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Dexamethasone-induced stress exacerbates shedding, tissue antigen distribution, and pathology of caprine Brucellosis

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ABSTRACT

This study investigated dexamethasone-treatment, shedding routes, tissue antigen distribution, and pathology of caprine Brucellosis. Eighteen non-pregnant goats were randomly grouped into A, B, and C. Group A was administered dexamethasone for 7 days at 2 mg/kg before inoculating 0.5 mL *B. melitensis* at 10⁷ CFU ocularly while group B was inoculated 0.5 mL *B. melitensis* only, and C as control negative. Blood samples, ocular, nasal, and vaginal swabs were obtained for evaluation. Three goats were sacrificed from each group at days 21 and 42 post-inoculation (pi) and selected tissues collected for PCR, histopathology, and immunohistochemistry. *Brucella melitensis* was detected in the ocular swabs of group A significantly higher than group B. Shedding was prolonged in group A compared to B. The overall shedding was 22.2% in group A and 9.4% in group B. The uterus of both groups A and B revealed mild inflammation and microgranuloma, extensive necrotic lesions in lymph nodes. Liver showed multifocal necrosis predominantly in group A. Lesion scoring showed significantly higher scores in A compared to B. Strong immunostaining was observed in the liver, lungs, and spleen, predominantly at day 21 pi. This study demonstrated dexamethasone prolonged shedding, tissue antigen distribution, and pathology in dexamethasone-treated goats.

KEYWORDS

Brucellosis; dexamethasone; goats; immunohistochemistry; tissue antigen distribution

Introduction

Brucellosis is one of the most important zoonotic disease worldwide, particularly in developing countries.^[1] *Brucella melitensis* is the primary etiologic agent responsible for ovine and caprine Brucellosis, and it is the most pathogenic species for humans.^[2–4] Currently, diagnosis of Brucellosis depends upon serological and microbiological methods with limitations.^[5] Bacteriological

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methods on the other hand have limited sensitivity,^[2,5] hence the need to also use shedding/swabs to enhance detection of infected animals.^[3]

Studying how stress influences the pathology of infectious diseases is invaluable for assessing the biological importance of stress-enhanced pathology of certain diseases.^[4,6,7] Currently, there is enough proof from studies of animals that the degree of stress-induced pathology is substantial enough to have a deleterious impact on the health of these animals.^[4,8] Studying the course of stress-enhanced pathology of diseases may offer vital information that could be useful in developing pharmacological therapeutics as well as management strategies that are capable of mitigating pathological consequence associated with such diseases.^[9,10]

Dexamethasone treatment at 0.2–2 mg/kg body weight has been reported by several authors to induce stress in animals similar to natural occurrence.^[4,8,11,12] Even though the effect of dexamethasone treatment prior to *Brucella melitensis* infection was evaluated in our previous study,^[7] the effect of dexamethasone treatment before *Brucella melitensis* infection in nonpregnant goat is not known. These data if available could enhance the understanding of the pathology and/or pathogenesis of Brucellosis in non-pregnant stressed does. This study seeks to elucidate the influence of dexamethasone treatment on the shedding routes, tissue antigen distribution, and the pathology of caprine Brucellosis or rather pathological dynamics of brucellosis in dexamethasone-stressed goats.

Materials and methods

Ethical statement

The design and execution of this experiment were approved in compliance with the humane methods recommended by the Animal Care and Use Committee (ACUC) of the Faculty of Veterinary Medicine, Universiti Putra Malaysia. The animals were carefully handled to minimize additional stresses during treatment and sampling. The pens were cleansed thoroughly and disinfected for days before the experimental animals were introduced to the pens.

Animals

In this study, 18 healthy non-pregnant Boer goats aged 2–3 years old, with body weight ranging from 20 to 25 kg was utilized. The said goats were subjected to clinical examination and apparently healthy does were selected. The selected goats were subjected to further screening using PCR (using DNA extracted both from blood and bacterial colonies cultured from nasal, ocular, and vaginal swabs), Rose Bengal Plate Test (RBPT), and iELISA. The goats

were all negative for brucellosis. Following the final selection, the goats were kept for three weeks for acclimatization. During this period the goats were given concentrates, fresh grass and water *ad libitum*. Following acclimatization, the goats were grouped at random into three groups as A, B, and C with each having six goats. The first group (A) was administered dexamethasone for seven days at 2 mg/kg body weight intravenously,^[4] before inoculation with 0.5 mL of 10^7 CFU *Brucella melitensis* antigen intraocularly. The second group (B) was not administered dexamethasone but they were treated with a similar dose of *Brucella melitensis* antigen intraocularly, as control positive and the third group (C) were similarly treated with normal saline, as control negative group.

Inoculum preparation

A field strain of *B. melitensis* isolated and characterized earlier from *Brucella* outbreak in Malaysia,^[13] was obtained from the Bacteriology unit of the Faculty of Veterinary Medicine, Universiti Putra Malaysia. The isolate was sub-cultured on Brucella agar and subjected to incubation for 96 hours at 37°C. This was followed by biochemical analysis to confirm the isolate.^[14] This was followed by further subculturing of the colonies in Brucella broth for yet another 96 hours at 37°C in the incubator shaker. The cultured broth was then subjected to centrifugation and the supernatant was discarded while the sediment was further subjected to dilution to the required 10^7 CFU using McFaland standards.

Rabbit anti-Brucella melitensis primary antibodies preparation

The preparation of immune serum against *B. melitensis* antigen (referred to as primary antibody) was performed following the technique described earlier by Tanko et al.^[7]

Collection of samples

Blood samples

Collection of blood samples was made into sodium heparin vacutainer tubes prior to and on days 3, 6, and 8 during the dexamethasone administration. Blood samples were similarly made on days 0, 7, 14, 21, 28, 35, and day 42 postbacteria inoculation (pi). The obtained samples were immediately taken to the laboratory and they were divided into portions which were then utilized to RBPT, iELISA, and PCR analysis. Three animals from each group were sacrificed at days 21 and day 42 pi. Postmortem examination was performed while tissue samples from lymph nodes, mammary gland, liver, uterus, kidney,

lungs, and the spleen were collected and used in the detection of *B. melitensis* DNA utilizing PCR technique. The tissues were similarly employed for histological and immunohistochemistry evaluation.

Detection of bacterial shedding (nasal, ocular, and vaginal swabs) using PCR

Dry and sterile cotton wool swabs were used to collect the ocular, nasal, and vaginal swabs prior to inoculation and on days 3, 6, and 8 during the dexamethasone administration and on days 0, 7, 14, 21, 28, 35, and day 42 postbacteria inoculation (pi). The vulva was cleansed with clean cotton wool before the swab stick was inserted into the vagina to a depth of approximately 3 cm and gently rotated several times before slowly pulling it out. A similar procedure was done for the nasal swab. The swab stick was then gently inserted into the Eppendorf tube containing 0.5 ml Brucella broth and immediately transported to the laboratory for culturing on brucella agar as described above under “inoculum preparation section.” The colonies obtained thereafter were tested for *Brucella* using PCR at the similar condition as described below under the “PCR section.” Positivity was reported based on the routes per sampling time in each group.

Serology (iELISA and RBPT)

Sera were harvested from an aliquot of the blood and stored at -20°C until screened using RBPT and iELISA. In running the RBPT, minor alterations were made according to the recommendation by Blasco et al.,^[15] to enhance the sensitivity of the test and to minimize inconsistencies. Briefly, 75 μL from each serum was placed on a white plastic board, followed by the addition of 25 μL of Rose Bengal suspension. These were gently mixed appropriately and the mixture was allowed to stay for 10–15 minutes. In this test, a conventional agglutination of the mixture was taken as sero-positive. On the other hand, the iELISA assay was carried out in accordance with the earlier reported technique.^[3] The cutoff point used in determining positive samples was computed utilizing optical density of known positive and negative serum samples which were run alongside the test sera.

Bacterial detection from blood using PCR

The DNA extraction from the blood was done using the Promega DNA extraction kit (Promega, USA) in accordance with the instructions provided. The DNA extracted was used for the detection of *B. melitensis* using PCR with primers specific to *B. melitensis* as earlier described.^[3] The DNA extracted from goats with bacteriologically proven infection were used as the gold standard while known negative DNA samples were used as negative controls to evaluate the sensitivity and specificity of the test. Tissues that were kept at -20°C were thawed

at room temperature and subjected to genomic DNA extraction using Promega DNA purification kit, following the manufacturer's protocol. Following this, PCR was done using the said primers (5'-AGTGTTTCGGCTCAGAATTAATC-3') and (5'-CATGCGCTATGTCTGGTTAC-3') following the earlier described amplification condition.^[3]

Histopathology

Post mortem examination was conducted with a gross examination of the reproductive organs, lymph nodes, lungs, liver, spleen, and kidney of the sacrificed goats. Tissues collected from these organs were routinely processed and subjected to staining with hematoxylin and eosin (H&E) and the lesions were graded using a semi-quantitative tissue scoring technique. For every slide, five different microscopic fields were examined. The mean was taken and scored as; 0: no significant changes, 1: mild changes, 2: moderate pathological changes and 3: marked pathological change as earlier described by Tanko et al.^[7] The mean for each slide and group were computed and recorded for the group at days 21 and 42 accordingly.

Tissue antigen distribution (Immunohistochemistry)

Sections of 4 µm thickness were made from formalin-fixed, paraffin-embedded tissues onto silane-coated grease-free glass slides. Immunohistochemistry (IHC) was done following instructions reported earlier.^[14] A semiquantitative scoring technique was employed to grade the immunoreactivity.^[14] The immunostaining was graded as no immunoreactivity (0), mild (1), moderate (2), and strong staining (3) according to the intensity and distribution of the golden-brown color deposition.

Statistical analysis

Analysis of data obtained from the study was done utilizing MedCalc Statistical Software version 13.0.6 (MedCalc Software bvba, Ostend, Belgium) and the level of significance was set at 5%. Analysis of the data obtained from lesion scoring was done using the Kruskal-Wallis test. Correlations between immunohistochemistry and histopathologic lesions were also analyzed. All data are written as a standard error means.

Results

Detection of bacterial shedding (nasal, ocular, and vaginal swabs) using PCR

The generality of the rates of shedding showed that the proportion of bacterial detection and the frequency from the three routes investigated was significantly ($P < .05$) less in group B as compared to group A. In group A, the ocular and the vaginal routes were the first to shed the bacterium at day 10 pi with the vagina having a higher proportion of shedding (33%) while the ocular had 17% at day 10 pi. Surprisingly, the proportion of shedding from the nasal route was higher (33%) as compared to the ocular and vaginal routes (17% each) at day 14 pi. Peak shedding was observed in the first group (A) at the 21st day pi where there were 50% bacterial detection from the vaginal and nasal routes while the ocular route had 33%. Unlike in group A where the peak shedding was at day 21 pi, the peak shedding from group B was observed at day 10 pi. There was no shedding from group C throughout the study.

Bacterial detection relationship between the three routes

The bacteria were detected in at least two routes at a time in group A from day 10 to day 42 pi with the exception of day 28 pi. There was more relationship between the routes at days 14, 21, and day 35 pi with the peak at day 21 pi in group A. In group B on the other hand, the bacteria were only detected in two routes at days 10 and 14 pi. Group C, however, recorded no shedding throughout the study.

Summary of bacterial detection in percentages

Peak bacterial detection was observed in A on the 21st day pi, where there was 50% bacterial detection from the vaginal and nasal routes while the ocular route had 33%. The proportion of bacterial detection from the three routes investigated in this study revealed significantly less ($P < .05$) detection from the B group in comparison to group A. The nasal route especially from group A appeared to have the highest percentage (28.2%) of bacterial detection which is significantly higher ($P < .05$) compared to the ocular and vaginal routes. This is followed by the ocular route while the vaginal route had the least percentage (17.9%). These percentages in group A are significantly higher ($P < .05$) compared to group B. The overall percentage of shedding was 22.2% for group A while group B had only 9.4%. There was no shedding from group C throughout the study.

Independent Enzyme Linked Immunosorbent Assay (iELISA)

The iELISA utilized showed significantly less ($P < .05$) serologic response in A in comparison to B (Figure 1). At day 7 pi, group A recorded 17% positivity

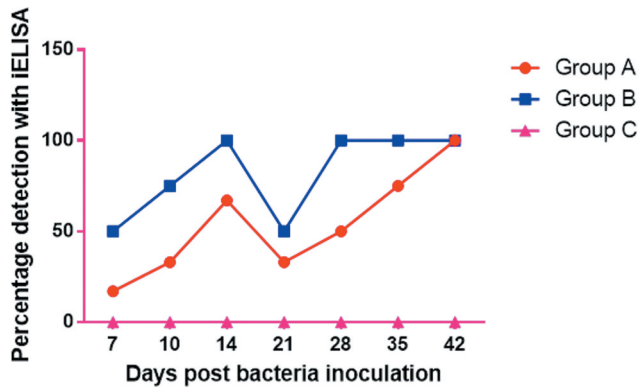


Figure 1. iELISA following bacteria inoculation for both the dexamethasone-treated group, positive and negative control from day 0 through day 42 post *B. melitensis* inoculation. The result is presented in percentages of positives animals from each group per sampling time.

in comparison to the 75% positivity observed in B group. Positivity was 100% in B group at day 14 pi while A group had 67%. Group B only reached 100% positivity on the 42nd day pi. Goats from C however, had no positivity throughout the study.

Rose Bengal Plate Test

The RBPT test utilized in this study recorded positivity within the first 10 days pi during which A recorded 33% positivity in comparison to the 67% recorded in B (Figure 2). The goats in A recorded 67% positivity at day 14 pi in comparison to the 100% positivity recorded in B. It was observed that group A did not record 100% positivity throughout the study period while group B consistently recorded 100% positivity. Group C recorded no positivity throughout the study.

***Brucella melitensis* detection from the blood using PCR**

In this study, PCR performed using DNA extracted from the blood showed all the goats from A were positive only at days 10 and 14 (Figure 3). The animals in this group were 50% and 87% positive at day 3 and day 7 pi correspondingly. Thereafter, the positivity declined to 83% in days 21 and 28 pi and the positivity further declined as the infection progresses and reached the lowest (33%) at day 42 pi. These percentages are significantly different from group B. In group B however, it was only at day 10 that the animals were all positive (100%) and subsequently, the number of animals positive declined significantly ($P < .05$) in comparison to group A. No *B. melitensis* detected in C as they were not infected.

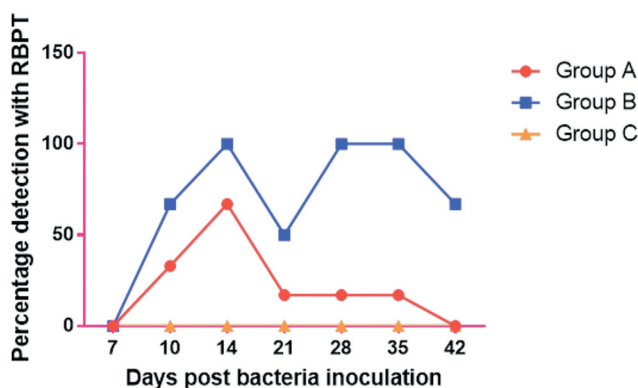


Figure 2. Rose Bengal Plate Agglutination Test (RBPT) following *B. melitensis* inoculation for both the dexamethasone-treated group, positive and negative controls from day 0 through day 42 pi. The result is presented in percentages of positives animals from each group per sampling time.

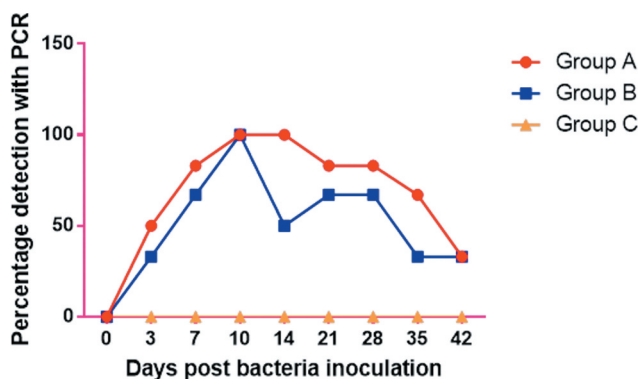


Figure 3. Percentage detection of *B. melitensis* DNA with PCR following *B. melitensis* inoculation for both dexamethasone-treated group, positive and negative controls from day 0 through day 42 pi. The result is presented in percentages of positives animals from each group per sampling time.

Histopathology (Lesion scoring for tissues obtained at days 21 and 42 pi)

The grading of the tissues using semiquantitative scoring is presented in Table 1. The uterus, ovary, vagina, and mammary glands of both groups A and B revealed mild infiltration of inflammatory cells and microgranuloma, and locally extensive necrosis was observed in the lymph nodes, most predominantly in the mandibular and prescapular lymph nodes (Figure 4–9). Predominant histopathological alterations observed in the liver consist of diffused necrosis, lymphocytic infiltrations at the periportal areas, microgranuloma, and congestion, predominantly in A which showed significant difference ($P < .05$) from group B at days 21 and 42 pi. The predominant lesions observed in the lungs were congestion, broncho-interstitial pneumonia, focal necrosis and mononuclear cell infiltration and the difference between the groups at days

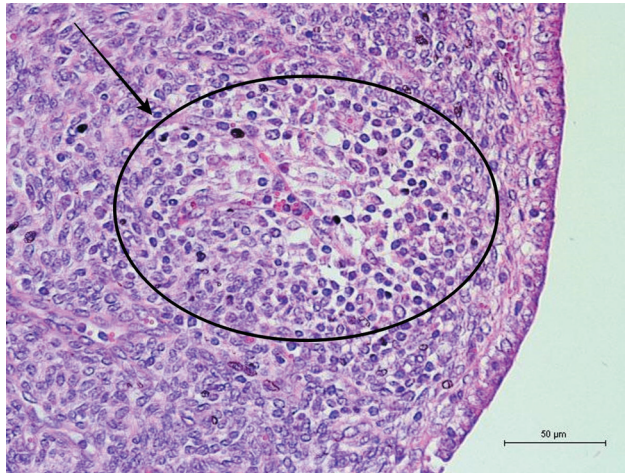


Figure 4. Photomicrograph of representative histopathological lesions of the uterus of group **A** at day 21 pi, showing microgranuloma in the endometrium (area under the circle).

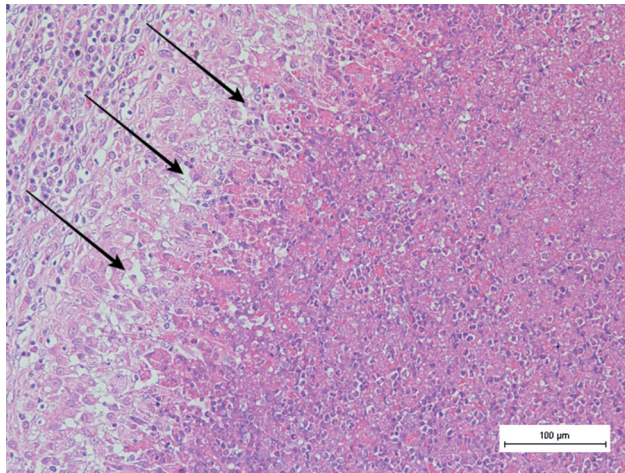


Figure 5. Photomicrograph of representative histopathological lesions of Mandibular lymph node of group **A** at day 21 pi, showing locally extensive necrosis (black arrows).

21 and 42 pi were significant ($P = .001$). Alveoli macrophages were also seen in the alveoli lumen. Few lymphocyte aggregates were seen around bronchioles.

The lesions observed in the spleen were mainly congestion, lymphoid depletion, and mononuclear cell infiltration with significant difference ($P = .001$) in the lesions between groups at day 21 and day 42 pi. In the kidneys, mild congestion and focal mononuclear leucocytic infiltration were the main lesions observed with a significant difference ($P = .001$) in each of the lesions between the groups. The lesion scoring from both A and B generally decreased toward the 42nd day pi as there was reduced significance between

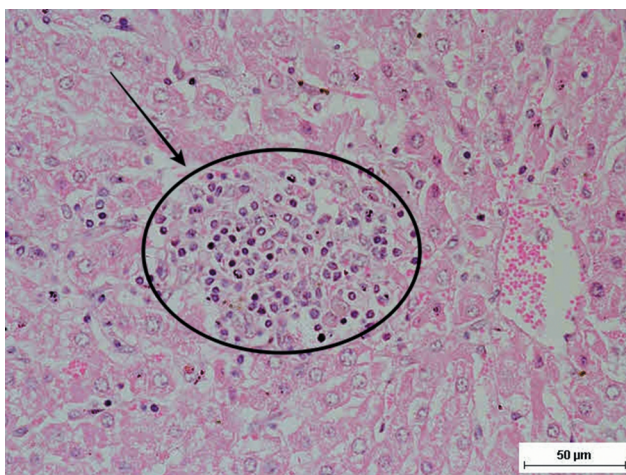


Figure 6. Photomicrograph of representative histopathological lesions of Liver from group A at day 21 pi showing lymphocyte aggregate disrupting the cellular architecture of the hepatocytes (black arrow) with mild congestion at the central vein.

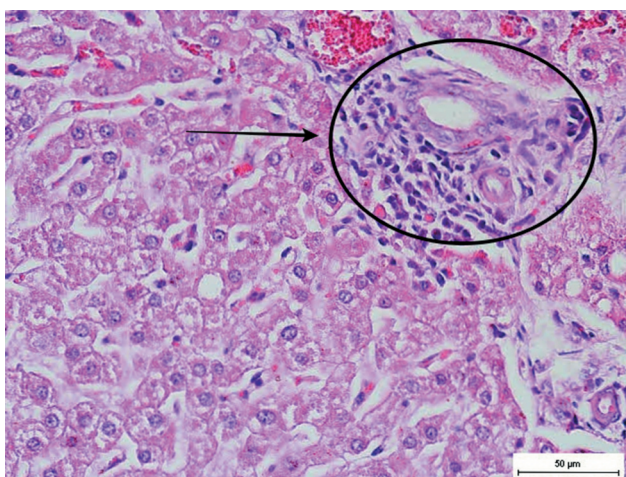


Figure 7. Photomicrograph of representative histopathological lesions of Liver from group B at day 42 pi showing periportal leukocytic infiltration (arrow) and mild congestion.

groups A and B which correlated with the mild lesions seen during the postmortem examination.

Tissue antigen distribution (Immunohistochemistry)

The appearance of a golden-brown stain, signifying site of the antigen-antibody reaction was considered positive. Positive reactions were recorded in the uterus, lymph node, lung, spleen, kidney, and liver [Table 2](#). In the uterus, immunoreactivity was observed in the perimetrium, myometrium, and

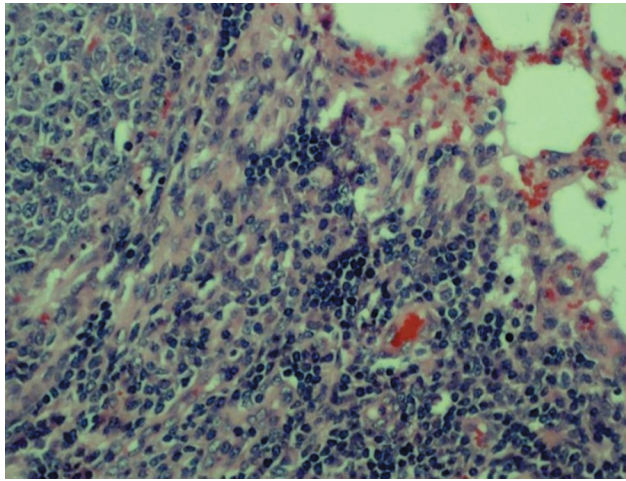


Figure 8. Photomicrograph of representative histopathological lesions of Lungs from group A at day 21 pi showing lymphoid nodules, bronchopneumonia and thickened alveolar septa.

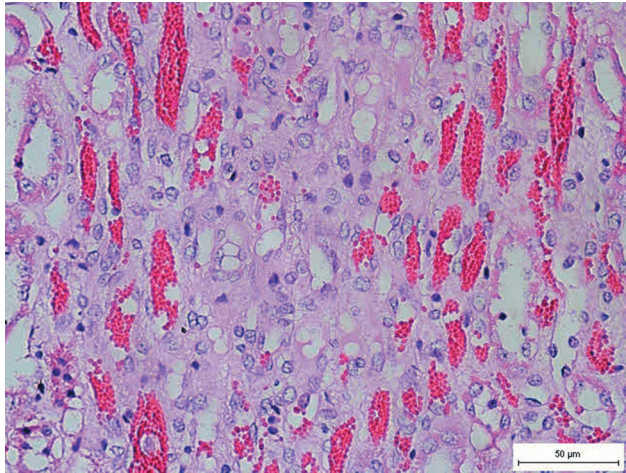


Figure 9. Photomicrograph of representative histopathological lesions of Kidney from group A at day 42 showing congestion at the sinusoids and mild hemorrhage and mononuclear cell infiltration.

endometrium while in the lymph nodes, immunoreactivity was seen in the splenic and white pulps. Positive reactions in the lung were seen intracellularly, predominantly in foamy macrophages, neutrophils, and extracellularly in the debris of cells in alveoli spaces (Figure 10–15). Immunostaining was also observed in the interstitium of the alveoli. In the liver, immunoreactivity was seen in the periportal areas while in the spleen, immunostaining was seen both in the white and red pulps. In the kidney, immunostaining was seen at the paracortical and medullary areas. Group A recorded strong immunostaining in the lungs and spleen while group B recorded moderate to mild staining in

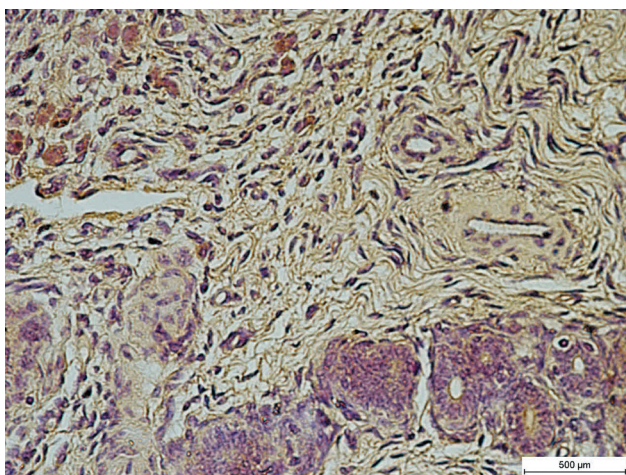


Figure 10. Photomicrograph of immunostaining of Uterus from group A showing moderate immunoreactivity in the myometrium at day 21 pi. Hematoxylin counter stain, 40X.

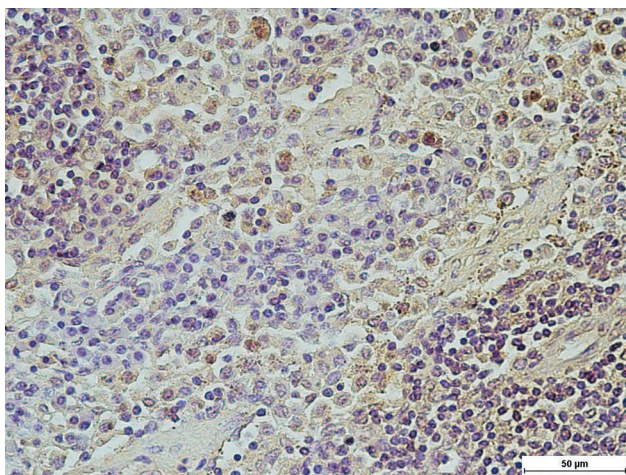


Figure 11. Photomicrograph of immunostaining of Mandibular lymph node from group A showing moderate immunoreactivity at day 42 pi. Hematoxylin counter stain, 40X.

these organs while group C showed no immunoreactivity. Moderate to mild immunostaining was equally observed in the hepatocytes of group A day 21 pi. The intensity of the staining was found to be less at day 42 pi compared to day 2 in both group A and group B.

***Brucella melitensis* DNA detection from tissues using PCR**

Brucella melitensis DNA detection from the tissues collected from sacrificed animals on days 21 and 42 pi are shown in Table 3. More organs from the dexamethasone-treated animals (group A) were positive for *Brucella* and the

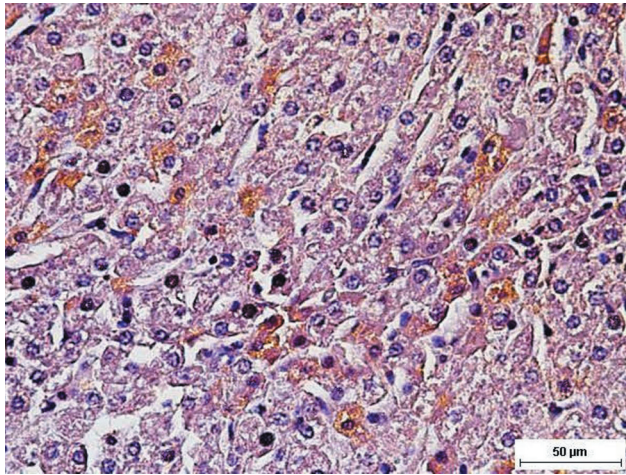


Figure 12. Photomicrograph of immunostaining of Liver from group A at day 21 pi showing moderate hepatocyte immunoreactivity. Hematoxylin counter stain, 40×.

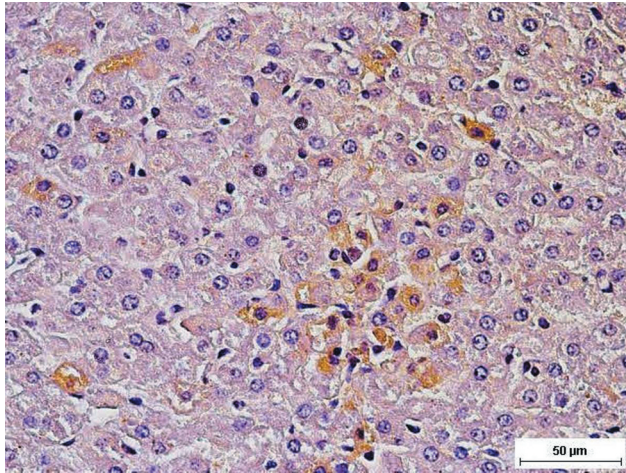


Figure 13. Photomicrograph of immunostaining of Liver from group B at day 21 pi showing mild hepatocyte immunoreactivity. Hematoxylin counter stain, 40×.

bacteria persisted in these organs for a relatively longer period compared to group B as these organs were positive days 21 and 42 pi. There was a direct relationship between bacterial detection and histopathological lesions. Although *B. melitensis* was detected from all the organs collected, the frequency of detection was significantly higher from the lungs and spleen of group A compared to group B. However, no *B. melitensis* was detected from group C.

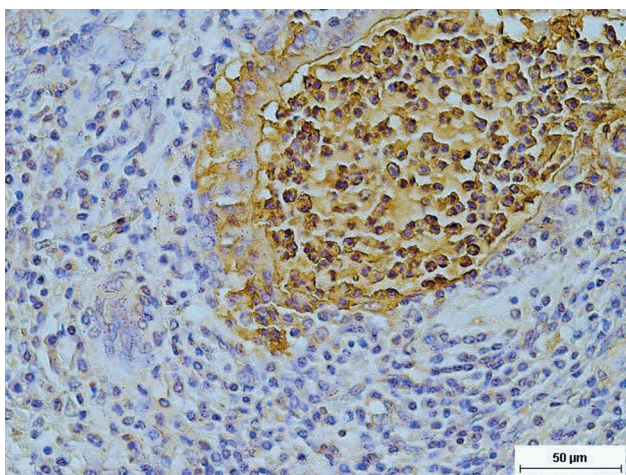


Figure 14. Photomicrograph of immunostaining of Lungs of group A at day 21 pi showing strong immunoreactivity involving both the epithelial cells of the bronchiole and infiltrated inflammatory cells. Hematoxylin counter stain, 40 $\times$.

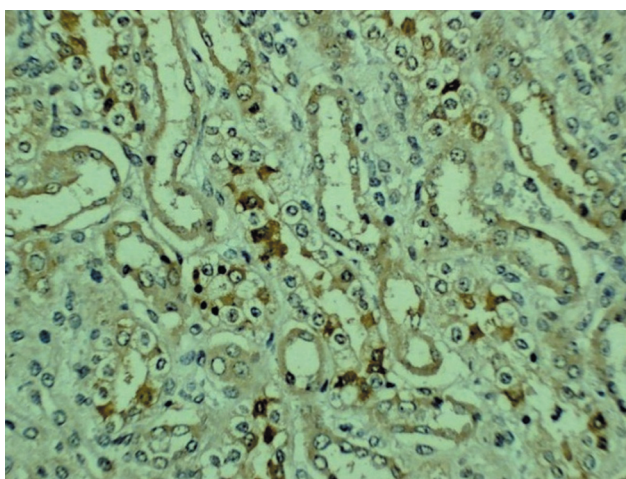


Figure 15. Photomicrograph of immunostaining of Kidney of group A at day 21 showing intense immunoreactivity in the epithelial cells. Hematoxylin counter stain, 40 $\times$.

Correlation between lesion score and tissue antigen distribution (immunohistochemistry)

There was a strong positive correlation between histopathological lesion score and IHC in the spleen ($r = 0.866$) and kidney ($r = 0.753$) in group A. There was also a moderate positive correlation between histopathological lesion score and IHC in the lungs and liver ($r = 0.618$) and ($r = 0.600$) respectively in group A. Group B similarly showed moderate positive correlations between histopathological lesion score and IHC in lungs, spleen, liver, and kidney.

Table 1. Lesion scoring for tissues obtained at days 21 and 42 pi.

Organ	Lesion	Group B		Group B		Group C	
		D21	D42	D21	D42	D21	D42
Liver	Focal necrosis	1.1 ± 0.02	2.2 ± 0.03*	1.0 ± 0.01	1.1 ± 0.02	0.0	0.0
	Congestion	2.3 ± 0.03*	2.3 ± 0.04*	1.1 ± 0.02	1.0 ± 0.02	0.0	0.0
	PPLI	1.1 ± 0.02	0.00	1.2 ± 0.03	0.00	0.0	0.0
Lungs	Bronchointerstitial pneumonia	2.2 ± 0.04*	2.15 ± 0.03*	0.00	1.2 ± 0.03	0.0	0.0
	Congestion	3 ± 0.05*	1.1 ± 0.02	1.2 ± 0.03	1.1 ± 0.02	0.0	0.0
	Focal necrosis	1.1 ± 0.02	2.4 ± 0.05*	0.00	1.1 ± 0.02	0.0	0.0
	MN infiltration	1.3 ± 0.02	1.3 ± 0.02	0.00	1.0 ± 0.01	0.0	0.0
Spleen	Congestion	2.3 ± 0.04*	0.00	1.3 ± 0.02	0.00	0.0	0.0
	Lymphoid depletion swp	2.3 ± 0.04*	1.7 ± 0.03*	0.00	1.1 ± 0.02	0.0	0.0
	Mononuclear infiltration srp	1.3 ± 0.02	2.3 ± 0.04*	1.1 ± 0.02	0.00	0.0	0.0
Kidney	Congestion	1.3 ± 0.02	0.00	1.1 ± 0.02	1.1 ± 0.02	0.0	0.0
	Focal interstitial mononuclear leukocytic infiltration	1.7 ± 0.03*	1.1 ± 0.02	0.00	1.1 ± 0.02	0.0	0.0
Uterus	Congestion	1.6 ± 0.2	1.2 ± 0.1	0.3 ± 0.03	0.00	0.0	0.0
	Inflammation	1.3 ± 0.1	1.0 ± 0.1	0.6 ± 0.02	0.3 ± 0.02	0.0	0.0
	microgranuloma	1.3 ± 0.01	1.3 ± 0.1	0.00	0.3 ± 0.02	0.0	0.0
Lymph nodes	Necrosis	2.7 ± 0.02*	2.3 ± 0.3*	1.3 ± 0.02	1.2 ± 0.02	0.0	0.0
	microgranuloma	1.7 ± 0.03*	1.1 ± 0.02	1.0 ± 0.1	1.0 ± 0.02	0.0	0.0
Total score		29.6*	22.45*	11.2	12.6	0.0	0.0

PPLI: Periportal leukocytic infiltration. MN: Mononuclear cell. SWP: splenic white pulp. SRP: splenic red pulp. Data are expressed as the mean ± standard error of the mean. *: the mean difference is significant at $P < 0.05$ when compared with group B

Table 2. Immunostaining scoring for liver, lungs, kidney, spleen, uterus, and lymph nodes at days 21 and 42 pi.

Organs	Group A		Group B		Group C	
	D21	D42	D21	D42	D21	D42
Lungs	2.88 ± 0.2*	2.21 ± 0.3*	1.32 ± 0.01	1.13 ± 0.01	0.00	0.00
Liver	1.83 ± 0.01*	1.31 ± 0.02	0.00	1.12 ± 0.03	0.00	0.00
Spleen	3.00 ± 0.04*	2.22 ± 0.01*	1.13 ± 0.01	1.14 ± 0.23	0.00	0.00
Kidney	2.41 ± 0.04*	2.12 ± 0.02*	1.21 ± 0.03	0.00	0.00	0.00
Uterus	1.13 ± 0.1	1.30 ± 0.1	0.30 ± 0.1	0.00	0.00	0.00
Lymph nodes	2.72 ± 0.04*	2.72 ± 0.02*	1.31 ± 0.03	1.00	0.00	0.00
Total score	13.97*	11.88*	5.27	4.39	0.00	0.00

0: No immunoreactivity, 1: Mild immunoreactivity, 2: Moderate immunoreactivity, 3: Intense immunoreactivity. Data are expressed as the mean ± standard error of the mean. *: the mean difference is significant at $P < 0.05$ when compared with group B

Table 3. *Brucella melitensis* DNA detection from tissues using PCR.

Organ	Group A		Group B		Group C	
	Day 21	Day 42	Day 21	Day 42	Day 21	Day 42
Liver	+(2)	+(2)	+(1)	+(1)	-	-
Lungs	+(2)	+(1)	+(1)	-	-	-
Kidney	+(2)	+(2)	-	+(1)	-	-
Spleen	+(2)	+(2)	+(1)	+(1)	-	-
Uterus	+(1)	+(1)	+(1)	-	-	-
Lymph nodes	+(2)	+(1)	+(1)	+(1)	-	-
Total	11*	9*	5	4	-	-

The numbers in bracket represent the number of positive samples per group per sampling time

Discussion

This study investigated the shedding routes and immune-pathological changes associated with *Brucella* infection in immunosuppressed goats. The major source of brucellosis in humans is the contamination of the environment and animal products with *Brucella* antigen from domesticated animals; hence, endemic areas have a higher public health risk.^[3,5] The increased detection of the bacteria from the investigated routes in group A in comparison to group B in this study is indicative of stress enhancing shedding of the bacteria as documented in earlier studies.^[3,7] The bacteria were detected in at least two routes at a time in group A from day 10 to day 42 pi with the exception of day 28 pi. That the goats did not shed the bacteria on day 28 pi could only be explained in the context of the undulant fever nature of the bacteria as documented in other studies.^[8,16] There was more relationship between the routes at days 14, 21, and day 35 pi with the peak at day 21 pi in group A. In group B on the other hand, the bacteria were only detected in two routes at days 10 and 14 pi. This also stresses the influence of stress on the shedding pattern as similarly documented in earlier studies,^[3,7,17] who found that stress is linked to increased disease susceptibility, bacterial load, and subsequently discharging the pathogen. The substantial difference in the summary of bacterial detection from the investigated routes in group A (22.2%) as against group B (9.4%) is also an indication that stress has influenced the shedding of the bacterium in the infected animals significantly. The dominance of the nasal route in the shedding of the bacterium is suggestive of its proximity with the route of inoculation.^[3]

Immunosuppression as a consequence of acute and chronic stress can increase the susceptibility of ruminants to a range of infectious diseases.^[9,10] Studies linking stress with the pathology of disease conditions have revealed that stress enhance immunopathology and/or decrease immunoprotection.^[6,7,10] The reduced serological responses as evidenced by the iELISA and the RBPT used in this study agree with our earlier findings,^[3] where both transport-induced stress and dexamethasone-induced stress were found to suppress serological responses. The consistent result of 100% positivity recorded at days 10 and 14 pi with PCR utilizing DNA extracted from blood was an indication that it is a good diagnostic method for the detection of brucellosis as earlier observed.^[2,14] Nevertheless, the inconsistency observed starting from day 21 pi may be as a result of the bacteria relocating from the vascular system to phagocytic and nonphagocytic cells in order to keep away from immune responses as documented in related studies.^[8,16] This implies the absence of the bacteria agent in the blood does not necessarily mean the animal is negative of *Brucella*. Therefore, there is a need to always test several biological samples, especially during the screening exercise.^[3] Dexamethasone, a synthetic corticosteroid, an anti-inflammatory agent

could suppress the immune function thereby increasing susceptibility to infectious diseases.^[3,14] The increased gross lesions seen in A in comparison to B which had mild lesions could be due to the stress induced by dexamethasone administration. The absence of these lesions in group C, in addition to the increased severity of the lesions in group A when compared with lesser severity reported in other related studies on the pathogenesis of *B. melitensis* further gave credence to this fact.^[7,14] These findings further consolidate the observations documented by scholars earlier,^[3,14] who opined that stress influences the pathology of infectious diseases as it enhances disease progression and severity.

The significant difference in the histopathological lesions on tissues of group A compared to group B which had relatively mild lesions implicated dexamethasone treatment as been responsible. This also agreed with the gross lesions earlier observed during postmortem examination. The increased lesions in group A imply that stress could enhance the pathological derangements resulting from *B. melitensis* infections in goats negatively via immunosuppression. This was similarly documented in earlier studies,^[6,14] where road-transported goats and dexamethasone-treated goats showed a significant difference in lesions of caprine pneumonia and brucellosis, respectively, compared to the control groups. The reduced lesion scoring toward day 42 pi in group B as compared to A could imply that lesions associated with brucellosis in goats reduce in chronic infection with stress possibly prolonging the period of the severity of the lesions as observed in this study. The histopathological lesions seen in this study are in accord with earlier studies.^[7,14] This appears to be the first study to determine the pathological dynamism of *Brucella* infection in goats pretreated with dexamethasone.

The immunohistochemistry technique employed in this study revealed intense immunostaining from tissue sections of group A on the 21st day pi and this is attributed to the immunosuppression induced by dexamethasone treatment.^[6,7] The intensity of the immunostaining decreased at day 42 pi and this could imply that in chronic brucellosis, the bacterial load could be reduced by immune cells.

The strong positive correlation observed between the histopathological lesions and IHC in the uterus, lymph nodes, spleen, and kidney, and the moderate positive correlation in the lungs and liver in group A further affirmed that the bacterium is responsible for the lesions observed. The mild to moderate immunostaining in the lungs, lymph nodes, uterus, liver, spleen, and kidney of group B animals which were less compared to those of group A may not be unconnected with the stress induced by dexamethasone treatment as reported in other small ruminant diseases.^[4,6] These immunostainings imply colonization of the organs by the *Brucella* pathogen as earlier reported by,^[1] that bacteremic phase of infection results in colonization of

the spleen and liver. The presence of intense immunostaining in the alveoli space of the lungs of group A animals further gave credence to an earlier study,^[3,7] who reported that stress increased the shedding of *Brucella* antigen through the nasal route. The specificity of the immunohistochemistry technique used is satisfactory as all tissues from the control negative animals were negative through days 21 and 42 pi. This technique has also been reported to be accurate and rapid in detecting *Brucella* antigen in formalin-fixed, paraffin-embedded tissues.^[14,18,19]

The detection and/or isolation of *B. melitensis* from tissues of suspected or infected animals is one of the techniques employed in the diagnosis of brucellosis.^[4,20] Compared to group B, the bacterium was detected from more tissues (liver, lungs, kidney, spleen, uterus, and lymph node) of group A. This could be due to the immunosuppression which might have impaired the immune system thereby reducing its ability to curb the infecting bacteria. The reduced ability of the immune system to curb the infecting bacteria leads to the increased susceptibility to infection as earlier revealed.^[3,4,7] Although the bacterium was detected in the tissues of both groups A and B, the rate of bacteria detection from these organs decreased over time as there were only a few detections on day 42 pi. Even though this appears to be the first report in goats that are immunosuppressed by dexamethasone treatment prior to infection with *B. melitensis*, this finding is in accord with the reports of Nwunuji et al.,^[3] and Tanko et al.,^[7] who reported that stress increased the rate at which infected animals shed bacterium and that over time, the rate of shedding decreased. It was suggested that recovery from the stress and the gradual return to normalcy of the immune system as the disease progressed toward chronicity is responsible for the decline observed in bacteria detection.

The findings of the strong positive correlation between lesion score and tissue antigen distribution in both groups A and B show the bacterium is responsible for the lesions. That group A has strong and moderate positive correlations while group B had only moderate correlations between lesions and tissue antigen distribution could perhaps explain the effect of dexamethasone-induced stress prior to *B. melitensis* infection. This, to the best of our knowledge, is the first study to evaluate the correlation between lesions and tissue antigen distribution in dexamethasone-induced stress prior to *B. melitensis* infection.

Conclusion

In conclusion, this study revealed the shedding routes, tissue antigen distribution, and pathology during *Brucella* infection in dexamethasone-treated goats. Although the pathology of *Brucella* infections has been a subject of extensivity study, the extent of the shedding and pattern, tissue antigen distribution, and the extent of pathological changes associated with this disease

following dexamethasone-induced stress has not been investigated. This seems to be the first study associating stress with bacteria shedding, tissue antigen distribution, and pathological changes in goats experimentally inoculated with *B. melitensis* following dexamethasone administration. The study demonstrated that dexamethasone treatment induced a significantly higher rate of shedding, tissue antigen distribution, and pathology in experimental *Brucella* infection in dexamethasone-treated goats compared to non-treated goats.

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