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Original Research

Impact of brucellosis disease on interleukin 12 concentration and blood parameters in the Awassi sheep of Iraq

Authors:
Inam Nayyef A

Institution:
Department of Animal Production, Faculty of Agriculture, University of Baghdad, Iraq.

Corresponding author:
Inam Nayyef A

ABSTRACT:

This research was conducted to study the effect of some factors such as the percentage incidence of brucellosis, level of interleukin 12 (IL-12) and blood parameters for Awassi sheep. Overall mean rate of incidence was 0.42% and concentration of IL-12 to 0.23 ng/ml. Higher percentage incidence was found to be 0.52% and increasing of IL-12 to 0.25 ng/ml for animals with ages 3-5 years. More infections occurred in winter (0.59%) with decreasing IL-12 to 0.22 ng/ml and females susceptible to infection with higher percentage reached to 0.44%. On the other side, increasing of IL-12 for twin infants to 0.35 ng/ml witness less infections (0.27%) compared with those of single infants which received 0.46%. Age and season have a significant effect (P>0.05) on the occurrence of disease which is not affected by sex and birth type. Levels from IL-12 was influenced significantly (P>0.05) by birth type and were not affected by age, season and sex. Comparisons of blood parameters and concentrations for infection and non-infection cases were investigated, thereby, levels of IL-12, globulin, albumin / globulin raised to 0.45 ng/ml 3.44 g/dl and 2.00 while concentration of albumin, total protein declined to 3.77 g/dl and 6.80 g/dl respectively for the infected animals. Correlation coefficient between infection and each of IL-12, age, birth type, albumin, globulin total protein were negative and significant (P>0.05) while positive and significant between infection and both of sex and albumin/globulin and positive (P>0.01) between IL-12 and birth type (0.45) and with globulin reached to 0.34 was negative (P>0.01) with albumin/globulin received to -0.28 and negative (p>0.05) and with albumin was -0.23 and positive and insignificant with total protein 0.19. Also insignificantly negative with sex which reached to -0.08. Estimation of heritability for brucellosis, IL-12 and total protein were 0.33, 0.21 and 0.15 respectively, while repeatability for brucellosis was 0.64.

Keywords:
Brucellosis, Interleukin 12, Blood proteins, Awassi sheep

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INTRODUCTION

Sheep is a part of animal resources and one of the most consumption sources; therefore, care for them is necessary especially in the health side. When animals are infected with a disease, they influence on the performance and production. Brucellosis is a widespread disease caused by *Brucella* a gram negative bacteria with rod shape that include twenty species which vary according to the kind of host that infects. *B. ovis and B. abortus* are seen in our studies while *B. melitensis* are also more common in the sheep (Dermott *et al*., 2013) causing unhealthy cases like decreased fertility such as abortion, placenta retention, weak lambs, metritis and damages in male congenital tract which results in sterility (Lonea *et al*., 2013). Infectious organisms reproducing intra mammary glands perform to the reduction in quality and quantity of milk production (Scholz *et al*., 2010). In chronic infection the virulence of organisms confirm colonies that cause damages in liver and spleen which induce defects in their functions and this increase or decrease enzymes release depending on the infection stage (AL-Majali, 2005).

Sun *et al*. (2015) found that cytokines have important role in auto and acquired immune activities to respond the disease and to recognize the pathogens. Auto immune cells send signals about infectious agents to alarm immune system. T cells then receive these signals to get rid of organism and cytokines organize functions of various immune cells. Thus, interleukin 12 is essential as targets in many of the disorders and this type of interleukin organize signal paths to immune cells with other types of cytokines such as IL-23, IL-27 and IL-35 which activate pre immune response (Trinchieri *et al*., 2003). IL-12 was produced mainly by Antigen Producing Cells (APC) and have a key role in treating many diseases and become critical to develop Th1 response during brucellosis infection (Hamza *et al*., 2010). Infection frequently depend on the virulence of pathogens, in addition, to the host immunity. Brucellosis symptoms include sweating, fever, fatigue and arthritis (Grifths *et al*., 2010). Microorganisms are found in embryonic membrane and gets translated to the new born and infect after parturition (Regasa *et al*., 2009). The disease occurs among ruminants especially in intensive breeding system (Hassanain and Ahmed, 2012). Several factors participate to favour brucellosis like climate, geographic area, kind, sex and age of the animals (Bhat *et al*., 2017). Muma *et al*. (2006) indicated that a relationship between brucellosis and animal sex was seen in the infected sheep, among ewes, more than rams, for Al-Rahmani sheep of Egypt; also older animals are more susceptible to this disease than youngsters, while lambs in age less than one year have high resistance to infection for Kabashi sheep in Sudan (Abdullah *et al*., 2015). Also there are parameters depending upon their genetic traits. Genetic studies showed relation among multi genes or two genes influence on two traits in the same animal type (Rodgers and Nicewander, 1988). Heritability is the percentage of genetic to phenotype. Repeatability represents regression of future performance on the past for animals (Edriss *et al*., 2010). Genetic parameters, IL-12, and blood parameters relationship are also found to be influencing the infection due to brucellosis belonging to the Iraqi Awassi sheep, so we conducted this study to highlight these factors for the outbreak of brucellosis.

MATERIALS AND METHODS

This study was conducted in the farm of animals at the Agriculture College, University of Baghdad from 1.9.2016 to 15.1.2017. The study included national Awassi sheep with different ages which breeds at the open houses and management of herd depends on the preparation program of mating, time, season and births happen mostly on October and April, and natural suckling is dominant. A total of 48 animals were surveyed mostly through veterinarians and breeding records were maintained according to the infection frequency with

brucellosis.

Blood samples

Blood samples were collected from the jugular vein under sterile condition in tubs free of anti-agglutination and then let to clot for twenty minutes until serum gets separated at 3000 rpm for five minutes and frozen at -20ºC till the time of analysis.

Serological analysis

Rose Bengal Plate test (RBP) was used to expose brucellosis antibodies (Alton *et al*., 1988). Levels of IL-12 in serum were determined using ELISA kit, and the total protein was estimated by biuret method (Kaplan and Szalbo, 1983). Bromocresol green method was used to estimate albumin concentration in the serum (Rodkey, 1965). Globulin concentration was calculated by adding the quantity of non-albumin proteins. Albumin/globulin ratio was assessed manually.

Statistical analysis

General Linear Model (GLM) was constructed using Statistical Analysis System (SAS, 2012) which is used to estimate fixed factors and obtain least square means for brucellosis disease as per the following model:

$y_{iJklm} = \mu + A_i + M_J + S_k + B_l + e_{iJklm}$

where, y_{isklm}: observation value; μ: general mean; A_i: age; M_J: season; S_k: sex; B_l: Birth type; e_{iJklm}: random error value (Patterson and Thompson, 1971).

The same model was used to assess the means of IL-12, globulin, globulin/ albumin (glo/alb) and total protein concentrations with the same factors. Significant differences between means were seen and were also tested by Duncan's multiple test. Variance for random effects were estimated by maximum likelihood method through the paternal half sib across sires who have more than five offspring's to estimate heritability for brucellosis infection and levels of IL-12 in addition to the total protein repeatability values. Correlation coefficient was calculated between the studied factors. Health was predicted through the statistical analysis to estimate blood parameters which restarted with every trait as given in the following model:

$y_{ij} = \mu + D_i + e_{ij}$

where, D_i : represents health status

RESULTS AND DISCUSSION

Factors effect on incidence of disease

Overall mean for the rate of incidence of brucellosis disease is 0.42% while concentration of IL-12 reached to 0.23% ng/ml (Table 1). The incidence percentage in this study is less than that found in Awassi sheep of Iraq and was 20% (Mohamed *et al*., 2015) and those in Ethiopian sheep was from 1.17% to 3.29% (Dabasa and Tefera, 2013); (Ashnafi *et al*., 2007), whereas the incidence percentage reached to 3.23% in the Kashmir sheep in India (Lonea *et al*., 2013). The reason of different percentage may be referred to the variance of sample size and mixing among animals from different regions in addition to the changes of sites and management systems.

Table 1. Means squares for Brucellosis and IL-12

S. No	Source of variance	Brucellosis		IL-12	
		df	Mean squares	df	Mean squares
1	Age	2	0.84934411*	2	0.00455607^{NS}
2	Season	3	0.68692439*	3	0.00635609^{NS}
3	Sex	1	0.08315317^{NS}	1	0.00614051^{NS}
4	Birth type	1	0.3683630^{NS}	1	0.13175536**
5	Standard error	85	0.21864587	63	0.01029919

* (p>0.05); ** (p>0.01); NS: Not significant

Table 2. Mean squares ± standard error for brucellosis incidence rate and interleukin 12 concentrations

S. No	S.O.V	Brucellosis (%)		IL-12 (ng/ml)	
		n.	Mean square± standard error	n.	Mean squares ± standard error
	Overall mean	93	0.42 ± 0.05	71	0.23 ± 0.01
	Age (year)				
1	1 - < 3	24	0.38 ± 0.12[a]	17	0.22 ± 0.03[a]
2	3 - < 5	42	0.52 ± 0.09[a]	34	0.25 ± 0.02[a]
3	>5	27	0.29 ± 0.10[b]	20	0.21 ± 0.02[a]
	Season				
1	Winter	27	0.59 ± 0.11[a]	20	0.22 ± 0.03[a]
2	Spring	11	0.55 ± 0.17[a]	9	0.19 ± 0.04[a]
3	Summer	11	0.18 ± 0.14[b]	7	0.27 ± 0.04[a]
4	Autumn	44	0.34 ± 0.07[a]	35	0.25 ± 0.02[a]
	Sex				
1	Male	44	0.38 ± 0.09[a]	33	0.24 ± 0.03[a]
2	Female	49	0.44 ± 0.08[a]	38	0.22 ± 0.02[a]
	Birth type				
1	Single	71	0.46 ± 0.06[a]	59	0.21 ± 0.01[b]
2	Twin	22	0.27 ± 0.11[a]	12	0.35 ± 0.03[a]

Means with the same letters are not significant

Age

Age has significant effect on infectious disease, but does not influence on concentration of IL-12 in serum (Table 1). Recent study realized that animals with ages 3-5 years have higher percentage of brucellosis and was 0.52% with increases of IL-12 concentration at 0.25 ng/ml while these values declined to 0.29% for infection with 0.21 ng/ml to IL-12 among adult animals with age more than five years (Table 2). On contrast to this study, Rajala *et al*. (2016) reported that brucellosis increased with increase in age but agreed with the significant effect for the age on the incidence rate in Asian sheep in Tajikistan (Abdullah *et al*., 2015) and variance with the result on Kabashi sheep in Sudan where higher percentage in adults is found to be 2.30%. In the same side, Mohamed *et al*., (2017) indicated that there are no infections among animals in ages older than four years while appeared in group with ages 3-4 years and declined for those less than two years of age which reached to 2.55% and 1.41% correspondingly in the black Ogden sheep of Somalia. Raise of brucellosis at the ages 3-5 years maybe referred to rise of birth number with frequency of abortions that correlated with the occurrence of brucellosis which perform more susceptible to this disease.

Season

Brucellosis is affected by the season significantly (Table 1). Higher incidence occurred in winter and spring which reached to 0.59% and 0.55% that accompanied with less concentrations of IL-12 and was 0.22 ng/ml and 0.19 ng/ml respectively. On the other hand, incidence rate fell down to 0.18% with the higher level of IL-12 and came up to 0.27 ng/ml (Table 2). On contrary, Alfonso (2013) in France recorded that occurrence of brucellosis increased throughout the hot season and this is caused by climate changes like temperature and humidity which spread pathogen among the affected animals and these environmental factors influence on

Table 3. Concentration of blood parameters in infected and healthy sheep

S. No	Factors Mean ± Standard error	Total number	Infected Mean± Standard error	Healthy Mean ± Standard error
1	IL-12 (ng/ml) 0.32 ± 0.09	71	(28) 0.45 ± 0.16[a]	(43) 0.25 ± 0.13[b]
2	Albumin (g/dl) 3.80 ± 0.17	76	(36) 3.77 ± 0.24[a]	(40) 3.84± 0.23[a]
3	Globulin (g/dl) 2.87 ± 0.25	76	(37) 3.44 ± 0.35[a]	(39) 2.32 ± 0.34[b]
4	Glo/alb 1.75 ± 0.24	76	(38) 2.00 ± 3.35[a]	(38) 1.49 ± 0.35[a]
5	Total protein (g/dl) 7.09 ± 0.17	76	(36) 6.80 ± 0.24[a]	(40) 7.36 ± 0.23[a]

Means with the same letters are not significant

bacterial resistance in the host which also change the ability to get transform among the animals. The reason for increased infections among animals in cold weathers is because of keeping them nearer to the door for a long periods of time. In addition, the time of most births occur in October and April and throughout the season, there exists hypothermia and hyper humidity which help to widespread *Brucella* and causes the disease .

Sex

Sex is not a factor that affects the brucellosis incidence and also IL-12 in serum (Table 1). Females infected with this disease was in the percentage of 0.44%; at the same time, concentration of IL-12 decreased to 0.22 ng/ml which increased to 0.24 ng/ml among the infected male with a less incidence rate of 0.38% (Table 2). This study agree with Boukary *et al*., (2013) who showed that the effect of sex on the disease is not significant on brucellosis. On the other hand, this result is similar to Hirsh and Zee (1999) who published that males are less susceptible to infection than females. Rahman *et al*. (2011) in Bangladesh who reported that incidence among females increased to 2.61% while no infection is found on males in Asian sheep. Our study is in disagreement with the study on Awassi sheep in Iraq. Higher incidence rate was seen on males with 3.17% and 1.34% on females (Mohamed *et al*., 2015).

Birth type

There is no significant effect of birth type on brucellosis disease but concentration of IL-12 was positively affected by different birth types ($p>0.01$) (Table 1). Infection increased for single neonatal to 0.46% with the reduction of IL-12 concentration to 0.21ng/ml which raised to 0.35 ng/ml markedly for the infected twins with a percentage of 0.27%. The cause of increasing incidence of single births maybe due to the infected dams. They are more susceptible to pathogens that may be transmitted through placenta while twin infants mostly came from the healthy dams. This is in agreement with the study of Abdullah *et al*. (2015) in decreasing infections among the twin infants Kabashi sheep which reached to 7.7% in Sudan.

Blood parameters

Interleukin 12

Animals were divided into two groups *viz*., healthy and infected without considering the age, to compare the blood indicators in serum for the both groups. Concentration of interleukin 12 for the infected sheep reached to 0.45 ng/ml higher than those of healthy group (0.25 ng/ml) (Table 3). The rising of IL-12 for brucellosis infections is due to pre-immune response activity against bacteria which cause the disease.

Albumin

Concentration of albumin is reduced in the infected animals to 3.77 g/dl while raised to 3.84 g/dl for the healthy group (Table 3). This result is similar to those recorded by Kumar *et al.* (2015) in India where level of albumin declined for the infected animals compared to other healthy animals as 2.24 and 2.78 g/dl respectively. In Chokla sheep, it is also recorded as 2.10 and 3.03 for healthy and infected Nali sheep respective-

Table 4. Hereditary parameters for traits

Correlation coefficient	IL-12	Alb	Glo	glo/alb	TP	Sex	Birth type	Age
Brucellosis	-0.13 ^{NS}	-0.03 ^{NS}	-0.08 ^{NS}	0.11 ^{NS}	-0.07 ^{NS}	0.07 ^{NS}	-0.14 ^{NS}	-0.08 ^{NS}
1L-12	-	-0.23 (P>0.05)	0.34 (P>0.01)	-0.28 (P>0.01)	0.19 ^{NS}	-0.08 ^{NS}	0.45 (P>0.01	-0.02 ^{NS}
	Brucellosis		**IL – 12**		**Total protein**			
Heritability	0.33		0.21		0.15			
Repeatability	0.64		-		-			

NS: Non-significant

ly (Kishore *et al*., 2017). Cause of albumin reduction on infections is seen in the cases of anorexia infected animals which has a decline in the albumin production due to the damaged liver as a result of infection with less urea because of kidney damage (Al-Hussary and Zuhairy, 2010).

Globulins

Immune globulins raised in the serum of the infected animals to 3.44 g/dl and reduced to 3.32 g/dl among the healthy group (Table 3). This result was in accordance with Kumar *et al.* (2015) and Kishore *et al*. (2017) in India who noticed that levels of globulins tend upward for infections while fall down accordingly, in the healthy animals with 5.02 g/dl, 4.39 and 3.92 g/dl and 3.34 g/dl respectively. Reason of globulin increase in the serum of infected animals may be due to the activation of immune system as a reaction to *Brucella* bacteria which is found in the blood (Abenga and Anasa, 2005).

Globulin/albumin (glo/alb)

The ratio of globulin to albumin for the infected group is more than those of non-infected animals and reached to 2.00 and 1.49 consecutively (Table 3). On contrast to this result, the ratio raised in healthy animals while reduced among the infected animals as 0.88 and 0.53 respectively for Nali sheep (Kishore *et al*., 2017). The increasing values of glo/alb for unhealthy animals with higher incidence rate is referred to the increase of immunoglobulin in these animals.

Total protein

Recent studies recorded the reduction of the level of total protein in the serum for infected animals to 6.80 g/dl compared with high concentration which reached to 7.36 g/dl for the healthy group (Table 3). This result is in agreement with many of the previous studies that showed up to 6.03 g/dl and 6.46 g/dl for infected and healthy Nali sheep respectively (Kishore *et al*., 2017). Also, levels of total protein in infected and non-infected was 5.96 g/dl and 6.26 g/dl in Chokla sheep of India (Kumar *et al*., 2015). Reduction of total protein concentration was due to kidney damage as a result of infection (Hamada *et al*., 2013).

Genetic parameters

Correlation coefficient between brucellosis and all factors are weak and insignificant. Association of infection with each of sex and glo/alb were 0.07 and 0.11 and is insignificantly positive also it was insignificant and negative between infection and IL-12 concentration, birth type, age, albumin, globulin and total protein with values of -0.13, -0.14, -0.08, -0.03, -0.08 and -0.07 respectively in association with IL-12 and both birth types were 0.45 (P>0.01) and globulin was 0.34 (P>0.01) respectively and insignificantly positive with total protein that reached up 0.19. On the same hand, relationship between IL-12 concentrations with the first glo/alb were negatively significant (P>0.01) (-0.28) and the second relationship with albumin appeared negative and significantly by equal to -0.23; also negative and unimportant with both of the sexes and age with the

values of -0.08 and -0.02 consecutively (Table 4). This study proved that correlation between IL-12 and each of the birth type and globulins were with medium values. Hence, increasing globulins conjugated tend to upgrade in the immune response which reflect levels of IL-12 in serum. On the other side, birth type have significant relation with IL-12 in the incidence of brucellosis. Immune response occurs at the time of infection directly and after that reduces to some extent. Heritability for brucellosis disease increased IL-12 and total protein at the value of 0.33, 0.21 and 0.15 respectively (Table 4). These evaluations indicated the genetic effect of participation in the appearance of these traits so that, improvement of environmental conditions surrounding the animals could reduce infection incidence, interleukin 12 reflect body immunity and the level of its response to the bacterial infection which cause this disease and relatively, depend on the genetic diversity with ratio of 21%. Therefore, feeding and vaccinating also played important role to increase the tolerance against bacteria.

Estimation of repeatability for incidence rate of brucellosis was 0.64 and this high value can be dependent in predicting the animal capability to infection in the future, accordingly, the record for animal is enough to give an idea about infection frequency.

CONCLUSION

This study concluded that brucellosis disease occurs partly by the effect of genetic dependency on heritability estimation which was medium with the high value of repeatability so that, selection can be followed for individuals who have less infection frequency record. On the other side, increase in IL-12 concentration and globulins for infected animals indicate the reaction of body immunity against this disease.

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