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HAEMATOLOGICAL PICTURE OF RABBIT IMMUNIZED BY *CORYNEBACTERIUM BOVIS*

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Abstract

This study was conducted to investigate the immunization role of *Corynebacterium bovis* in the local breed adult male rabbits. For this purpose, 15 local breed rabbits were divided into 3 groups; the first group immunized with *C. bovis* sonicated antigen bacterium and challenged with pathogenic *C. bovis*, the second group (control negative) treated with phosphate buffer saline and the third group was given pathogenic *C. bovis* and named as control positive group. The results reveal that the first group immunized with *C. bovis* sonicated antigen and challenged with pathogenic *C. bovis* higher levels in the blood picture represented by elevation in the total leucocytes number over controls plus an increase in the lymphocytes and granulocytes compared with controls. Also, there was an increase in the total erythrocyte numbers and haemoglobin concentrations in the first group compared with the controls. on the other hands, there was a decrease in the platelets (thrombocytes) total numbers in the first group by comparison with the controls. In conclusion, *C. bovis* sonicated antigen is a potent immunogen model which can be used to study the blood picture response in rabbits.

Keywords: rabbits, *Corynebacterium bovis*, immunisation, blood picture.

Introduction

Corynebacterium bovis is a gram-positive rod shape non-motile, non spore forming, pleomorphic bacteria. It causes bovine mastitis in dairy cattle and cream rancidity which is responsible for much economic losses in the world (Watts *et al.*, 2000). *C. bovis* can cause acute inflammation of kidneys (nephritis) in cattle (Bolton *et al.*, 1975). In mice, *C. bovis* infection could extend to hyperkeratotic dermatitis and acanthosis (Dole *et al.*, 2013). In humans, it might infect nervous system and bacterial endocarditis; or even otitis media; *C. bovis* could cause leg ulcer and may cause implant-associated infections such as shoulder prosthetic joint infection (Achermann *et al.*, 2009). However, this pathogen is mostly isolated from the infected milk of dairy cows which can be detected by severe infiltration of polymorphonuclear cells (neutrophils) in the mammary gland tissues (Blagitz *et al.*, 2013). In the past, *C. bovis* could was thought to cause no significant changes in the blood picture of mastitic cattle (Paape *et al.*, 1973). Later studies illustrated that *C. bovis* can cause impairment in the blood picture and might cause human septicaemia (Dalal *et al.*, 2008; Moore *et al.*, 2010). *C. bovis* is resistant to many antibiotic drugs as illustrated by (Watts and Rossbach, 2000).

Materials and Methods

Fifteen local breed adult male rabbits (1.5–2.0) Kg was fed on grass and pellets for 2 weeks for adaptation in the animal house of the College of Veterinary Medicine, University of Baghdad, Iraq from January to March 2019. The rabbits were divided into 3 groups; the first group immunized with *C. bovis* sonicated antigen (CSA) killed whole cell antigen bacterium (1000 ug/ml) 1 ml subcutaneously (S/C) at 14 days (Mitov *et al.*, 1992) and protein measured by (Henry *et al.*, 1974). A booster dose of (CSA) was given (1000 ug/ml) 1 ml (CSA) S/C at 28 days and then challenged with pathogenic *C. bovis* (9 X 10^8 cfu/ml) intraperitoneally (IP) (Al-Badrawi, 2016) at 42 days and blood collected (2.5 ml blood from heart puncture) after 14 days post challenge (56 days after the start of experiment), the second group (control negative) treated with phosphate buffer saline (PBS pH = 7.2) at 14 days, 28 days and 42 days S/C and 2.5 ml blood collected (from heart puncture) after 56 days of the start of experiment. The third group was given PBS (pH = 7.2) at 14 days and 28 days and named as control positive group. Then challenged by *C. bovis* (9 X 10^8 cfu/ml) intraperitoneally (IP) was given (Al-Badrawi, 2016) at 42 days and 2.5 ml blood collected (from heart puncture) after 14 days post challenge. Blood analyser machine (Mindray BC-2800, Auto Haematology Analyser Shenzhen Mindray Bio-Medical Electronics Co., Ltd, China) was used to analyse the blood picture (Complete Blood Count, CBC) which include the following parameters: WBCs = White Blood Cells (leucocytes), Lymph = Lymphocytes, Mid = Monocytes, Gran = Granulocytes, HGB = Haemoglobin, RBCs = Red Blood Cells (Erythrocytes), HCT (PCV) = Haematocrit (Packed Cell Volume), MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration, RDW-CV = Red Cell Distribution Width – Coefficient of Variance, RDW-SD = Red Cell Distribution Width – Standard Deviation, PLT = Platelets (Thrombocytes), MPV = Mean Platelet Volume, PDW = Platelet Distribution Width, PCT = Procalcitonin Test

Results

The results of this study revealed elevated WBCs, lymphocytes, granulocytes, RBCs (erythrocytes), and packed cell volume (Haematocrit), and Procalcitonin (PCT) levels of the group immunized with *C. bovis* compared with controls. Whereas, there was a decrease of the platelets (PLT) and mean platelet volume (MPV) in the immunized group compared with controls (tables 1, 2 and 3). However, non immunized and treated with PBS (Control negative group) showed normal values of the measured parameters (table 2). On the other hands, the non immunized and challenged group with pathogenic *Corynebacterium bovis* (Control positive group) showed significant increase in the lymphocytes (Lymph), and red cell distribution width – coefficient of variance (RDW-CV), while a sharp decrease in the granulocytes (Gran), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean platelet volume (MPV), platelet distribution width (PDW), and Procalcitonin (PCT) (Table 3)

Table 1 : Immunized and challenged group with pathogenic *Corynebacterium bovis*.

Parameter	Result	Reference range
WBCs	*15 X 10^9 ± 1.1 X 10^7 /L	4 – 12 X 10^9 /L
Lymph	*12.5 X 10^9 ± 0.87 X 10^7 /L	0.8 – 4 X 10^9 /L
Mid	0.8 X 10^9 ± 0.11 X 10^7 /L	0.1 – 1.5 X 10^9 /L
Gran	*1.7 X 10^9 ± 0.24 X 10^7 /L	2.0 – 7.0 X 10^9 /L
Lymph (%)	*83.3 ± 8.22 %	20 – 40 %
Mid (%)	5.4 ± 0.093%	3.0 – 15 %
Gran (%)	*11.3 ± 0.27 %	50 – 70 %
HGB	12.0 ± 0.31 g/dL	11.0 – 16.0 g/dL
RBCs	*6.18 X 10^{12} ± 1.8 X 10^{10} /L	3.5 – 5.5 X 10^{12} /L
HCT	*51.6 ± 4.69%	37.0 – 50.0 %
MCV	83.6 ± 7.05 fL	80 – 100 fL
MCH	*19.4 ± 1.68 pg	27.0 – 34.0 pg
MCHC	*23.2 ± 2.01 g/dL	32.0 – 36.0 g/dL
RDW-CV	*20.7 ± 1.95 %	11.0 – 16.0 %
RDW-SD	*56.9 ± 5.44 fL	35.0 – 56.0 fL
PLT	*68 X 10^9 ± 6.1 X 10^7 /L	150 – 400 X 10^9 /L
MPV	*6.2 ± 0.57 fL	6.5 – 12.0 fL
PDW	15.0 ± 1.41	9.0 – 17.0
PCT	*0.042 ± 0.0013 %	0.108 – 0.282 %

WBCs = White Blood Cells (leucocytes), Lymph = Lymphocytes, Mid = Monocytes, Gran = Granulocytes, HGB = Haemoglobin, RBCs = Red Blood Cells (Erythrocytes), HCT (PCV) = Haematocrit (Packed Cell Volume) , MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration, RDW-CV = Red Cell Distribution Width – Coefficient of Variance , RDW-SD = Red Cell Distribution Width – Standard Deviation , PLT = Platelets (Thrombocytes), MPV = Mean Platelet Volume, PDW = Platelet Distribution Width, PCT = Procalcitonin Test

* = presence of significant differences at $P<0.05$.

Table 2 : Non immunized and treated with PBS (Control negative group).

Parameter	Result	Reference range
WBCs	7.7 X 10^9 ± 0.62 X 10^7 /L	4 – 12 X 10^9 /L
Lymph	2.8 X 10^9 ± 0.17 X 10^7 /L	0.8 – 4 X 10^9 /L
Mid	0.6 X 10^9 ± 0.04 X 10^7 /L	0.1 – 1.5 X 10^9 /L
Gran	4.3 X 10^9 ± 0.37 X 10^7 /L	2.0 – 7.0 X 10^9 /L
Lymph (%)	36.4 ± 3.28 %	20 – 40 %
Mid (%)	7.8 ± 0.69 %	3.0 – 15 %
Gran (%)	55.8 ± 5.02 %	50 – 70 %
HGB	12.3 ± 1.56 g/dL	11.0 – 16.0 g/dL
RBCs	4.25 X 10^{12} ± 0.51 X 10^{10} /L	3.5 – 5.5 X 10^{12} /L
HCT	49.1 ± 4.33 %	37.0 – 50.0 %
MCV	78.7 ± 7.25 fL	80 – 100 fL
MCH	19.6 ± 1.88 pg	27.0 – 34.0 pg
MCHC	25 ± 2.17 g/dL	32.0 – 36.0 g/dL
RDW-CV	15 ± 1.26 %	11.0 – 16.0 %
RDW-SD	53.2 ± 5.01 fL	35.0 – 56.0 fL
PLT	371 X 10^9 ± 34.67 X 10^7 /L	150 – 400 X 10^9 /L
MPV	7.8 ± 0.62 fL	6.5 – 12.0 fL
PDW	17.0 ± 1.55	9.0 – 17.0
PCT	0.0269 ± 0.0011 %	0.108 – 0.282 %

WBCs = White Blood Cells (leucocytes), Lymph = Lymphocytes, Mid = Monocytes, Gran = Granulocytes, HGB = Haemoglobin, RBCs = Red Blood Cells (Erythrocytes), HCT (PCV) = Haematocrit (Packed Cell Volume) , MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration, RDW-CV = Red Cell Distribution Width – Coefficient of Variance , RDW-SD = Red Cell Distribution Width – Standard Deviation , PLT = Platelets (Thrombocytes), MPV = Mean Platelet Volume, PDW = Platelet Distribution Width, PCT = Procalcitonin Test

Table 3 : Non immunized and challenged group with pathogenic *Corynebacterium bovis* (Control positive group).

Parameter	Result	Reference range
WBCs	11.5 X 10^9 ± 1.24 X 10^7 /L	4 – 12 X 10^9 /L
Lymph	*9.8 X 10^9 ± 0.84 X 10^7 /L	0.8 – 4 X 10^9 /L
Mid	0.6 X 10^9 ± 0.02 X 10^7 /L	0.1 – 1.5 X 10^9 /L
Gran	*1.1 X 10^9 ± 0.09 X 10^7 /L	2.0 – 7.0 X 10^9 /L
Lymph (%)	*85.0 ± 8.11 %	20 – 40 %
Mid (%)	5.1 ± 0.47 %	3.0 – 15 %
Gran (%)	*9.9 ± 0.86 %	50 – 70 %
HGB	12.2 ± 1.3 g/dL	11.0 – 16.0 g/dL
RBCs	*6.11 X 10^{12} ± 0.59 X 10^{10} /L	3.5 – 5.5 X 10^{12} /L
HCT	42.5 ± 4.06 %	37.0 – 50.0 %
MCV	*69.7 ± 7.14 fL	80 – 100 fL
MCH	*19.9 ± 1.82 pg	27.0 – 34.0 pg
MCHC	*28.7 ± 2.55 g/dL	32.0 – 36.0 g/dL
RDW-CV	*20.5 ± 1.94 %	11.0 – 16.0 %
RDW-SD	51.3 ± 4.92 fL	35.0 – 56.0 fL
PLT	*85 X 10^9 ± 8.21 X 10^7 /L	150 – 400 X 10^9 /L
MPV	*5.8 ± 0.51 fL	6.5 – 12.0 fL
PDW	14.5 ± 1.22	9.0 – 17.0
PCT	*0.049 ± 0.0017 %	0.108 – 0.282 %

WBCs = White Blood Cells (leucocytes), Lymph = Lymphocytes, Mid = Monocytes, Gran = Granulocytes, HGB = Haemoglobin, RBCs = Red Blood Cells (Erythrocytes), HCT (PCV) = Haematocrit (Packed Cell Volume) , MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration, RDW-CV = Red Cell Distribution Width – Coefficient of Variance , RDW-SD = Red Cell Distribution Width – Standard Deviation , PLT = Platelets (Thrombocytes), MPV = Mean Platelet Volume, PDW = Platelet Distribution Width, PCT = Procalcitonin Test
* = presence of significant differences at P<0.05.

Discussion

The normal values of blood picture parameters was studied in a simplified way in the past before more than more than 80 years which included neutrophils (polymorphonuclear leucocytes) count, lymphocytes count, eosinophils, basophils total red blood cells (RBCs) and haemoglobin (Jackson J.W. and Stovall, 1930). In this study an extended blood parameters were recorded which include WBCs = White Blood Cells (leucocytes), Lymph = Lymphocytes, Mid = Monocytes, Gran = Granulocytes, HGB = Haemoglobin, RBCs = Red Blood Cells (Erythrocytes), HCT (PCV) = Haematocrit (Packed Cell Volume), MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration, RDW-CV = Red Cell Distribution Width – Coefficient of Variance, RDW-SD = Red Cell Distribution Width– Standard Deviation, PLT = Platelets (Thrombocytes), MPV = Mean Platelet Volume, PDW = Platelet Distribution Width, PCT = Procalcitonin Test.

The results of the control negative group (treated with PBS) in the current study is in line with the normal values of (Rosenthal, 2002, MediRabbit, 2019).

In the other ruminants (goats) the results of the control negative group is also partially compatible with (Odhah *et al.*, 2017) who studied the effect of *Corynebacterium pseudotuberculosis* in the female goats. They used 1×10^9 cfu/ml of pathogenic *C. pseudotuberculosis* intradermally. Their results were represented by a fall in the RBCs, haemoglobin, PCV and MCV in the treated groups.

In rabbits, complete blood picture was studied in response to the relationship between hypercholesterolemia (increase of cholesterol in the blood) and the inflammatory activity; the results showed significant increase of heterophils (Sharma *et al.*, 2018) which is in line to we found so far.

Corynebacterium bovis that infect other mammals such as cattle could cause significant increase in the total number of leucocytes in the mammary gland (increase in the somatic cell count) (Watts *et al.*, 2000, Dalal *et al.*, 2008, Gonçalves *et al.*, 2016, Manuel *et al.*, 2017), which is nearly similar to the increase of WBCs we noted in our research. In the same line, it was recorded that *C. bovis* was responsible for a sharp increase (doubled) in the total leucocytes count in the bovine mammary gland (Pankey *et al.*, 1985, Huxley *et al.*, 2003, Blagitz *et al.*, 2013) which is concomitant with what we found.

The strain *Corynebacterium bovis* was detected in the chronic testicular abscess and the pulmonary tissues of rabbits whereas an intravenous injection of *C. bovis* reproduced a inguinal and vulval infection in the rabbits which is caused an elevated count of WBCs (Arseculeratne and Navaratnam, 1975) which is similar to what we found in the control positive group and the immunization group.

The results of the current study illustrated that normal complete blood count (CBC) in the control negative group were within the normal range while the control positive group showed an increase in the lymphocytes and RBCs which are consistent with (Razook, 2018) who noticed severe infiltration of leucocytes in the histopathological sections in the internal organs of the infected rabbits with *Klebsiella pneumoniae* and *Eimeria tenella* antigens.

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