Coccidiosis in Awassi, Romanov, Charollais and Suffolk sheep breeds during the winter and summer seasons in Jordan

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Abstract

Subclinical infection of coccidiosis in adult sheep cause loses in animal production especially for animals under stress including susceptible animals imported to a new environment. Local breeds usually are adapted to local environmental stress and more resistant to local parasite species/strains than exotic breeds. In this study, we investigate; coccidia infection rate, oocyct output and differential blood cell count in four groups of local Awassi sheep and three exotic breeds (Romanov, Charollais and Suffolk) in the Summer and Winter seasons. About 63.4% of animals were infected with Eimeria spp. The highest infection rates were in Romanov (81.5%), and in winter (P<0.05) than in summer for all breeds. The lowest infection rate and oocyct per gram (OPG) were recorded for Awassi breed during the summer season (11.1%).A negative correlation (P <0.05) between the OPG and lymphocyte (L) blood level was detected; while a positive (P <0.05) correlation with neutrophil (N) was only detected in the Awassi breed. Repeatability estimates for OPG were 0.32, 0.22, 0.30 and 0.42 for Awassi, Charollais, Romanov and Suffolk breeds, respectively. The N/L ratio estimates of repeatability were ranged between 0.04 in Charollais to 0.25 in Awassi breed. The current results demonstrated within and between breeds variations that can be utilized in genetic selection and breeding programs to increase genetic resistance to coccidiosis.

Keywords: sheep; coccidia; genetic resistance; Repeatability

1. Introduction

Coccidiosis is a protozoan parasitic disease most commonly affects young animals. Lambs are more susceptible to the clinical form of coccidiosis at 4 to 7 weeks of age, as well as around the weaning time. Older animals are usually sub-clinically infected and serve as a source of infection to new born. Coccidiosis has a great economic impact on small ruminants due to the losses in weight gain and milk production as a result of diarrhea and mal absorption of the nutrients (Kaya 2004; Chartier and Paraud 2012). Excretions of *Eimeria* oocyst during or just after the rainy season have been observed (Woji et al. 1994; Harper and Penzhorn 1999).

Faecal oocyst outputs varied among different breeds (Gorski et al. 2004).Many researchers agreed that the breed of sheep and the genetic group or sheep line are among the major factors that influence the rate of genetic resistance to parasites (Yvore et al. 1992; Gauly et al.2001; Filippini et al.2006). Some interesting genetic parameters were also estimated for fecal egg count in sheep that indicates the great ability of improving the rate of genetic resistance to coccidia by means of genetic selection.

Repeatability analysis is a way to determine whether quantitative genetic analysis will be profitable. The technique uses repeated measurements of a character from the same individual, separated in time, to assess the magnitude of environmental variation resulting from measurement error or other changes in character value between measurement periods. The repeatability values can be used to set an upper limit to the coefficient of genetic determination (heritability) (Yvore et al. 1992).

Coccidia is an intra-cellular protozoan parasite. Exact immune responses needed to control the disease are not well defined yet, but a major contribution was found for lymphocytes, mainly T cells. To a lesser extent, innate immune response (Neutrophils, macrophages and NK cells) may have a role in the early control of the disease (Ovington et al. 1995).

Awassi is the most common sheep breed in the Arabian Peninsula and in many Middle Eastern countries (Yamoor et al. 1988; Galal et al. 2008). The breed is well adapted over centuries for extensive management systems and known as a breed that adapted to the harsh desert conditions and arid environments and also known for its ability to tolerate local diseases (Al-Jassim et al. 1999)

The aim of this study was to investigate the infection rate of *Eimeria* spp., oocyst per gram of feces (OPG), and differential white blood cell count in four groups of adult Awassi Romanov, Charollais and Suffolk breeds during the summer and winter seasons.

2. Materials and Methods

2.1. Study Area

The study was conducted at the research station of Jordan University of Science and Technology- Sekhra. The central geographical coordinates of the study area were latitudes: 32° 22' N, longitudes: 35° 50' E. Animals were kept throughout the study period confined at the research station. The study area is located in a hilly region, dominated by cold rainy winter and hot dry summer.

2.2. Animals

Romanov, Charollais and Suffolk breeds of sheep were imported from Czech Republic in 2003, along with the local Awassi breed, were reared in the research station. Pure breed ewes aged 3-4 years were selected for the study. Total number of ewes under study was 61; among which 15 Suffolk, 18 Romanov, 19 Charollais, and 9 Awassi.
Animals were reared under routine management practices, kept indoor all around the year. Feed was offered according to the physiological stage of the ewes as recommended by NRC (1985).

2.3. Fecal samples examination

Rectal fecal samples were collected from all ewes three times during summer and three times during winter. Samples were then transferred to the parasitology diagnostic lab at Jordan University of Science and Technology. Fecal egg count was determined by using a modified McMaster technique that is described by Ministry of Agriculture, Fisheries and Food (MAFF,1977).Shortly, 2grams of fecal samples were re-suspended in 30 ml saturated NaCl, and 0.1 ml of the suspension were transferred to the McMasters slide chambers after being thoroughly mixed. Two chambers for each sample were counted under light microscope for higher sensitivity.

2.4. Leucocyte differential count

At the time of fecal sample collection, blood samples were collected from all ewes. Blood smears were made from the blood sample and stained with Wright's stain. The differential count was conducted according to Seiverd (1973) method. Briefly; 100 cells were differentially counted for each sample and out of these the number of Neutrophils (N), Lymphocyte (L), Basophile (B), Eosinophil (E) and Monocyte (M) was determined. The number of N was divided by the number of L for getting the N/L ratio.

2.5. Statistical analysis

SAS (version 2010) was used for the analysis. The Freq procedure of SAS,(2010) was used to calculate the infection rate of Eimeria. The Effect of season on the monthly incidence of infection in the 4 breeds was analyzed using the GLM procedure with a logit link function. Based on the output of the GLM procedure, the odds ratios (OR) as the probability of being infected at one of the seasons were estimated (Kaps and Lamberson 2004).

Effects of season on number of oocyst excreted per gram of feces (OPG) were estimated with one-way ANOVA using proc GLM of SAS. Because the OPG data were not normally distributed (Kolmogorov-Smirnov, $P < 0.05$) and positively skewed (Skewness>0), OPG data (y) were log transformed using the following function; [log(y) =Log10 (y+10)].Transformed data of total oocyst excretion were subjected to analysis of variance to extract variance components attributable to differences between and within lambs using Restricted Maximum Likelihood (REML) procedure. Repeatability was calculated as the ratio between-lamb variance to the total of between-lamb variance and random error (Falconer 1997).Correlation was used to estimate the Pearson Correlation Coefficients between oocyst excretion and leucocytes blood count and nuetrophil and lymphocytes ratios.

3. Results

The overall breed and season dependent infection rate of *Eimeria* oocyst infection are shown in Table 1. The overall infection rate was 63.4% among all breeds. The overall infection rate was higher in winter (68%) than summer (54.1%) for all breeds. Seasonal differences in oocysts prevalence were significant only in Awassi breed ($P< 0.002$) (Table 1).

3.1. Breed

Effects of breed and season on number of oocyst excreted per gram of feces (OPG) are shown in Table 2. The lowest ($P< 0.05$) OPG was found in Awassi sheep, followed by Charollais that was significantly ($P< 0.05$) different from the OPG found in other breeds. The highest OPG number was counted in Romanov breed that was significantly different from the other breeds ($P< 0.05$)except Suffolk (Table 2).

3.2. Season

Awassi and Charollais breeds had the lowest ($P<0.05$) OPG in winter (Table 2). The studied breeds seemed to be significantly ($P<0.05$) different from each other in summer OPG count. The lowest OPG number was identified in Awassi sheep (31.3) while the highest was counted in Romanov breed (300) (Table 2).The only significant ($p<0.002$) differences (within each breeds) between summer and winter OPG number was observed in Awassi sheep. Summer and winter OPG numbers didn't significantly differ within each of Charollais, Romanov and Suffolk breeds (Table 2).

3.3. Repeatability of OPG, N, L and N/L

Repeatability estimate of OPG number that achieved by exploiting the transformation procedure (log(opg+100)) revealed that Suffolk breed had the highest repeatability estimate (0.42) followed by the local breed Awassi (0.35), while the lowest estimate was found for Charollais breed (0.22) (Table3). Awassi breed exhibited the maximum repeatability values of N and N/L ratio compared to other breeds.

A highly significant ($P<0.001$) positive correlation (0.50) was detected between Awassi sheep oocyst excretion and neutrophil (N), while asignificant negative (- 0.50) correlation with Lymphocytes(L) was found for the same breed (Table 4). No significant correlation between the N, L percentages and oocytes exertion was detected in the other three breeds, although the correlation in these breeds was negative with Lymphocyte (Table 4). Monocytes (M) percent were negatively ($P<0.05$) correlated (- 0.27) with oocytes number within Charollais breed only. Within Suffolk breed, Basophil (B) observed to be significantly ($P < 0.01$) correlated with oocyte exertion (Table 4). The neutrophil lymphocytic ratio (N/L) was positively ($P<0.05$) correlated with oocyts exertion in Awassi sheep (Table 4).

4. Discussion

4.1. Infection rate and OPG in the 4 breeds

The highest Eimeria spp. infection rate was observed in Romanov breed followed by the other exotic breeds that were significantly higher than that obtained for Awassi breed. This may be attributed to the lack of adaptation of exotic breeds compared the local breed (Awassi). Romanov was shown to have higher infection rate and oocycst count compared to Merino of Aries in a study was done on lamb in France (Yvore et al. 1992). The Romanov breed is known for its high prolificacy rate which may contribute to higher stress on the animal that makes it more susceptible to gastrointestinal parasitic infection (Ricordeau et al. 1990). Gruner et al. (1986) have shown a higher infection ratewith nematodes in Romanov compared to Laucone breed in France.

4.2. Season (infection rate and OPG in the 4 breeds)

The significant difference in infection rates during winter and summer found in this work agreed with previous findings(Woji et al. 1994;Harper and Penzhorn, 1999; and Taylor 2009).Previously, a significant breed effect on the number of excreted gastrointestinal nematode eggs was reported in five Polish sheep breeds(Gorski et al. 2004) and on parasite resistance (Yazwinski et al. 1979)

The confinement of the animals under our study may played a little role on the parasitic seasonal output, while in Awassi sheep, the difference seen might be due to more adaptation to heat stress in summer compared to other breeds of sheep (AbiSaab and Sleiman 1995) and the effect of natural breed selection to immune resistant animals.

In general the estimated repeatability values were moderate. Kelly and Gray (1995) reported repeatability estimates for nematodes EPG ranged between 0.42 and 0.63 in a group of Merino Ewe's infected naturally in the grazing pasture. While Filippini et al. (2006) reported coccidian OPG repeatability to be 0.62. In another study, Gauly et al.(2001) reported a range of repeatability estimates between 0.07 and 0.41 for oocyts count based on a two days sampling intervals in fourteen Rhön lambs (7-99 days of age). This variation in repeatability estimates compared to our study could be as a result of the differences in genetic and environmental factors.

Since the repeatability is the upper limit of heritability, we could expect the heritability estimates of OPG to be less than the obtained repeatability values. This may point out the selection method that can be used for increasing the ability of obtaining some individuals that have the ability for coccidian resistance. Genetic selection based on OPG, could be recommended for improving the resistance to coccidiosis, which agrees with previous findings of Reeg et al. (2005).

Repeatability estimate of the neutrophil to lymphocyte ratio (N/L) ranged between 0.25 to 0.04 for Awassi and for Charollais sheep breeds, respectively (Table 3). The estimated repeatability values were low in the exotic breeds for L, N and N/L while they were moderate in Awassi sheep. The only study that we could find dealing with the genetic parameters of the neutrophil and lymphocyte ratio (N/L ratio) was published by Al-Murrani et al. (2000). In that study, they estimated the heritability of the N/ L ration in different Awassi cross breeds to be 0.38, 0.14, 0.44 and 0.44, respectively for Local Awassi, Turkish Awassi and Turkish Awassi x Local Awassi. The higher estimate of heritabilities that was obtained by Al-Murrani et al.(1997 &2000) may indicate the ability to include the N/L ratio in selection programs for improving the tolerance of stress and diseases in sheep. Although Awassi breed is known for its low productivity, it seems to have some resistance to coccidiosis and possibly other endemic diseases. We expect that cross breeding of Awassi breed with other high producing exotic breed could improve their adaptation to local area environmental conditions and resistance to diseases. As coccidian infection is an intracellular parasite, the control of the infection depends on a cell mediated immune response, mainly T lymphocyte (Ovington, et al. 1995), it is expected to have this negative correlation in the resistant breed between the OPG and the Lymphocyte. Aleksanersen et al. (1995) reported a high number of lymphocytes in the intestinal epithelium of lamb infected with coccidian.

5. Conclusions

This is the first study on ovine coccidiosis in Awassi sheep compared with three exotic breeds. Awassi sheep was found to have the lowest infection rates and OPG numbers that may reflect a resistance to the Jordanian Eimeria spp. strains supported by a negative correlation between OPG and lymphocyte count, while the Romanov breed had the lowest infection rates and OPG numbers that may reflect a susceptibility to the Jordanian Eimeria spp./strains. Repeatability estimates of OPG were moderate in all studied breeds reflecting a possible positive outcome of genetic selection procedure for increasing the resistance to ovine coccidiosis in a cross-breed, using the low producing Awassi and the high producing exotic breeds.

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Table 1: Showing the percent infection rate of *Eimeria* in 4 sheep breeds during the winter and summer seasons and the Chi-squared results

Breed	Infection rate		OR	*P*
	Winter	Summer		
Awassi	3.2	72.2	11.1	0.0027
Charollais	1.3	63.2	52.6	0.4451
Romanov	0.5	77.8	88.9	0.3218
Suffolk	1.5	60.0	40.0	0.2049

OR: Odd Ratio, P: P-values

Table 2: The (LSMean±SE)* of oocyst per gram of feces excreted by 4 sheep breeds during the winter and summer

Breed	Winter	Summer	Season effect (Pr>F)
Awassi	144.7 ±22.1 [a]	31.3 ±12.6 [a]	0.0020
Charollais	113.4 ±21.6 [a]	116.8 ±30.5 [b]	0.9280
Romanov	369.3 ±58.7 [b]	300.0 ±83.0 [c]	0.4987
Suffolk	307.9 ±144.1 [b]	203.9± 90.0 [b]	0.3896

*: LSMeans and SE represent untransformed data, P- values are based on the transformed data.
[a, b, c] Means in the same column with different superscripts are significantly (P < 0.05)different

Table 3: The estimated repeatabilities of mean OPG, neutrophil (N), lymphocyte (L), and Nuetrophil/ lymphocytic ratio (N/L) of the different studied sheep breeds

Breed	OPG	N	L	N/L
Awassi	0.35± 0.036	0.38	0.19	0.25
Charollais	0.22± 0.086	0.06	0.04	0.04
Romanov	0.30 ± 0.089	0.01	0.01	0.07
Suffolk	0.42 ± 0.0928	0.11	0.24	0.10

Table 4: Relations (Pearson Correlation Coefficients) between oocyst excretion and leucocytes blood count and neutrophils and lymphocytes ratios

OPG within Breed	Neutrophils (N)	Lymphocyte (L)	Monocyte (M)	Eosinophil (E)	Basophile (B)	N/L
Awassi	0.50 ***	-0.50 ***	-0.25 NS	0.01 NS	-0.20 NS	0.43 *
Charollais	0.13 NS	-0.09 NS	-0.27 *	-0.09 NS	0.12 NS	0.10 NS
Romanov	0.07 NS	-0.08 NS	0.06 NS	-0.07 NS	0.18 NS	0.10 NS
Suffolk	0.09 NS	-0.08 NS	-0.18 NS	-0.06 NS	0.36 **	0.02 NS

NS: not significant p > 0.05., * p < 0.05.,** p < 0.01., *** p < 0.001.