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# Haematological, Biochemical And Serum Electrolyte Changes In Non-Pregnant Boer Does Inoculated With *CorynebacteriumPseudotuberculosis* Via Various Routes

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***Abstract:*** *Caseous lymphadenitis is a chronic disease characterized by internal and external abscesses and is caused by corynebacterium and pseudotuberculosis.Thisstudy is designed to measure the hematological, biochemicaland serum electrolyte changes in experimental non-pregnant doesinoculated withcorynebacterium pseudotuberculosisvia variousroutes. Little is known about the changes in these parameters through different routes of infection.A total of 20 healthy does (n=20) were divided into 4 groups (intradermal, intranasal, oral and control) of5 goats each. The three groups were inoculated with $10^7$cfu/1ml of live corynebacterium pseudotuberculosis, while control is kept unexposed. Following infection, blood samples were collected from the jugular veinfor hematological, biochemical and serum electrolyteanalysis. A significantdecrease was observed in RBC count ($p<0.05$) in the intradermal group, whilenochangesobservedin PCV, Hb, MCV and MCHC parameters. Significant increasein WBC were observed in intradermal, intranasal and oral groups ($p<0.05$). Slight increase ($p<0.05$) in monocyte count was observed in intranasal group. A significant reduction ($p<0.05$) in lymphocytic count was observed for the intranasally inoculated group($p<0.05$) and a slight increase ($p<0.05$) in neutrophil was observed in intranasal and intradermal groups. Biochemically, decrease in albumin, increase in creatininelevelsinintranasal group($p<0.05$) and GGT levels were elevated in all the three infected groups ($p<0.05$).However,there were no significant changes in AST, T.Protein and APT. Serumelectrolyte, revealed a decrease in Calcium ($Ca^+$) concentration in intradermal group with concentration of 2.22mmol/L,intranasal 2.23mmol/L group ($p<0.05$), and no changes were observed in potassium ($K^+$) and sodium ($Na^+$). The study, therefore, observed increase in WBCs, neutrophils, monocytes, creatinine, GGT, levels and decrease in RBCs, lymphocytes, albumin and calcium concentrations on different route of infections.*

***Keywords:****corynebacterium pseudotuberculosis, haematology, biochemical, serum electrolyte, does, routes.*

## I. Introduction

Caseous lymphadenitis (CLA) otherwise known as cheesy gland [1] isachronicdisease that usually affect sheep and goats. It is characterized by the formation of abscesses in superficial and internal lymph nodes [1,2]. CLA infection cause a great economic losses to ovine and caprine farmers, such as decreased in wool, meat and milk production, culling of affected animals and condemnation of carcasses and skin in slaughter houses [1,3].Haematology, biochemical and serum electrolyte parameters are very important in monitoring animals health during disease conditions. Many of the available information on these parameters on blood have been studied during CLA infection on different species and doses[4, 5, 6].

There is lack of information on the effects of *C.pseudotuberculosis* infection on haematological, biochemical and serum electrolyte parameters on non-pregnant does through various routes of infection. Therefore, this study was undertaken to determine the possible changes in these parameterson various routes and toidentify which route has greater effectof*C.pseudotuberculosis* infection in goats.

## II. Material And Methods.

**Ethical consideration**

The experimental procedure was conducted under the approval of the Animal Care and Use Ethics Committee,Universiti Putra Malaysia as required in Malaysia by the Animal welfare Act (2014) and with reference number UPM/IACUC/AUP-R029/2014.

**Animals and management**

Twentyadult healthy non-pregnant Boer does, with average weight of 30 ±5kg were used in this study. The animals were acclimatized for 2 weeks prior to the experiment and were fed with commercial goat pellets (300g/goats/day) with cut Napier grass. Bloodandswab samples (nasal, oral mucosa and vaginal) were collectedfor the screening of *C.pseudotuberculosis*infection. The animals were randomly divided into four groups (A, B, C and D) of five goats each.

**Estrus Synchronization**

In order to avoid variations cyclic changes, all the does were synchronized by insertion of anintra-vaginal sponge containing 30mg flurogesterone acetate (FGA) for 9 day. At 48 hours before the sponge was removed Cloprostenol (50ug) and pregnant mare serum gonadotrophin (PMSG; 750IU) was injected intramuscularly[7]. After synchronization, inoculation proceeded immediately on the same day.

**Preparation of inoculum**

*Corynebacterium pseudotuberculosis* that was previously isolated from an outbreak ofclinical CLAcases among goatsatUniversiti Putra Malaysia [8] was used in this study, the bacterium was inoculated into brain heart infusion (BHI) broth and followed by incubation in shaker incubator at 150 x g at $37^0$ C for 48hrs. The cultured colonies were then harvested and diluted using the 10 fold serial dilution method and 1ml of each of the serial dilution were inoculated into agar blood plate. Plate count method as described by Alcamo [9] was used to determined the bacteria concentration.

**Inoculation**

Animals of group A,B and C were inoculated with 1ml of the inoculum containing $10^7$cfu/ml of live *C.pseudotuberculosis*through the intradermal (on the neck region), intranasal and oral route respectively.While group D (control) were kept unexposed andweregiven 1ml of phosphate buffer saline (PBS) orally. Clinical signs were observed daily for 30 days post inoculation.

**Sampling**

The blood samples were collected from jugular vein between three days interval periods from the control and infected goats using a 1.2x38mm (21G1.5'') venoject needle (Precision GlideTM, Becton Dickinson, UK) With a venoject holder (Vacutainer®, BD Vacutainer TM, USA) in 5ml tubes containing EDTA anticoagulant (Vacutainer®, BD Vacutainer, USA) for complete blood count analysis and in 5ml plain tubes (Vacutainer®, BD Vacutainer, USA) where the serawas extracted and kept at $-20^0$ C for biochemical and serum electrolyte analysis.

**Analysis of samples**

Animal blood counter (ABC) 112AB8105 (France) machine was used for red blood cell and white blood cell counts, PCV was determined using a microhaematocrit technique. An automatic analyser machine (HITACHI 902 Japan) was used for biochemical and electrolytes analysis.

**Statistical analysis**

Data were analysed using statistical software JMP (version 9.0.1 SAS Institute Inc., Cary, NC, USA). Two -way analysis of variance (ANOVA) was used to test the differences between specific pairs. The differences were considered as significant when $p \leq 0.05$.

## III. Results

The hematological studies showed a significant decrease in RBC counts inthe intradermal with a mean value of $11.78x10^{12}$/L ($p<0.05$), no changes in RBC havebeen observed in oral and intranasal groups compared to the control group.No significant changes in PCV, Hb, MCV, and MCHC, compared to the control group (Table.1).

There were significantincrease ($p<0.05$) in WBC countfor intradermal, intranasal and oral groups with the mean value of $13.68x10^9$/L, $9.68x10^9$/L, $8.67X10^9$/L, respectively, compared to the control group. Neutrophils was slightly elevated ($p<0.05$) in the intranasal and intradermal groups with a mean of $11.87x10^9$/L, $8.48^9$/L, respectively and a significant increase ($p<0.05$) in monocyte count from the intranasal group have been observed with a mean of $0.75x10^9$/L compared to other groups, while no significant changes ($p>0.05$) in eosinophil, basophiland plasma protein parameters.However, a slight reduction ($p<0.05$) in lymphocyte count from intranasal group have been observed with a mean value of $3.37x10^9$/L compared to other groups (Table. 2 ).

For biochemical analysis, there were significant decrease ($p<0.05$) inthe concentration of albumin( 28.74g/L ) and increase increatinine concentration (91.33umol/L) for intranasalgroup. Significant increase in

GGT concentration for intranasal, intradermal and oral groups with the mean value of 48.00U/L, 46.52U/L, and 36.62U/L respectively (Table. 3).

Serum electrolytes analysis showed significant decrease ($p<0.05$)in the concentration of calcium from intradermal and intranasal groups with the mean value of 2.22mmol/L and 2.23mmol/L, respectively (Table.4).

**Table:1.Changesin Red bloodcells in infected and control groups (Mean ±SD).**

| Parameters | | | | | |
|---|---|---|---|---|---|
| **GROUP** | **RBC($x10^{12/L}$)** | **Hb(g/L)** | **PCV(L/L)** | **MCV(f/L)** | **MCHC(g/L)** |
| **Control** | **$11.78±1.53^{a}$** | **$80.56±11.01^{a}$** | **$0.22±0.03^{a}$** | **$18.49±1.85^{a}$** | **$371.80±24.18^{a}$** |
| **Intradermal** | **$11.33±0.99^{b}$** | **$77.25±8.87^{a}$** | **$0.21±0.03^{a}$** | **$18.33±1.71^{a}$** | **$373.61±24.33^{a}$** |
| **Intranasal** | **$11.44±0.84^{a,b}$** | **$78.33±9.14^{a}$** | **$0.21±0.03^{a}$** | **$18.20±1.38^{a}$** | **$377.49±21.44^{a}$** |
| **Oral** | **$11.78±0.63^{a}$** | **$82.88±8.06^{a}$** | **$0.22±0.03^{a}$** | **$18.66±1.45^{a}$** | **$378.48±20.03^{a}$** |

**Note: all values were expressed as Mean ± SD and $^{a, b}$ within the columns with different superscripts differed significantly ($p<0.05$).**

**Table: 2.Changes in White blood cellsof infected and control groups (Mean ± SD).**

| Parameters | | | | | | | |
|---|---|---|---|---|---|---|---|
| **GROUP** | **WBCS ($x10^{9}$/l)** | **Neutrophil ( $x\ 10^{9}$/L)** | **Lymphocyte ( $x\ 10^{9}$/L)** | **Monocyte ($x\ 10^{9}$/L)** | **Eosinophil ( $x\ 10^{9}$/L)** | **Basophil ( $x\ 10^{9}$ /L)** | **Plasma protein ( $x\ 10^{9}$/L)** |
| **Control** | **$8.67±0.09^{d}$** | **$3.74±2.66^{b}$** | **$3.56±1.09^{a,b}$** | **$0.59±0.15^{b}$** | **$0.29±0.27^{a}$** | **$0.18±0.09^{a}$** | **$72.41±5.70^{a}$** |
| **Intradermal** | **$13.68±5.43^{a}$** | **$8.48±4.83^{a}$** | **$3.88±1.13^{a}$** | **$0.50±0.15^{b}$** | **$0.63±3.56^{a}$** | **$0.19±0.09^{a}$** | **$73.20±5.73^{a}$** |
| **Intranasal** | **$11.22±1.78^{b}$** | **$11.87±55.92^{a}$** | **$2.66±0.66^{c}$** | **$0.75±0.36^{a}$** | **$0.26±0.27^{a}$** | **$0.13±0.09^{a}$** | **$72.13±7.67^{a}$** |
| **Oral** | **$9.68±2.95^{c}$** | **$4.32±1.13^{b}$** | **$3.37±1.20^{b}$** | **$0.51±0.14^{b}$** | **$0.21±0.17^{a}$** | **$0.13±0.09^{a}$** | **$72.83±7.87^{a}$** |

**Note: all values were expressed as Mean ± SD and $^{a,b,c,d}$within the columns with different superscripts differed significantly ($p<0.05$).**

**Table: 3.**Changes in **Biochemical parametersof the infected and control groups (Mean ± SD).**

| Parameters | | | | | |
|---|---|---|---|---|---|
| **GROUP** | **ALB(g/L)** | **AST(U/L)** | **GGT(U/L)** | **Urea(mmol/L)** | **Creatinine(umol/L)** |
| **Control** | **$30.63±3.62^{b}$** | **$99.56±18.61^{a}$** | **$43.07±9.43^{b}$** | **$8.71±2.42^{a}$** | **$83.86±20.23^{b}$** |
| **Intradermal** | **$30.11±4.19^{b}$** | **$102±29.82^{a}$** | **$46.52±8.85^{a}$** | **$8.49±2.20^{a}$** | **$88.51±14.83^{a,b}$** |
| **Intranasal** | **$28.74±3.43^{a}$** | **$98.86±22.46^{a}$** | **$48.00±8.52^{a}$** | **$9.23±2.12^{a}$** | **$91.33±13.46^{a}$** |
| **Oral** | **$29.56±4.72^{a,b}$** | **$107.48±37.60^{a}$** | **$36.62±5.78^{c}$** | **$9.26±2.01^{a}$** | **$89.88±14.88^{a,b}$** |

**Note: all values were expressed as Mean ± SD and $^{a,b,c}$ within the columns with different superscripts differed significantly ($p<0.05$).**

**Table: 4. Changes in Electrolytes parameters in infected and control groups of non-pregnant Boer does inoculated with *C.pseudotuberculosis* (Mean ±SD).**

| Parameters | | | | | | |
|---|---|---|---|---|---|---|
| **GROUP** | **Ca(mmol/L)** | **Ck(UL/L)** | **Totalprotein(g/L)** | **$Na^{+}$(mmol/L)** | **$K^{+}$(mmol/L)** | **$Cl^{-}$(mmol/L)** |
| **Control** | **$2.33±0.23^{a}$** | **$206.86±98.05^{a}$** | **$71.47±9.69^{a}$** | **$145.67±8.31^{a}$** | **$4.78±0.52^{a}$** | **$118.11±109.95^{a}$** |
| **Intradermal** | **$2.22±0.25^{b}$** | **$215.47±85.38^{a}$** | **$70.22±8.15^{a}$** | **$145.08±5.24^{a}$** | **$4.70±0.40^{a}$** | **$104.90±4.24^{a}$** |
| **Intranasal** | **$2.23±0.24^{b}$** | **$231.89±104.91^{a}$** | **$69.39±9.34^{a}$** | **$145.42±6.64^{a}$** | **$4.82±0.46^{a}$** | **$105.27±4.72^{a}$** |
| **Oral** | **$2.25±0.23^{a,b}$** | **$278.00±329.87^{a}$** | **$71.92±9.86^{a}$** | **$145.41±5.11^{a}$** | **$4.80±0.40^{a}$** | **$104.87±4.15^{a}$** |

**Note: all values were expressed as Mean ± SD and$^{a,b}$within the columns with different superscripts differed significantly ($p<0.05$).**

## IV. Discussions

From the study, the red blood cellsshowed significantdecrease innumber.This may be due to the harmful effect of the bacterial toxin on the blood cell where *C.pseudotuberculosis* exhibit properties of exotoxin[10] This finding is in agreement with [11, 12]. [13]reported a severe hemolytic anaemia, macrocytic

hypochromic and hypochromic normocytic aneamia were observed in sheep experimentally infected with *C.pseudotuberculosis.* However, inthis study the findings of [13] was not observed andthis may be due to the used of different species in the present study. Changes in Hb, MCV, and MCHC were not observed in the current study, however, this result is not in agreement with [4]which may be as a result of differentdoses ofinfection and species used. The former author used male sheep on natural infection and in the current study goats were used which is one ofthe natural hosts for CLA disease. There was increase in WBC count for all the treatment groups and the result of this study was in agreement with the outcome obtained by [12].The increasein WBC count may be due to the infection caused by *C.pseudotuberculosis* and this bacteria is able to stimulate the white blood cells to have reaction towards the infection. The infected goats showed slight increase in neutrophils, monocyte and sightdecrease inlymphocyte counts and no changes have been observed in basophil and eosinophil counts. This observationhowever, are in agreement with [5], who observed increase in neutrophils, lymphocytes and monocyte counts in mice inoculated with *C.pseudotuberculosis* and its exotoxin (PLD) via intraperitoneal route.

The significant increase in the concentration of creatinine in the present study isin agreement with [5, 14]. The increase in these parameters may be due to the infection of *C.pseudotuberculosis*which may leadtomuscle damage dueto the formation of abscesses and also its effect towards the renal system. The significant increase in GGT level observed in the current study, might be as a result of oxidative stress and presence of bacterial toxin in the liver. Similar observation has been obtained by [4, 5]. The slight decrease in albumin level observed in this study could be as a result of the bacteria toxin in the liver. On the other handthe hypocalcaemia observed in this study and the decreased in albumin concentration due to the diseased liver may lead decrease in calcium concentration in the blood. [15]observed in a study of human patient that albumin – bound calcium varied inversely with the absolute albumin concentration.

Therefore, changes observed in the concentration in GGT, creatinine, albumin and calcium may be due to the presence of bacterial in the liver and kidney, which affect the activities of this enzyme. *C.pseudotuberculosis* was isolated in these organs of sheep and similarly observed a ceseating tubercule, giant multinucleated cells, necrosis, micro abscess, haemorrhage, infilteration of neutrophil and macrophages [16].

## V. Conclusion

This study has shadelight on the effect of early stage of *C. pseudotuberculosis* infection on the haematogical, biochemical and serum electrolyte parameters in non-pregnant does. This will therefore, assist in the diagnosis and consequently control of caseouslymphadenitis (CLA).

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