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Association between Brucellosis with interleukin 12 and immune cells in native sheep.

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ABSTRACT

Research conducted to study relationship between immunological cells and interleukin 12 concentration (1L – 12) with Brucellosis in Awassi sheep, Neutrophils increased to more than 50% with incidence rate up to 1.00% (P<0.05), as well as monophils and eosinophils which rising to more than 6% and 3% associated to incidence rate received to 1.00% and 0.95% (P<0.01) respectively contrary, lymphocyte raised up to 50% (P<0.01) while basophils and erythrocytes not affected by Brucellosis whereas, 1L – 12 level tend to 2.14 ng/ml markedly (P<0.01) with increasing monophils to 6% and incidence rate 1.00% according to 1L – 12 concentration reached to 1.29 ng/ml with prevalence rate 1.00% with upgrade number of neutrophils. On the other side, 1L – 12 not affected by lymphocyte, eosinophils, basophils and erythrocyte however, higher correlation between Brucellosis and each of monophils and eosinophils were 0.499 and 0.406 (P<0.01) respectively, when association between neutrophils, monophils with 1L – 12 level were similar values received to 0.303 and 0.302 (P<0.05) correspondingly, relation between infection and both of monophils -0.456 (P<0.05) and neutrophils with small value was 0.279 (P<0.05). Study was concluded that is a positive relation between Brucellosis and white blood cells while 1L – 12 has a significant importance with neutrophils and monophils.

Keywords: Brucellosis, 1L – 12, immune cells, sheep.

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INTRODUCTION

The mostly common and widespread disease around the world because of facility and acceleration of its transition, occurs by Brucella which related to genus *brucella spp* refer to family *Brucellaeae* which considered threating for health, parasitic, intra cellular not lysis the blood also stay for a long period inter the body then translated to human directly throughout bio vector as insects (Fayyadh, 2010). Incubation period of infection continued to less than a week until several months, this long time reduce the activity of innate immunity during early phase of disease (Digpas, 2011). Ali et al. (2017) demonstrated the negative effect of infection on animal performance and productivity that resulting economic losses and they membered that incidence rate among Itamadani sheep received to 31.7% in Iraq.

Relationship between pathogen and host immunity system is important to attack bacteria or for developing its parasition inter cell when invades erythrocyte and survival there without replication to protect against immune system and antibiotics (Vitry et al., 2014). Virulance of pathogen associated with its ability to invade and replicate inter cell so that disease will be chronic. Broucella has a lipo poly saccharide as a mean defense which surrounding outer wall that persist cellular enzyme digestion to prevent expression antigen on surface of the cell which fail presenting antigen to lymphocytes and resulting accumulation bacteria inter phagocyte (Smith, 2018). In the same side, body resistance to pathogens depend upon phagocytes, dendritic cells activation and increase lymphocytes to produce antibodies, this interaction with Brucella for enabling to live intra cell (Nielsen and Duncan, 2018).

Cytokines produced from T helper 1 cells which eradicate lymphocytes to secret antibodies which strength phagocytosis by completing activity of antigen presenting, therefore lymphocytes have a fundamental role in immune response (Skendros and Baura, 2013). 1L – 12 stimulate interferon gamma to accelerate immune response and this depend on equilibrium between 1L – 12 and the cell so, this cytokine act as bridge between pro persistence unspecific and adaptive immunity for antigen (Sikder et al., 2012). Limited studies reported about relationship between Brucellosis with immune cells, thus, this study conducted to highlight on this faculty in sheep.

MATERIALS AND METHODS

This research detected in animal farm at Agriculture College/ University of Baghdad for periods from January to July 2017 includes local Awassi sheep which kept nearer the door, births occur in October and April while natural suckling conducted. Veterinarian and breeding records used for studying prevalence of Brucellosis and reveals.

Serological analysis

Blood samples collected in sterile with anti-agglutination tubs which clotted for 20 minutes at room temperature then serum separated at 3000 r.p.m for 5 minutes and stored at -20C° until tests time, these samples used for Rose Bengal plate test used for presence of Brucella antibodies (Alton et al., 1988). 1L – 12 concentration estimated by using ELISA kit provided by shanghai yekua biological technology, China, Co. Ltd. Blood collected in vacutainers coated with EDTA and at once translated to laboratory for counting differential leukocytes by covering glass slides with smears of fresh blood, Leishman staining checking through microscope and erythrocytes counted manually.

Statistical analysis

General Linear Model within SAS (2012) used to know means of incidence rate and 1L – 12 level for each type of leukocytes in addition to erythrocytes which divided into groups according to this model:

$$Y_{ijklmno} = \mu + N_i + L_j + M_k + E_l + B_m + R_n + e_{ijklmno}$$

Where, μ: overall mean, N_i: neutrophils, L_j: lymphocytes,
M_k: monophils, E_l: eosinophils, B_m: basophils,
R_n: erythrocytes, $e_{ijklmno}$: random error.

The same model used again to produce relationship between 1L – 12 concentration and hematological markers, differences between means considered by Duncan test. In the same side, Correlation coefficient between all factors estimated to know contribution of genetic effect on brucellosis incidence rate and immune response in sheep (Patterson and Thompson, 1971).

RESULTS AND DISCUSSION

Concentration of IL – 12 with immune cells and brucellosis incidence rate Neutrophils influenced by brucellosis (P<0.05), percentage of neutrophils raised to more than 50% in the same time with increasing incidence rate to 1.00% as well as 1L – 12 level which raised to 1.29 ng/ml (P<0.05) (table 1). This result disagree with Raj et al., (2017) findings which recorded that neutrophils decreased to 30% among infected cattle in India. Increasing neutrophils with brucellosis may be refer to that neutrophils more spreading and faster response for Brucella which eradicate this kind of white blood cells by toll like receptor to become enough for producing 1L – 12 from natural killer cells besides pathogens and this interleukin responsible on communication between white blood cells that found with special receptor on cell membrane (Dah, 2016).

This study indicated that animals with higher prevalence rate was 1.00% have less rate of lymphocytes 21 – 31 % while increased to 50% for infections which received to 0.50% (P<0.01). In the same hand, 1L – 12 concentration was 0.63 ng/ml for animal with incidence rate 0.95 % and lymphocytes was 31 – 50 % (table 1). This study illustrated rising of lymphocyte with declined incidence rate of brucellosis while lymphocyte reached to 51% for infected cattle in India (Raj et al., 2017). A Positive effect of disease on lymphocyte which raising with lower incidence antibodies that surrounding and destroy pathogens then release chemical materials bring phagocytes to break down infectious cells. After invasion of Brucella, INF-ɣ produced and natural killer cells in addition 1L – 12 because of weakend signals between cells perform to reduction of lymphocyte as well as lowered INF-ɣ and 1L – 12 resulted disequilibrium between lymphocyte and Antigen Presenting Cell (Higgins, 2015).

Monophils tend up to 6% at higher incidence rate received to 1.00% according to 1L – 12 level raised markedly to 2.14 ng/ml (P<0.01) (table 1). Increasing values of monophils and 1L – 12 with highly incidence rate because of monophils preparing immunity system to rapid reaction when proposed to pathogen also still for a long time in blood thus, they increased with rising 1L – 12 production resulting from high activity in presenting pathogen to lymphocyte which contribute in 1L – 12 secretion in blood, on the other side, dendritic cells eradicated by bacterial lipo-polysaccharides and that increasing monophils number and 1L – 12 concentration (Smith, 2018).

Brucellosis effect on eosinophils significantly (P<0.05) which was 0–3 % with prevalence rate was 0.63 % whereas, 1L – 12 concentration received to 0.84 ng/ml. In the same side, incidence rate upgrade to 0.95 % with reduction of 1L – 12 level to 0.24 ng/ml non-significantly (table 1). High values of eosinophils with up percentage for brucellosis induction may be back to sensitivity which resulted from infection intracellular for a long period.

Brucellosis not effect on basophils and 1L – 12 level. Basophils rate 0–1 % at higher incidence rate to 1.00% with 0.41 ng/ml for 1L – 12 level and basophils increased to 2% associated with 0.79 % for incidence rate and 0.51 ng/ml for 1L – 12 level (table 1), this result contrast that of Dah (2016) who said that basophils influenced by Brucella for their role in removing bacteria from the body.

This disease not effect on erythrocytes and 1L – 12 level which reached to 5×10^6/mm and 0.98 ng/ml for both of them respectively at percentage of disease was 0.85 % (table 1). Recent result demonstrated there is no significant for infection on erythrocyte, disagreed with this study another one which clarified that infection transmitted via erythrocytes that invaded by brucella which survival in the cytoplasm (Martirose et al., 2011).

Table 1: Concentration of 1L – 12 and incidence rate ± standard error for blood cells

Blood cells (%)	No.	1L – 12 ± standard (ng/ml) error	No.	Incidence rate(%) ± standard error
Neutrophils	39	P<0.05	41	P<0.05
32 - < 50	30	0.27 ± 0.25 b	31	0.74 ± 0.07 b
> 50	9	1.29 ± 0.46 a	10	1.00 ± 0.12 a
Lymphocyte		NS		P<0.01
21 - < 31	6	0.39 ± 0.60 a	6	1.00 ± 0.14 a
31 - < 50	20	0.63 ± 0.33 a	21	0.95 ± 0.07 a
>50	13	0.34 ± 0.41 a	14	0.50 ± 0.09 b
Monophils		P>0.01		P<0.01
0 - < 3	13	0.30 ± 0.36 b	14	0.50 ± 0.09 b
3 - < 6	21	0.24 ± 0.29 b	21	0.95 ± 0.07 a
>6	5	2.14 ± 0.59 a	6	1.00 ± 0.14 a
Eosinophils		NS		P<0.01
0 - < 3	17	0.84 ± 0.34 a	19	0.63 ± 0.09 b
>3	22	0.24 ± 0.30 a	22	0.95 ± 0.08 a
Basophils		NS		NS
0 – 1	34	0.41 ± 1.03 a	6	1.00 ± 0.07 a
2	5	0.51 ± 0.24 a	35	0.79 ± 0.06 a
Erythrocyte		NS		NS
(3-5)X10^6 /mm	24	0.28 ± 0.29 a	25	0.76 ± 0.08 a
>5 X 10^6 /mm	15	0.93 ± 0.40 a	16	0.85 ± 0.11 a

Means with the same letters are not significant

Correlation coefficient

Correlation coefficient between 1L – 12 concentration and both of neutrophils and monophils (P<0.005) positive were 0.303 and 0.302 respectively, while correlation between 1L – 12 level with both of lymphocyte was 0.245 and erythrocyte was 0.215 non-significantly, whereas, association was negative non-significant between 1L – 12 with eosinophils -0.212 and basophils -0.127.

Higher relation between brucellosis and monophils 0.279 (P<0.05) and lymphocyte -0.456 (P<0.05) in addition both of basophils and erythrocyte were 0.214 and 0.100 non-significantly correspondingly (Table 2). This study appeared that correlation between 1L – 12 and each of neutrophils and monophils are weaked with the same value so, depending on interleukin 12 and other kinds of cytokines, neutrophils and other white blood cells determine the path to infection site which remain for limited time to prevent damage that occur in immflamable region, also secretion of 1L – 12 for short period acting on eradication INF-γ for immunity response. However, Brucellosis associated with both of monophils and eosinophils in medium estimation received to 0.499 and 0.406 significantly, this reflect high reaction and activation of these cells with infection which have top phagosis towards Brucella infection that increased in the same direction, relation of brucellosis with lymphocyte is negative and medium and this explain reduction relation of brucellosis with increasing infection that is refer to un sufficient of these cells production but destroyed with the virulence of pathogens then transfer to other part of the body.

Table 2: Correlation coefficient between 1L – 12 level and brucellosis incidence rate with blood cells.

Blood cells	1L – 12	Brucellosis
Neutrophils	0.303 (P<0.05)	0.279 (P<0.05)
Lymphocyte	0.245 NS	- 0.456 (P<0.05)
Monophils	0.302 (P<0.05)	0.499 (P<0.01)
Eosinophils	- 0.212 NS	0.406 (P<0.01)
Basophils	- 0.127 NS	0.214 NS
Erythrocyte	0.215 NS	0.100 NS

(P<0.01): high significant; (P<0.05): significant; NS: non-significant

CONCLUSSIONS

Recent study proved association between white blood cells except basophils and brucellosis, according to 1L – 12 founded a significant correlation with neutrophils and monophils. On previously, we can depend upon correlation values between white blood cells and brucellosis also between 1L – 12 with neutrophils and monophils although, these enabling breeder to select animals whom have higher values of these indicators to determine the resistance to pathogens and this partly depending on inheritance of these indicators.

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