolia. *Emerging Infectious Diseases*. 9: 1509-1515 [doi: 10.3201/eid0912.020520]

05. Dean AS, Crump L, Greter H, Schelling E and Zinsstag J (2012). Global burden of human brucellosis: a systematic review of disease frequency. *PLoS Neglected Tropical Diseases* 6(10): e1865 [doi: 10.1371/journal.pntd.0001865]

06. Hull NC and Schumaker BA (2018). Comparisons of brucellosis between human and veterinary medicine. *Infection Ecology and Epidemiology* 8(1): 1500846 [doi: 10.1080/20008686.2018.1500846]

07. Zhang WY, Guo WD, Sun SH, Jiang JF, Sun HL, Li SL, Liu W and Cao WC (2010). Human brucellosis, inner Mongolia, China. *Emerging Infectious Diseases* 16: 2001-2003 [doi: 10.3201/eid1612.091081]

08. Forbes LB and Steele TB (1989). An outbreak of *Brucella abortus* biovar 2 in Canadian cattle. Canadian Veterinary Journal 30: 888-893 [PMID: 17423457]

09. Alvarez J, Saez JL, Garcia N, Serrat C, Perez-Sancho M, Gonzalez S, Ortega MJ, Gou J, Carbajo F Goyache J and Dominguez L (2011). Management of an outbreak of brucellosis due to *B. melitensis* in dairy cattle in Spain. *Research in Veterinary Science* 90: 208-211 [doi: 10.1016/j.rvsc.2010.05.028]

10. Lemos TS, Cequinel JC, Costa TP, Navarro AB, Sprada A, Shibata FK, Gondolfo R and Tuon FF (2018). Outbreak of human brucellosis in Southern Brazil and historical review of data from 2009 to 2018. *PLoS Neglected Tropical Diseases* 12(9): e0006770. [doi: 10.1371/journal.pntd.0006770]

11. Herriman R (2016). India: Brucellosis outbreak in Kolar, Karnataka. outbreaknewstoday.com/ indiabrucellosis-outbreak-kolar-karnataka-78159/ [Accessed on 3 December 2023]

12. Deka RP, Magnusson U, Grace D and Lindahl J (2018). Bovine brucellosis: prevalence, risk factors, economic cost and control options with particular reference to India- a review. *Infection Ecology & Epidemiology* 8:1, 1556548 [doi: 10.1080/20008686.2018.1556548]

13. Rahman MS (2019). Brucella abortus killed vaccine: The achievement of 52 years (1967-2019) in Bangladesh. *EC Veterinary Science* ECO.02: 14-21

14. Franc KA, Krecek RC, Hasler BN and Arenas-Gamboa AM (2018). Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action. *BMC Public Health* 18, 125 [doi: 10.1186/s12889-017-5016-y]

15. WHO (2019) Neglected zoonotic diseases. World Health Organization. https://www.who.int/neglected_ diseases/zoonoses/infections_more/en/ [Accessed on 2 December 2023]

16. Olsen SC and Palmer MV (2014). Advancement of knowledge of Brucella over the past 50 years. *Veterinary Pathology*, *51*(6), 1076-1089 [doi: 10.1177/0300985814540545]

17. Maurin M (2015). Antibiotic susceptibility testing of Brucella species- old and new drugs. intechopen.com/chapters/49399 [doi: 10.5772/61706]

18. Deka RP, Lindahl J and Grace D (2017). Epidemiological research on brucellosis in India: knowledge generated and gaps. Workshop on One Health approach for brucellosis control in India, New Delhi, India 26 October 2017. Pp.1-20, slideshare.net/slideshow/brucellosis-india/86963864 (https://www.slideshare. net/ILRI/brucellosis-india)

19. Mia AS and Islam H (1967). A preliminary study on the incidence of bovine infertility and the economic loss caused by it. Pakistan Journal of Veterinary Science 1: 5-10

20. Islam MA, Khatun MM, Were SR, Sriranganathan N and Boyle SM (2013). A review of Brucella seroprevalence among humans and animals in Bangladesh with special emphasis on epidemiology, risk factors and control opportunities. *Veterinary Microbiology* 166: 317-326 [doi: 10.1016/j.vetmic. 2013.06.014]

21. Rahman MS, Sarker RR, Melzer F, Sprague LD and Neubauer H (2014). Brucellosis in human and domestic animals in Bangladesh: A review. *African Journal of Microbiology Research* 8: 3580- 3594 [doi: 10.5897/AJMR2014.7074]

22. Samad MA (2019). A 50-year review on the prevalence of clinical diseases and disorders of cattle in Bangladesh. *Journal of Veterinary Medical and One Health Research* 1: 1-16 [doi: 10.36111/jvmohr. 2019.1(1).0001]

23. Islam MS, Garofolo G, Sacchini L, Dainty AC, Khatun MM, Saha S and Islam MA (2019). First isolation, identification and genetic characterization of *Brucella abortus* biovar 3 from dairy cattle in Bangladesh. *Veterinary Medicine and Science* 5: 556-562 [doi: 10.1002/vms3.193]

24. Bowden RA, Racaro GC and Baldi PC (1999). Effect of early antibiotic treatment on the antibody response to cytoplasmic proteins of *Brucella melitensis* in mice. *Clinical and Diagnostic Laboratory Immunology* 6: 440-443

25. Harun A and Gulin E (2012). The relationship between Standard Tube Agglutination titers in brucellosis and biochemical and hematologic parameters. *Journal of Clinical and Analytical Medicine* 3: 432-434

26. Hall WH and Manion RE (1970). *In vitro* susceptibility of Brucella to various antibiotics. *Applied Microbiology* 20: 600-604

27. Hall WH (1990). Modern chemotherapy for brucellosis in humans. *Review of Infectious Diseases* 12: 1060-1099

28. Khan MZ and Zahoor M (2018). An overview of brucellosis in cattle and humans and its serological and molecular diagnosis in control strategies. *Tropical Medicine and Infectious Diseases* 3: 65[doi: 10.3390/tropicalmed3020065]

29. Saxena HM and Raj S (2018). A novel immunotherapy of brucellosis in cows monitored noninvasively through a specific biomarker. *PLOS Neglected Tropical Diseases* 12 (4): e0006393 [doi: 10.1371/journal.pntd.0006393]

30. Roushan MRH, Mohraz M, Hajiahmadi M and Ramezani A (2006). Efficacy of gentamicin plus doxycycline versus streptomycin in the treatment of brucellosis in humans. *Clinical Infectious Diseases* 42: 1075-1080 [doi: 10.1086/501359]

31. Martin CM, de Bagues MPJ, Barberan M and Blasco JM (1989). Efficacy of long acting oxytetracycline alone or in combination with streptomycin for treatment of *Brucella ovis* infections in rams. *American Journal of Veterinary Research* 50: 560-563 [PMID: 2712423]

32. El Azab MFA (2015). Evaluation of serum enzyme activities and protein fractions in Brucella infected cows. Turkish Journal of Veterinary and Animal Science 39: 480-484 [doi: 10.3906/vet1504-31]

33. Kushwaha N, Rajora VS, Mohan A, Singh JL and Shukla SK (2014). Assessment of hematobiochemical parameters and therapeutics on Brucella-infected cattle. *Journal of Microbiology and Experimentation* 1:1-5 [doi: 10.15406/jmen.2014.01.00012]

34. Maruf AA, Yasmin F, Yeasmin F, Alam MN, Rahman MM, Hasan MM, Alam M, Alam MR, Rahman AK and Rahman MS (2019). Assessment of haemato-biochemical and therapeutic responses of chronic brucellosis in crossbred dairy cows in Bangladesh. *Journal of Veterinary Medical and One Health Research*. 1: 211-29 [DOI: 6111/jvmohr.2019.1(2).0013].

35. Alton G, Jones L, Angus R, Verger J (1988). Techniques for the Brucellosis Laboratory, Institute National de la Recherché Agronomies, Paris.

36. Untergasser A, Nijveen H, Rao X, Bisseling T, Geurts R and Leunissen JAM (2007). Primer3 Plus, an enhanced web interface to Primer3. Nucleic Acids Research 35.2: W71-74 [doi: 10.1093/nar/gkm306]

37. Khan MZ and Zahoor M (2018). An overview of brucellosis in cattle and humans and its serological and molecular diagnosis in control strategies. *Tropical Medicine and Infectious Diseases* 3: 65[doi: 10.3390/tropicalmed3020065]

38. Islam MA, Khatun MM, Were SR, Sriranganathan N and Boyle SM (2013). A review of Brucella seroprevalence among humans and animals in Bangladesh with special emphasis on epidemiology, risk factors and control opportunities. *Veterinary Microbiology* 166: 317-326 [doi: 10.1016/j.vetmic.2013.06.014]

39. Rahman MS, Sarker RR, Melzer F, Sprague LD and Neubauer H (2014). Brucellosis in human and domestic animals in Bangladesh: A review. *African Journal of Microbiology Research* 8: 3580- 3594 [doi: 10.5897/AJMR2014.7074]

40. Singh SV, Gupta VK, Kumar A, Gupta S, Tiwari R and Dhama K (2014). Therapeutic management of bovine brucellosis in endemically infected dairy cattle herd of native Sahiwal breed. *Advances in Animal and Veterinary Sciences* 2(1S): 32-36. [doi: 10.14737/journal.aavs/ 2014/2.1s.32.36]

41. Samad MA (2019). A 50-year review on the prevalence of clinical diseases and disorders of cattle in Bangladesh. *Journal of Veterinary Medical and One Health Research* 1: 1-16 [doi: 10.36111/jvmohr. 2019.1(1).0001]

42. Ahmed BS, Osmani GM, Rahman AKMA, Hasan MM, Maruf MA, Karim MF, Karim SMA, Asaduzzaman M, Hasan MR, Rahman MH and Rahman MS (2018). Economic impact of caprine and ovine brucellosis in Mymensingh district, Bangladesh. *Bangladesh Journal of Veterinary Medicine* 16: 193-203 [doi: 10.33109/bjvmjd1805]

43. Bowden RA, Racaro GC and Baldi PC (1999). Effect of early antibiotic treatment on the antibody response to cytoplasmic proteins of *Brucella melitensis* in mice. *Clinical and Diagnostic Laboratory Immunology* 6: 440-443

44. Domingo S, Diaz R and Gamazo C (1995). Antibiotic treatment induces an increase of the specific antibody levels in *Brucella melitensis* infected mice. *FEMS Immunology and Medical Microbiology* 12: 91-96

45. Chifiriuc MC, Holban AM, Curutiu C, Ditu LM, Mihaescu G, Oprea AE, Grumezescu AM and Lazar V (2016). Antibiotic drug delivery systems for the intracellular targeting of bacterial pathogens. https://www.intechopen.com/books/smart-drug-delivery-system-for-theintracellulartargeting-of-bacterial-pathogens [doi: 10.5772/61327]

46. Poester F, Samartino L and Santos R (2013). Pathogenesis and pathobiology of brucellosis in livestock. *Review of Science and Technology* 32: 105-115

47. Currin J (2019). Abortions in dairy cattle I: Common causes of abortions. https://www.pubs.ext.vt.edu/ 404/404-288/404-288.html

48. Di A, Kiya T, Gong H, Gao X and Malik AB (2017). Role of the phagosomal redox-sensitive TRP channel TRPM2 in regulating the bactericidal activity of macrophages. *Journal of Cell Science* 130: 735-744

49. CRL (2018). Cell-penetrating antibiotics: Targeting intracellular pathogenic bacteria. Chemielewski Research Lab = CRL. https://www.chem.purdue.edu/chml/research/cellantibiotics.html

50. Gomarasca M, Martins TFC, Greune L, Hardwidge PR, Schmidt AA and Ruter C (2017). Bacterium-derived cell-penetrating peptides deliver gentamicin to kill intracellular pathogens. *Antimicrobials Agents and Chemotherapy* 61(4): e02545-16 [doi: 10.1128/AAC.02545-16]

51. Ariza J, Bosilkovski M, Cascio A, Colmenero JD, Corbel MJ, Falagas ME, Meish ZA, Roushan MRH, Rubinstein E, Sipsas Nv, Solera J and Young EJ (2007). Perspectives for the treatment of brucellosis in the 21st century: the Loannina recommendations. PLoS Medicine 4(12): e317 [doi: 10.1371/journal.pmed.0040317]

52. Ranjbar M (2015). Treatment of brucellosis. https://www.intechopen.com/books/updates-onbrucellosis/ treatment-of-brucellosis [doi: 10.5772/61093]

53. Omer SA, Al-Megrin WAI, Alagaili AN, Elobeid MA and Mohammed OB (2011). A new protocol for the treatment of *Brucella melitensis* in Neumann‟s gazelle (*Gazella erlangeri*) from Saudi Arabia using oxytetracycline and streptomycin. *African Journal of Biotechnology* 10: 11075-11080

54. Richardson M and Holt JN (1962). Synergistic action of streptomycin with other antibiotics on intracellular *Brucella abortus in vitro. Journal of Bacteriology* 84: 638-646

55. Kushwaha N, Rajora VS, Mohan A, Singh JL and Shukla SK (2014). Assessment of haematobiochemical parameters and therapeutics on Brucella-infected cattle. *Journal of Microbiology and Experimentation* 1:1-5 [doi: 10.15406/jmen.2014.01.00012]