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Seroprevalance of brucellosis in rams in NTR & Krishna district, Andhra Pradesh

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Abstract
Brucellosis is a Zoonotic disease that occurs world wide. It causes abortions in the last trimester of pregnancy of the effected animals leading to many economic losses in the livestock sector. The aim of the present work is to study the Seroprevalance of Brucellosis in Rams of NTR and Krishna Districts of Andhra Pradesh. A total of 1655 sera samples were collected from 116 villages and 53 mandals of NTR and Krishna Districts. The samples were screened for Brucellosis by Rose Bengal Plate test (RBPT). RBPT is a rapid slide agglutination test used for screening of animals against Brucellosis and i-ELISA for confirmation. 332 (20%) samples were found positive on RBPT and 406 (24.5%) samples were observed to be positive on ELISA. Based on the results of the biochemical analysis, there were decreased levels of protein and calcium, along with elevated levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The hematological analysis also showed a decrease in mean values of hemoglobin levels, as well as leucocytosis and monocytosis. These findings indicate potential health concerns that may require further evaluation and treatment.

Keywords: Seroprevalance, Krishna & NTR Districts, Andhra Pradesh, Brucellosis, Rams, RBPT, i-ELISA

Introduction

Small ruminants, which account for more than half of the domesticated ruminants in the world, are an important component of the farming systems in most developing countries. Despite the importance of small ruminants in the livelihoods of producers, the current productivity of goats and sheep in developing countries remains low, mainly due to under-feeding, poor management system and diseases. Brucellosis is one of the infectious diseases considered as most constraints for sheep and goats productivity. Brucellosis is an economically important and widespread zoonosis in the world caused by bacteria of the genus Brucella, which tend to infect specific animal species (Awah-Ndukum *et al*., 2018) [4]. The pathogen causes severe disease in livestock and has an enormous impact on the economy of developing countries (Ebid *et al*., 2020) [2].

Brucella melitensis is the most important cause of brucellosis which primarily affects sheep and goats and also very pathogenic for human beings. The disease is also caused by B. ovis which severely affects sheep. Although the disease has preferred hosts, the bacteria can cross-infect other domestic animals. Hence, sporadic infections in small ruminants can also be caused by B. abortus or B. suis, but such cases are rare. It is also defined as a contagious systemic bacterial disease primarily of ruminants, characterized by inflammation of the genital organs and fetal membranes, abortion, sterility, and formation of localized lesions (Awah-Ndukum *et al*., 2018) [4]. Additionally, the disease also poses a major constraint to international trading of animal and animal products.

Typically, Rose Bengal Plate Test (RBPT) is used for field screening for brucellosis, sometimes along with indirect enzyme-linked immunosorbent assay (i-ELISA).

The present study mostly focuses on the seroprevalance rate of brucellosis in small ruminants especially Rams which are the main source of transmission of the disease in the flock. The study proceeds by collecting the sera samples from rams targeting the sheep flocks of Krishna and NTR Districts of Andhra pradesh, screening of samples by a rapid agglutination test, RBPT and confirming them by i-ELISA.

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Materials and Methods
Study area and Period
The study is performed in the districts of Krishna and NTR of Andhra Pradesh. The sheep and Goat population of Andhra Pradesh is 23.74 (17.63 million) and 3.71 (5.52 million) respectively.

The period of study is from April 2022 to March 2024 i.e., for a period of 2 years.

Sample collection
Sera Samples: The Rams are selected randomly from the sheep flocks of NTR and Krishna Districts of Andhra Pradesh. Whole blood is collected from the jugular vein of each animal using 10 ml sterile Vacutainer tubes without anticoagulant for serological tests. The samples collected are neatly labeled with codes for identification of the animal. Blood samples were kept in an upright position for 30 min in a cool place and then centrifuged at 3000 rpm for 20 min. The serum was collected by a micropipette and placed in Eppendorf tubes. Serum samples and blood samples were kept at −20 °C until used, as recommended by OIE. (Ebid *et al.*, 2020) [2].

Table 1: Showing Mandal wise No of Samples collected from Both the Districts

District	Mandal	No. of Samples Collected
Krishna	Athukur	25
	Machilipatnam	130
	Bantumulli	35
	Challapalli	45
	Ghantasala	25
	Gudlavalleru	25
	Guduru	25
	Kankipadu	53
	Koduru	30
	Mopidevi	25
	Movva	12
	Nagayalanka	45
	Nandiwada	25
	Pamarru	26
	Pamidimukkala	21
	Pedana	56
	Unguturu	18
	Total	621
NTR	Chandralapadu	40
	G.konduru	20
	Gampalagudem	90
	Mylavaram	205
	Nandigama	135
	Penuganchiprolu	50
	Tiruvuru	150
	Vastavai	92
	Veerullapadu	40
	Jaggaiahpeta	172
	Kanchikacherla	40
	Total	1034
Grand total		1655

Rose Bengal Plate Test
Sera samples were tested using the RBPT antigen, according to Ebid *et al.*, (2020) [2]. Briefly, 30 µL of serum and 30 µL of RBPT antigen combined on the white ceramic plate and carefully mixed. The plate was agitated for 4 min, and the degree of agglutination was recorded as a grade from 0, +, ++, and +++. Grade (0) indicates the absence of agglutination, grade (+) indicate barely visible agglutination, grade (++) indicates finely dispersed agglutination, and grade (+++) indicates coarse clumping. The samples with grade (+, ++, and +++) were considered positive. Positive and negative control sera for comparison of the results were used.

i- ELISA
All samples that were tested positive by RBPT were further analyzed by i-ELISA for confirmation. i-ELISA was performed following ELISA kit manufacturer's instructions.

Results and Discussion
The Rams are selected randomly from the sheep flocks where few animals exhibiting symptoms like abortion, still birth, delivery of weak offspring and placenta retention in females and orchitis and epididymitis in males.

RBPT
A total of 1655 Rams sera samples, 1034 and 621 from NTR and Krishna districts respectively were collected for screening against Brucellosis. All the samples were subjected to a simple plate agglutiation test RBPT, where the antigen is prepared using Rose Bengal Reagent as a coloring agent, which helps in visualising the positive reaction i.e., agglutination.

Out of 1655 samples, 332 samples were found positive on RBPT. The % positivity on RBPT is 20%.

i-ELISA
All the 1655 sera samples were subjected to i-ELISA for confirmation. Out of 1655 samples, 406 samples were observed to be positive on ELISA. The percent positivity observed is 24.5%.

Haemotological and biochemical profile: 30 Brucella positive blood and sera samples and 10 normal blood and sera samples of rams were used used to determine mean values of some haematological parameters (Haemoglobin, Total WBC and Monocytes.), mean values of SGOT, SGPT, Calcium and Total Protein in examined Rams.

Table 2: The mean values of hematological parameters were given

	Control Healthy (N=10)	Seropositive animals (N=30)
Haemoglobin (g/dl)	12.2 + 0.3	8.1 + 0.2
PCV(%)	34.1 + 0.7	25.6 + 0.5
TRBC 10^6/ul	8.1 + 0.2	6.9 + 0.1
TWBC 10^3/ul	9.6 + 0.6	13.5 + 0.5
Granulocytes	48.9 + 1.4	39.6 + 3.0
Lymphocytes	45.9 + 1.5	13.4 + 0.5
Monocytes	5.2 + 0.5	47 + 3

Table 3: The mean values of Serum Biochemical parameters were given

	Control Healthy (N=10)	Seropositive animals (N=30)
Total Protein	7.1 + 0.1	5.9 + 0.2
SGOT	61.1 + 0.4	126.4 + 0.8
SGPT	23.9 + 0.1	45.4 + 0.5
Calcium	11.3 + 0.1	7.1 + 0.2

The biochemical analysis of the Brucella positive sera samples indicated decreased mean levels of protein and calcium, along with increased enzymatic activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The hematological analysis showed a decrease in hemoglobin levels, as well as leucocytosis and monocytosis. Tizard *et al.*, 1992 stated that bacterial lipopolysaccharides released from disrupted bacteria are readily adsorbed onto erthythrocytes and the subsequent immune response against the bacterium and its products results in erythrocyte destruction causing anemia. Earlier studies stated that anemia and leukocytosis is evident in in brucella-infected ewes (Aida *et al.*, 2009, Hashem *et al.*, 2020) [6, 8]. Darwish *et al.*, 2023 [9] proposed that leukocytosis is an important reaction from the host as neutrophils are the first line of defense against bacterial infection and monocytes serve as scavengers of uterine tissue debris, observed in cases of retained placenta and delayed natural uterine cleaning associated with Brucella infection.

The decrease in serum protein mean values in this study were in accordance with the study conducted by Rajiv *et al.*, 2017 [5]. The decrease in serum total protein can be attributed to Liver damage, leads to the decreased production of proteins (Aida etal., 2009) [6]. In the present study the mean values of ALT and AST in *Brucella* affected rams were higher compared to healthy rams. The increase in transaminase enzyme activities were due to the activation of oxidative and phosphorylation enzymes (NAD N- Diaphorase, LDH-ase and ATPase) in liver mitochondria by inducing liver dysfunction. Decreased mean values of calcium compared with healthy animals observed similar to the findings of Hussary and Al-Zuhairy (2010) [11].

In conclusion, Brucellosis can cause a wide range of disturbances in the biochemistry and blood parameters of infected sheep rams. These can vary widely and contribute to the economic losses associated with the disease. Early diagnosis through testing, removal of infected animals via a slaughter program, strict sanitary practices, and vaccination program for healthy young animals can reduce the prevalence of brucellosis in sheep rams.

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