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ORIGINAL ARTICLE

Haematological Variations in *Brucella abortus* Antibody Positive Cross-bred Cattle at Chittagong, Bangladesh

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SUMMARY

A hematological study was carried out to determine the considerable variations in blood parameters for brucellosis seropositivity in commercial dairy cattle in the Chittagong region of Bangladesh from January to May 2012. The study population comprised of 250 commercial cross-breed dairy cattle, randomly selected from 7 commercial farms. Milk Ring Test (MRT) was done as a screening test. The MRT positive 50 cows were subjected to blood collection for hematological and serological tests. After separation of sera, two serological tests specifically indirect Enzyme Linked Immuno Sorbent Assay (iELISA) and Rose Bengal Plate Test (RBPT) were done for confirmation. Hematological tests like hemoglobin (Hb), packed cell volume (PCV), erythrocyte sedimentation rate (ESR), red (TEC) and white (TLC) blood cell count, differential leukocyte count (DLC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined to observe whether any significant variation between the brucellosis positive and negative group was exist. The results revealed that no significant variations were found among the parameters ($p<0.05$). However, slightly increased values of TLC, monocytes, eosinophil, MCV and MCH were recorded in the positive group. In addition, a little decline in the values of TEC, and neutrophil were found in the same group. The values of Hb, PCV, ESR, lymphocytes, basophils and MCHC were remained unchanged. The results showed that *Brucella* organisms are not responsible for a significant change in the hematological values, underscoring the need for further studies including chemical and structural changes in the serum or tissue or cellular or molecular level.

Key Words *Brucellosis, iELISA, TEC, DLC, MCHC*

Chittagong, Bangladeşte *Brucella abortus* Antikor Pozitif Melez Sığırlarda Hematolojik Varyasyonlar

ÖZET

Mayıs 2012 den Ocak ayına kadar Bangladeş'in Chittagong bölgesinde bruselloz seropozitif bulunan ticari sağmal ineklerin kan parametrelerinde önemli farklılıkları belirlemek amacıyla hematolojik bir çalışma yürütüldü. 250 ticari melez süt sığırından oluşan çalışmada, rastgele 7 çiftlik seçildi. Bir tarama testi olarak Milk Ring Testi (MRT) yapıldı. MRTde pozitif sonuç veren 50 inekten hematolojik ve serolojik testler için kan alındı. Serumların ayrılmasından sonra, konfirmasyon amacıyla Enzyme Linked Immuno Sorbent Assay (iELISA) ve Rose Bengal Plate Testi (RBPT) yapıldı. Brusella pozitif ve negatif gruplar arasında anlamlı bir varyasyon mevcut olup olmadığını gözlemek için hematolojik testlerden hemoglobin (Hb), paketlenmiş hücre hacmi (PCV), eritrosit sedimantasyon oranı (ESR), kırmızı (TEC) ve beyaz (TLC) kan sayımı, diferansiyel lökosit sayısı (DLC), eritrosit hacmi (MCV), ortalama eritrosit hemoglobini (MCH) ve eritrosit hemoglobin konsantrasyonu (MCHC) belirlendi. Sonuçlar parametreler arasında anlamlı farklılıklar bulunmadığını gösterdi ($p<0.05$). Ancak pozitif grupta, TLC, monosit, eozinofil, MCV ve MCH arasında hafif bir değer artışı kaydedildi. Buna ek olarak, TEC, ve nötrofil değerlerinde ufak bir azalma bulundu. Hb, PCV, ESR, lenfositler, bazofiller ve MCHC değerleri aynı kaldı. Sonuçlar Brucella etkenlerinin hematolojik değerlerde önemli bir değişiklikten sorumlu olmadığını gösterdi. Öte yandan serum, doku, hücresel veya moleküler seviyede kimyasal ve yapısal değişiklikler de dahil olmak üzere daha fazla çalışmaların yapılmasının gerekli olduğu kanaatine varıldı.

Anahtar Kelimeler *Brusellozis, iELISA, TEC, DLC, MCHC*

INTRODUCTION

Brucellosis is one of the most important and widespread re-emerging zoonotic disease in the world (Mustafa & Nicoletti, 1995). The disease affects cattle, swine, sheep, goats, camels, equines, dogs. It may also infect other ruminants and marine mammals. Humans can become infected indirectly through contact with infected animals or by animal products consumption. Brucellosis in cattle is usually caused by biovars of *Brucella abortus.* It causes abortion, infertility, retention of placenta, stillbirth and calf loss in animals and huge economic losses to dairy farmers (Franco *et al.,* 2007, Singh *et al*., 2002).

Brucellosis occurs worldwide but it is well controlled in

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most developed countries. It has been eradicated from Japan, Canada, some European countries, Australia, New Zealand and Israel (OIE, 2010).

In Indian subcontinent, Imperial Veterinary Research Institute (now Indian Imperial Veterinary Research Institute), Muketswar, first investigated contagious abortion in livestock associated with Brucellosis. In Bangladesh, brucellosis was first identified in cattle by Mia and Islam (1967).

In Bangladesh, prevalence of brucellosis has been reported in cattle from different areas. For example, prevalence of brucellosis was determined in buffaloes, cattle, sheep and goats of five different districts viz. Bagerhat, Bogra, Gaibangha, Mymensingh and Sirajgonj (Rahman *et al*., 2006). The overall seroprevalence of brucellosis in Bangladesh was 2% in Mymensingh district, 16.66% in Tangail district, 11.52% in Pabna district, 2.92% in Faridpur district, 2% in Bogra district (Rahman and Rahman, 1982).

Normal hematological parameters of exotic or exotic cross cow was demonstrated by Research Animal Resources (RAR), University of Minnesota that is Hb: 8-15 gm/dl, PCV: 24-48%, TLC: 4-12 Thousand/µl, DLC (Neutrophil: 20-40, Lymphocyte: 40-70, Monocyte: 1-6, Eosinophil: 0-4, Basophil: 0-2), MCV: 40-60 fl, MCH: 11-17 pg, MCHC: 30-36 g/dl (RAR, 2011).

Milk ring test, serological test like Rose Bengal Plate test (RBT), slow agglutination Test (SAT), Tube agglutination Test (TAT), mercaptoethanol test and/or ELISA (indirect, competitive, Avidin-Biotin), Fluorescent antibody test (FAT) are commonly execute for recognition of Brucella infections in cattle (OIE, 2010). But there were limited research on hematological diagnosis of brucellosis in cattle. Considering the above facts the present work was intended to determine whether there are any significant diagnostic variations in the hematological parameters in *Brucella* positive cows.

MATERIALS and METHODS

Study area and population

The study was conducted on commercial dairy cows at Chittagong region which is the south-east part of Bangladesh. The type of animals kept under commercial farming system were all cross of local (*Bos indicus*) with different exotic breeds (Friesian mostly, *Bos taurus*). The Dept. of Livestock Services of Chittagong maintains the register of commercial dairy farms at Chittagong. From that register 7 farms having a total of 250 cows were selected by simple random sampling method using the Excel software (Microsoft Office, 2007).

Questionnaire design and data collection

Information about each herd and the animals kept was collected by means of a structured questionnaire, which was completed at all the selected herds on a single visit. The questionnaire was designed to comprise mostly closed ended (categorical) questions to ease data processing, minimize variation, and improve precision of responses (Thrusfield, 2005). The questionnaire was filled up by repeated questioning to the farmers and also farm manager and attendant, taking records from register book by the author. Important herd and animal level data includes cattle location, total number of animals, breed, history of abortion and other reproductive disorders.

Samples and serological tests

Approximately 5ml of milk was collected from four quarters (after disinfection of udder with potassium-permanganate solution) of each cow into sterile screw capped vial (Becton Dickson, UK). Then the vials labeled the ID and stored in the ice box. Within 6 hours of collection the samples were screened by MRT as recommended by Sharma *et al.* (2003).

The cows that shown positive result to MRT were subjected to blood collection (within 2 days of MRT) for separation of sera. After disinfection of the jugular furrow using Tr. Iodine, 10 ml of blood was collected from jugular vein using disposable sterile syringe (12 ml). About 5 ml of blood then immediately transferred to vacutainer tube (Becton Dickson, UK) and rest 5 ml to EDTA vial (Becton Dickson, UK) and labeled. The vacutainer tubes kept inclined position for about 30 minutes to allow clotting and maintained at app. +4°C in refrigerator until they were processed. In the laboratory, sera were separated by centrifugation at 2500 rpm for 15 min and stored in 1.5 ml eppendrof tubes at -20°C until serological tests were performed. The EDTA vials were tilted without delay for proper mixing. The hematological tests were made within 6 hours of collection. The Hb, ESR, TEC, TLC and DLC values were determined as recommended by Campbell (1995) and PCV value was measured by the procedure described by Howlett *et al* (2002). The MCH, MCV and MCHC values were made from values of Hb, PCV and TEC thereafter.

Antibodies to *Brucella* spp. were detected by sequential testing of samples using the indirect ELISA and RBPT for confirmation. The indirect (i)ELISA kit was obtained from Svanova Biotech AB, art. No. 10-2700-10, SE-751 83 Uppsala, Sweden. The test procedure followed as suggested by Shafee *et al.* (2011). The RBPT antigen was supplied by VLA Weybridge, UK. The test procedure recommended by Alton *et al.* (1975) was followed. A cow was considered to be positive if it tested positive on all three tests: the MRT, iELISA and RBPT.

Data analyses

Data from the laboratory results and questionnaires were stored in personal computer, using Microsoft Excel spreadsheet program. Descriptive statistical analyses of various risk factors and dependent variables were done using Intercooled STATA 9.0 (Stata Corporation 2008). Proportional analysis and multinomial logistic regression was used to interpret the data.

RESULTS

Serological test results

The milk and sera test results are presented in the Table 1. Of the 250 sampled animals, serological results were available from 50 animals as the animals shown negative reaction with MRT were considered as negative to brucellosis. Again, an animal was considered as positive if it became positive in all three tests (MRT, iELISA and RBPT). Here, among the 250 samples cows 21 were shown positive reaction with all three tests.

Table 1. The cows' response to different immunological tests

Tests	Total Sample	Test	
		Positive	Negative
MRT	250	50	200
iELISA	50	40	10
RBPT	50	21	29
MRT + iELISA + RBPT	250	21	29

Haematological test results

The hematological tests exhibit a little diminution in the TEC, percentages of neutrophils and basophils in the *Brucella* positive group of cow. On the contrary, moderate augmentation of TLC was found in positive group and a slight increase in percentages of monocytes and eosinophils was found in the same group of cow though the results were not statistically significant. The values of Hb, PCV, ESR and lymphocytes were unchanged. Details of the comparative hematological tests result given the Table 2.

Table 2. Haematological parameters of Brucellosis positive and negative group of cattle

Variables	Positive (N=21)		Negative (N=29)		P value
	Mean ± SD	95% CI	Mean ± SD	95% CI	
Hb (gm/dl)	7.462 ± 0.532	7.219-7.704	7.238 ± 0.532	7.036-7.441	0.09
PCV (%)	29.714 ± 6.034	26.967-32.461	29.207 ± 6.304	26.809-31.605	0.75
ESR (mm in first hour)	0.00	0.00	0.00	0.00	-
TEC ($x10^6$ cells/µl)	4.867 ± 1.571	4.153-5.583	5.081 ± 1.746	4.416-5.745	0.56
TLC ($x10^3$)	9141.429 ± 2584.839	7964.824-10318.03	8487.931 ± 3288.906	7236.898-9738.964	0.39
Lymphocyte (%)	64.333 ± 8.212	60.595-68.071	64.931 ± 8.594	61.662-68.199	0.82
Monocyte (%)	5.286 ± 3.243	3.809-6.762	4.276 ± 2.389	3.367-5.185	0.16
Neutrophils (%)	22.333 ± 7.438	18.947-25.719	23.586 ± 8.842	20.223-26.949	0.58
Eosinophil (%)	7.572 ± 5.644	5.002-10.141	5.966 ± 3.191	4.752-7.179	0.27
Basophil (%)	0.238 ± 0.436	0.039-0.437	0.379 ± 0.494	0.192-0.567	0.35
MCV (fl)	66.073 ± 21.094	56.470-75.675	61.184 ± 22.879	52.483-69.887	0.13
MCH (pg)	16.775 ± 6.323	13.897-19.654	15.942 ± 4.705	14.153-17.732	0.20
MCHC (%)	25.988 ± 4.938	23.741-28.236	25.585 ± 4.216	23.982-27.189	0.15

DISCUSSION and CONCLUSION

Haematological values of *Brucella abrotus* antibody positive cows showed variable degrees of discrimination. In brief, lowered values of Hb and MCHC were recorded compared to reference values (RAR, 2011). However, MCV, neutrophil, monocyte and eosinophil counts were found higher than the standard values. The values of PCV, ESR, TEC, TLC, lymphocytes, basophils and MCH were remain within the ranges of reference values though any of the values were not found statistically significant ($p>0.05$).

The hemoglobin value of the present study was found lower than the reference value and was in consistent with the findings of Dorgan, 2010 and Gurkan *et al.,* 2003 who worked on cattle and old women correspondingly. On the other hand, Cannella *et al.,* 2012; Kuperman *et al.,* 2010 recorded slightly higher and Tiller *et al.,* 2010; Abdollahi *et al.,* 2010 showed moderately higher values than the present study. Conversely, Lynch *et al.,* 1968 recorded a little lower Hb value in human with enteric fever. Intra-cellular position of the *Brucella* spp. might cause reduction of Hb percentage though the result is not significant. The distinct variations in Hb values might be due to poor sample size and variations in the test equipments and species diversification.

The hematocrit value of the current study was merged within the range of standard value and was in the line with the findings of El-Boshy *et al.,* 2009; Diaz *et al.,* 2000. Whereas, Arp *et al.,* 2011 found a bit higher and Gungor *et al.,* 2002; Kirk and George, 1970 found markedly elevated values. Though, Dogan, 2010; Dim *et al.,* 2009 recorded in some extent lesser than the present value. The standard PCV value might be indicated that it was not affected by brucellosis sero-positivity.

ESR value of the present study was found lower than the findings of Erbay *et al.,* 2009; Ayaslioglu *et al.,* 2005 who worked on human brucellosis. The TEC value was approved by Abdollahi *et al.,* 2010 though Forbes *et al.,* 1996 recorded a little lower in both male and female moose and El-Boshy *et al.,* 2009 found in some extent higher than this study in camel. Variation within a narrow range might not be associated with bovine brucellosis.

Increased TLC value was found close to the values recorded by Ayaslioglu *et al.,* 2005; Gurkan *et al.,* 2003. While, Kuperman *et al.,* 2010; Gungor *et al.,* 2002 showed quietly smaller values. Host defense mechanism activates in all types of infection and in bacterial infection infiltration of white blood cells increased which might be the reason behind increased WBC count (Radostitis *et al.,* 2000).

Percentages of neutrophil, monocyte and eosinophil were found at upper range of reference values in current study. The lymphocyte, monocyte and eosinophil percentages was found near to the findings of Forbes *et al.,* 1996 who worked on moose infected with brucellosis. Additionally, neutrophil and basophil values were found in consistent with the findings of Dim *et al.,* 2009 and El-Boshy *et al.,* 2009 subsequently. However, lowered lymphocyte values

were recorded by Erbay *et al.,* 2009. In addition, poorer and richer monocyte percentages were found by El-Boshy *et al.,* 2009 and Tiller *et al.,* 2010 correspondingly. Moreover, higher and lower neutrophil percentages were recorded by Forbes *et al.,* 1996 and Ayaslioglu *et al.,* 2005 consequently. Furthermore, lowered eosinophil and higher basophil values were showed by Erbay *et al.,* 2009 and Forbes *et al.,* 1996 accordingly. The higher neutrophil and monocyte values remain always higher in non-specific bacterial infection (Radostitis *et al.,* 2000). Mixed infection with different parasitic diseases especially helminthic disorders might be responsible for increased eosinophil percentages in this study.

The increased MCV value was found in parallel with the value recorded by Forbes *et al.,* 1996. Nevertheless, higher values of MCH and MCHC than the present study also found by the same author. Smaller and greater MCV values than the current study were recorded by El-Boshy *et al.,* 2009 and Gurkan *et al.,* 2003 subsequently. The reduced MCHC % might be indicated that a variable degree of normocytic normochromic to normocytic hypochromic anaemia is evidently associated with brucellosis.

Compared to iELISA, the sensitivity and specificity values of MRT were found as 97.9% and 96.8% and RBPT, 53.19% and 96.19% respectively. The same values for iELISA with Complement Fixation Test (CFT) were recorded as 99.4% and 98% correspondingly (Nielsen *et al.,* 2004).

This study reports that brucellosis is prevalent in cross-bred dairy cows at Chittagong. The hematological parameters of *Brucella* spp. antibody positive and negative cows were overlooked. This study will address the variations of blood parameters of brucellosis infected cross-bred dairy cows which will assist in hematological diagnosis of bovine brucellosis. Besides this, the results showed that *Brucella* organisms are not responsible for a considerable alteration in the hematological values. Further studies will be required including chemical, hormonal and molecular changes in the serum or tissue or cellular level.

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