

Natural co-infection and species composition of *Eimeria* in sheep in Al-Baha area, Saudi Arabia

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ABSTRACT

This work aimed to 1) identify *Eimeria* species of sheep 2) investigate the factors that might potentially influence their prevalence and parasite load and 3) study the associations between *Eimeria* species. This study was conducted in Al-Baha area, Saudi Arabia from March 2012 to May 2013. Out of 487 faecal samples examined, 227 (46.61%) were positive for *Eimeria* oocysts. The species composition of *Eimeria* consisted of eight species. They were *E. crandallis* (29.8%), *E. bakuensis* (27.9%), *E. weybridgensis* (23.4%), *E. ahsata* (12.3 %), *E. intricate* (9.9 %), *E. faurei* (7.6 %), *E. parva* (4.7 %) and *E. pallida* (2.9 %). The overall mean species richness of *Eimeria* species harbored per host was 1.27 ± 0.07 . Host age, season and location played a significant role in determining *Eimeria* species richness, prevalence and parasite load. Significant negative correlations were found between host age and both prevalence and oocysts' number per gram of faeces (OPG). Multiple-species infections were commonly present in all age categories and declined with host age. Some *Eimeria* species consistently co-occurred and positive pair-wise associations were found. In conclusion, the prevalence and parasite load are affected by host age and season.

Keywords: *Eimeria*, oocysts, sheep, co-infection, host age, season, Saudi Arabia.

INTRODUCTION

Coccidiosis is a worldwide distributed disease and one of the most economically important infections that threaten sheep production. Coccidia infect the intestinal tract with clinical manifestations that vary from asymptomatic to severe diarrhea with blood and gut lining tissue in the feces. Subclinical coccidiosis is the most common form of the disease. Subclinical coccidiosis cannot be readily identified, therefore has a significant impact on the flock's health and production efficiency (Wang *et al.*, 2010; Cavalcante *et al.*, 2012).

Co-infection by multiple parasite species is the normal state of animals (Griffiths *et al.*, 2011; Viney & Graham, 2013). Co-infection refers to a situation in which individuals harbour two or more infections simultaneously; this differs from mono-infection in which individuals harbour only one infection (Raso *et al.*, 2007). Co-infection has important ecological (Graham, 2008; Brooker & Clements, 2009), epidemiological and clinical consequences (Pullan & Brooker, 2008; Brooker & Clements, 2009). The distribution of co-infection is not only pervasive, but also heterogeneous. The heterogeneity is common among villages and among individual hosts in the number and diversity of infections borne (Foreyt, 1990; Viney & Graham, 2013). The co-infection is common with *Eimeria* species in sheep. There is evidence

for interactions between parasite species, highlighting the importance of understanding these relationships before devising control strategies against specific species in livestock systems (Lello *et al.*, 2004; Craig *et al.*, 2007). In assessment of the dynamics of co-infections (either naturally, or following external perturbation), measures of abundance will be more sensitive than prevalence. Furthermore, abundance will often be more informative for studies of infracommunity dynamics (Viney & Graham, 2013).

Fifteen *Eimeria* species have been documented from the sheep (Platzer *et al.*, 2005; Wang *et al.*, 2010). In Saudi Arabia, eleven *Eimeria* species have been found in sheep, namely *E. bakuensis* (formally, *E. ovina*), *E. weybridgensis*, *E. parva*, *E. faurei*, *E. crandallis*, *E. ninakohlyakimovae*, *E. ahsata*, *E. granulosa*, *E. intricate*, *E. pallida* and *E. arloingi* (Kasim & Al-Shawa, 1985; Toulah, 2007).

Few studies already have been carried out on total oocyst excretion of eimerians in different seasons in different age classes of sheep in Saudi Arabia (Toulah, 2007). The species composition and the importance of each species of *Eimeria* in Al-Baha area are unknown. Therefore, it was of interest to evaluate their prevalence and abundance in sheep. In addition to, study the interaction between *Eimeria* species.

MATERIALS AND METHODS

The study area

The study was conducted in Al-Baha area, west-south Saudi Arabia (20° N, 41° - 42° E) from March 2012 to May 2013. The study area is mainly hilly, with small areas of either mountainous or flat land and extends from 500 to 2500 m a.s.l. The climate in Al-Baha has two extremes. Mild winters and hot summers, with an average annual rainfall between 100 and 250 mm, prevail in the lowlands; cold winters and mild summers, with an average annual rainfall between 229 and 581 mm, prevail in the highlands (Ibrahim *et al.*, 2008).

Sample collection

A total of 487 sheep from three localities in Al-Baha area were chosen randomly from farms. There were no prophylactic treatments against coccidiosis. Faecal samples of approximately 3-5 g were collected directly from the rectum using examination gloves. All samples were placed into plastic bags and were classified into three age categories: young (< 6-months old), immature (6-12-months old) and adult (>12-months old) and two seasons (dry: September -January and wet season: February-July). The animals' ages were estimated by examining their teeth and asking the animals' owner. The structure of the sampled host population of sheep according to season, locality, host sex and age categories was shown in Table (1). The collected samples were stored at 4°C until being examined.

Table 1: The structure of the sampled host population of sheep.

Factors considered		No. of sheep examined
Season	Dry season	239
	Wet season	248
Location	Al-Baha	165
	Al-Aqiq	176
	Al-Mikhwah	146
Sex	Male	260
	Female	227
Age categories	Young (<6 months old)	166
	Immature (6-12 months old)	170
	Adult (>12 months old)	151
Total		487

Sample processing and recovery of Eimerian oocysts

Faecal samples were examined for the presence or absence of Eimerian oocysts by a flotation technique using saturated saline. Oocysts per gram of faeces (OPG) were quantified using a modified McMaster technique (MAFF, 1986). Oocysts in the two chambers of the McMaster slide were counted and the sum number of oocysts in both chambers was multiplied by the dilution factor (100) to obtain an estimation of the number of OPG of each faecal sample. Each faecal sample was examined three times using the same method (Wang *et al.*, 2010; Cavalcante *et al.*, 2012). The results expressed as the mean value of the three independent examinations.

Eimerian species identification

After examination, positive samples were filtered through sieves covered with folded gauze and centrifuged at $250 \times g$ for 10 min. Filtered material was placed into Petri dishes with 2.5% potassium dichromate solution ($K_2Cr_2O_7$) for sporulation at laboratory temperature. After sporulation, sporulated oocysts were recovered by centrifugation in saline saturated solution at $250 \times g$ for 5 min followed by washing with distilled water. Concentration of sporulated oocysts was performed by centrifugation at $250 \times g$ for 10 min, stored in potassium dichromate solution at 4 °C for subsequent study. The oocysts were identified based on their morphological characteristics (size, shape, colour, form index, presence or absence of micropyle and its cap, presence or absence of residual, polar and stieda bodies) of the oocysts and sporocysts (Pellérdy, 1974; Levine, 1985; Soulsby 1986; Wang *et al.*, 2010; Cavalcante *et al.*, 2012). To ensure that species identification is valid, when possible, at least 50 sporulated oocysts from each species were observed and measured (Wang *et al.*, 2010; Cavalcante *et al.*, 2012). One hundred oocysts were randomly selected and identified to determine the percentages of each *Eimeria* species present in the fecal samples and then OPG per *Eimeria* species was counted (Silva *et al.*, 2011). An estimate of intensity of each *Eimeria* species was achieved by relating their proportions to the McMaster counts (Craig *et al.*, 2007).

Data analysis

Prevalence was calculated according to Bush *et al.* (1997). Testing of effects of both individual and interacted factors (host age, sex, season and locality) on species richness of *Eimeria* species was statistically analyzed using the General Linear Interactive Model (GLIM) after normalization of the data by $\log_{10}(x+1)$ transformation (Crawley, 1993; Wilson & Grenfell, 1997; Behnke *et al.*, 1999). Differences in prevalence of *Eimeria* among different age categories of sheep, as well as among different localities were evaluated using a Chi square test. Comparisons of OPG numbers according to sex and season were tested using Mann-Whitney test (U-test) while according age categories and locality were tested by Kruskal Wallis. Correlations between host age and both prevalence and OPG number were examined by using the non-parametric, Spearman's rank correlation coefficients (r_s). Diversity of *Eimeria* species was measured using the Shannon-Weiner diversity index. All the statistical tests were performed by using the software packages SPSS 17.0 (USA) and a value of $P < 0.05$ was considered significant.

RESULTS

Out of 487 faecal samples examined, 227 (46.61%) were positive for *Eimeria* oocysts. The species composition of *Eimeria* consisted of eight species. *Eimeria* species were *E. crandallis* (29.8%), *E. bakuensis* (27.9%), *E. weybridgensis* (23.4%),

E. ahsata (12.3 %), *E. intricate* (9.9 %), *E. faurei* (7.6 %), *E. parva* (4.7 %) and *E. pallida* (2.9 %). The prevalence of these *Eimeria* species was shown in Fig. (1). *E. crandallis* (29.8 %), *E. bakuensis* (27.9%), and *E. weybridgensis* (23.4%) were the most prevalent *Eimeria* species. (Fig.1). The abundance and intensity of *Eimeria* species are shown in Table (2). The most abundant species was *E. crandallis*.

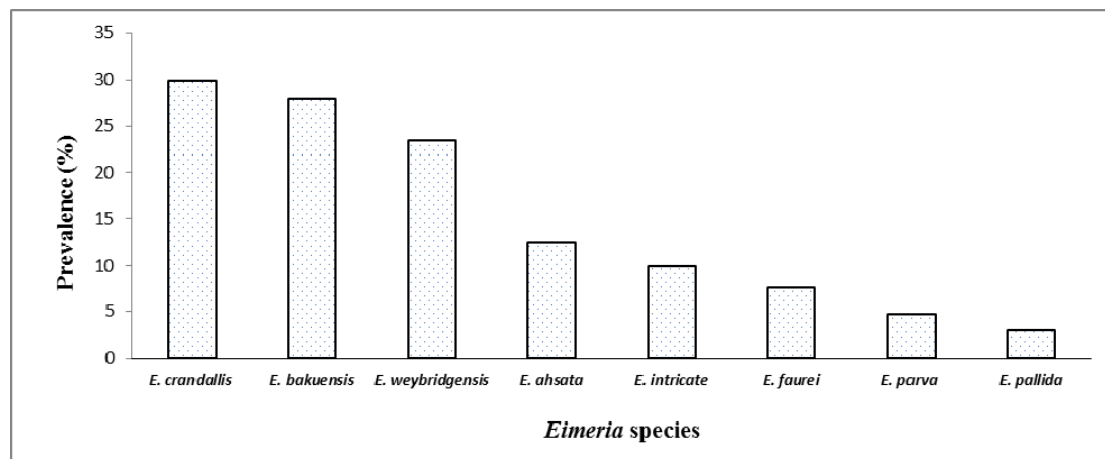


Fig 1: Prevalence of *Eimeria* species in sheep.

Table 2: Abundance (mean \pm SE) and intensity (mean \pm SE) of *Eimeria* species in sheep.

<i>Eimeria</i> species	Abundance \pm SE	Intensity \pm SE
<i>E. crandallis</i>	206.55 \pm 39.53 (0-1600)	274.78 \pm 20.83 (50-1600)
<i>E. bakuensis</i>	158.7 \pm 25.36 (0-1400)	189.44 \pm 17.41 (30-1400)
<i>E. weybridgensis</i>	145.77 \pm 21.09 (0-2023)	207.59 \pm 24.84 (30-2023)
<i>E. ahsata</i>	43.11 \pm 9.43 (0-1400)	118.66 \pm 15.58 (50-1400)
<i>E. intricate</i>	29.76 \pm 6.92 (0-1000)	100.70 \pm 13.88 (20-1000)
<i>E. faurei</i>	21.54 \pm 5.96 (0-600)	94.62 \pm 11.58 (20-600)
<i>E. parva</i>	15.18 \pm 5.6 (0-800)	70.28 \pm 12.89 (20-800)
<i>E. pallida</i>	12.68 \pm 3.78 (0-650)	50.13 \pm 18.39 (20-650)

The overall mean species richness of *Eimeria* species harbored per host was 1.27 ± 0.07 (S.E.M.). Analysis of these data with GLIM revealed that host age, season and location played a significant role in determining *Eimeria* species richness and there was interaction between season and location (Table 3). There is a negative correlation between host age and no. of *Eimeria* species ($r_s = -0.17$, $P < 0.001$). Diversity of *Eimeria* species was measured using the Shannon-Weiner diversity index. The overall diversity of *Eimeria* community was 2.381. Diversity in terms of prevalence significantly decreased with age ($F_{2,484} = 9.99$, $P < 0.001$) and showed significant differences between season ($F_{1,485} = 11.39$, $P = 0.001$) and location ($F_{2,484} = 7.23$, $P = 0.001$).

Table 3: Test of interaction (general linear model) between factors affecting species richness of *Eimeria* in sheep.

Overall <i>Eimeria</i> species	Factor considered	SS	df	X ²	F	Sig.
	season	12.272	1	12.272	5.337	.021
	Age	22.986	2	11.493	4.998	.007
	location	24.200	2	12.100	5.262	.006
	season * location	16.261	2	8.130	3.536	.030

The overall prevalence of *Eimeria* species in the three localities of Al-Baha area ranged from 35.15% to 55.48%. Statistically significant difference was found in prevalence ($X^2=12.98$, $df=2$, $P=0.002$) and OPG ($X^2=14.10$, $df=2$, $P=0.001$) among different localities of Al-Baha area. The highest prevalence (55.48%) and OPG (245.82±37.51) were observed in sheep in Al-Mekhwah locality. The lowest prevalence (35.15%) and OPG (163.56±25.58) were observed in sheep in Al-Baha locality (Table 4).

Table 4: Prevalence and parasite abundance (mean ± SE) of *Eimeria* oocysts in sheep in different localities of Al-Baha area.

Locality	Examined number	Infected number	Prevalence (%)	Significance among location	Mean no. of oocysts per gram of faeces (range)
Al-Baha	165	58	35.15	$X^2=14.10$, $df=2$, $P=0.001$	163.56±25.58 (0-1900)
Al-Aqiq	176	88	50		224.44±32.59 (0-2050)
Al-Mikhwah	146	81	55.48		245.82±37.51 (0-2400)

The overall prevalence of *Eimeria* oocysts in male was higher (48.08%) than that in female (44.93%). Also, the OPG number was higher in male (216.94±29.82) when compared to female (202.53±24.96). However there were no statistically significant difference in prevalence ($X^2=0.48$, $df=1$, $P=0.48$) and OPG ($U=28670.5$; $P=0.55$) between male and female (Table 5).

Table 5: Prevalence and abundance (mean ± SE) of *Eimeria* oocysts per host sex of sheep.

Host sex	Examined number	Infected number	Prevalence (%)	Significance between host sex prevalence	Mean no. of oocysts per gram of faeces (range)
Male	260	125	48.08	$X^2=0.48$, $df=1$, $P=0.48$	216.94±29.82 (0-2400)
Female	227	102	44.93		202.53±24.96 (0-2050)
Overall	487	227	46.61		210.22 ± 19.59 (0-2400)

The overall prevalence of *Eimeria* species in different age categories were 58.43%, 46.47%, and 33.77% in <6-months old, 6-12-months old and >12-months old respectively. OPG number was higher (386.45±43.54) in <6-months when compared to other age categories (Table 6). Significant difference was found in prevalence ($X^2=19.28$, $df=2$, $P<0.001$) and OPG number ($X^2=31.24$, $df=2$, $P<0.001$) among different age categories (Table 6). The overall prevalence, abundance and intensity decreased with increasing host age. Significant negative associations were found between host age and prevalence ($r_s=-0.20$, $P<0.001$), OPG load ($r_s=-0.28$, $P<0.001$) and intensity ($r_s=-0.32$, $P<0.001$).

Table 6: Prevalence and parasite load (mean ± SE) of *Eimeria* oocysts in different age categories of sheep.

Age categories	Examined number	Infected number	Prevalence (%)	Significance among age categories prevalence	Mean no. of oocysts per gram of faeces (range)
<6 months old	166	97	58.43	$X^2=19.28$, $df=2$, $P<0.001$	386.45±43.54 (0-2400)
6-12 months old	170	79	46.47		159.25±20.72 (0-1287)
≥12 months old	151	51	33.77		73.88±12.31 (0-832)
Overall	487	227	46.61		210.22 ± 18.52 (0-2400)

There was seasonal difference in prevalence of *Eimeria* oocysts in sheep. The prevalence of coccidial oocysts was significantly higher (54.03%) in wet season than that in dry season (38.91%) ($X^2=11.15$, $df=1$, $P=0.001$). OPG numbers were

significantly higher in wet season (224.24±29.23) when compared to that in dry season (Table 7; $U=25779$, $P=0.007$).

Table 7: Prevalence and parasite load of *Eimeria* oocysts in different seasons in Al-Baha area, Saudi Arabia

Season	Examined number	Infected number	Prevalence (%)	Significance among age categories prevalence	Mean no. of oocysts per gram of faeces (range)
Dry season	239	93	38.91		195.68±25.24 (0-2023)
Wet season	248	134	54.03	$\chi^2=11.15$, $df=1$, $P=0.001$	224.24±29.23 (0-2400)

Multiple-species infections (simultaneous infections with multiple parasite species in one individual host) of *Eimeria* species were commonly present in all age categories and declined with host age. 90.74% of positive sheep carried two to five species, and 78.85% of positive sheep had two to three species. Infections with 4 and 5 species were less common (11.89%); (Table 8). Some *Eimeria* species consistently co-occurred. Four positive pair-wise associations were found. These were between (i) *E. crandallis* and *E. bakuensis* ($r_s=-0.94$, $P<0.001$); (ii) *E. crandallis* and *E. ahsata* ($r_s=-0.29$, $P<0.001$); (iii) *E. ahsata* and *E. bakuensis* ($r_s=-0.28$, $P<0.001$) and (iv) *E. crandallis* and *E. weybridgensis* ($r_s=-0.20$, $P<0.001$). No negative associations were found.

Table 8: Percentage of single and multiple-species infections of different *Eimeria* species in sheep in Al-Baha, Saudi Arabia.

The number of <i>Eimeria</i> species in examined samples	0	1	2	3	4	5
Infected sheep	260	21	78	101	20	7
Percentage (%)	53.39%	4.31%	16.02%	20.73%	4.11%	1.43%

DISCUSSION

Information on the species composition and prevalence of *Eimeria* spp. is important to implement effective control programs. Previously, eleven *Eimeria* spp. had been identified from sheep in Saudi Arabia (Kasim & Al-Shawa, 1985; Toulah, 2007). In the present study, the species composition of *Eimeria* consisted of only eight species namely, *E. crandallis*, *E. bakuensis*, *E. weybridgensis*, *E. ahsata*, *E. intricata*, *E. faurei*, *E. parva* and *E. pallida*. The recorded species is similar to those recorded by Kasim and Al-Shawa (1985) and Toulah (2007) except *E. ninakohlyakimovae*, *E. granulosa* and *E. arloingi* which were not observed in the present work. In the present investigation, the overall prevalence of *Eimeria* species infection was 46.61%. This finding is higher than that recorded in Jeddah, Saudi Arabia (Toulah, 2007), Malayer suburb, Iran (Yakhchali & Rezaei, 2010) which revealed a prevalence of 41%, and 23.32% in sheep respectively. On contrast, the present recorded prevalence is lower than those reported in the Eastern (93.3%), Western (90.6%), Southern (89.8%), Central (97.4%) and Northern (77.5%) regions of Saudi Arabia (Kasim & Al-Shawa, 1985), northeastern China (92.9 %, Wang *et al.*, 2010), Nigeria (80%, Majaro & Dipeolu, 2012). These differences in prevalence may be due to various sanitation efforts in the management programs attempted by sheep producers to control coccidiosis or due to differences in ecological condition. *E. crandallis* (29.8 %), *E. bakuensis* (27.9%), and *E. weybridgensis* (23.4%) were the most prevalent *Eimeria* species. Similarly, Kasim and Al-Shawa (1985) and Craig *et al* (2007) found that *E. bakuensis* and *E. weybridgensis* were the most common species in sheep (*Ovis aries* L.) in Saudi Arabia and UK.

Statistically significant difference was found in prevalence and OPG load among different localities of Al-Baha area. The highest prevalence and OPG were observed in Al-Mekhwah locality. Kasim and Al-Shawa (1985) found variation in prevalence of infection in study sites and correlate it to difference in rainfall and humidity. The difference in prevalence and abundance of infection per location is probably due to difference in ecological parameters (such as rainfall, humidity and temperature).

Although, the overall prevalence and OPG of *Eimeria* oocysts in male were higher than that in female, there were no statistically significant differences. Similar finding was found by Maingi & Munyua (1994) and Yakhchali, & Rezaei, (2010). On the hand, Craig *et al.* (2007) found that males have significantly higher burdens of protozoan than females. They correlate this difference due to difference in reproductive fitness costs.

The prevalence as well as intensity and diversity of mixed *Eimeria* spp. declined with host age. All species were more prevalent in juveniles and all species were more intense in juveniles. This general juvenile bias in infection of *Eimeria* spp. is well documented in domestic sheep throughout the world (Maingi & Munyua, 1994; Arslan *et al.*, 1999). Acquired immunity has been shown to cause a decrease in infection of various *Eimeria* species with host age (Yun *et al.*, 2000). The prevalence and OPG of *Eimeria* oocysts in lower age category were significantly higher than that in adult sheep. These findings are consistent with previous observations (Maingi & Munyua, 1994; Arslan *et al.*, 1999; Wang *et al.*, 2010). This has been attributed to lower resistance or less immunity to *Eimeria* species in young animals compared to the older animals (Gregory *et al.*, 1980; Maingi & Munyua, 1994). Although acquired resistance is likely to contribute to the negative association of infection with host age, Ortega-Mora and Wright (1994) demonstrated an independent age-related resistance to infection in which a significant extension of the pre-patent period and decrease in intensity.

The prevalence and OPG of *Eimeria* species in wet season were significantly higher than that in dry season. Similarly, Majaro and Dipeolu (1981), El-Bahy *et al.* (2008) and Bakunzi *et al.* (2010) found higher *Eimeria* oocyst counts during the hot, rainy season than those during the cold, dry season. El-Bahy *et al.* (2008) reported that *Coccidia* sp. are abundant during April to June and their incubation period is about 1-2 week, so new infection could be occur in the same period as end of March till end of June. On the contrast, other researchers observed that there was no significant difference in OPG during seasons (Maingi & Munyua, 1994).

The present results demonstrated that multiple-species infections with two or three *Eimeria* species were more commonly seen than infection with a single *Eimeria* species. This finding is consistent with the findings of other researchers (Kasim & Al-Shawa, 1985; Craig *et al.*, 2007; Wang *et al.*, 2010; Majaro & Dipeolu, 1981). Four positive pair-wise associations were found. These were between (i) *E. crandallis* and *E. bakuensis*; (ii) *E. crandallis* and *E. ahsata*; (iii) *E. ahsata* and *E. bakuensis* and (iv) *E. crandallis* and *E. weybridgensis*. This finding is consistent with the finding of Craig *et al.* (2007). Craig *et al.* (2007) explained the positive pair-wise correlations between *Eimeria* species for one of two reasons. The first one is the overdispersion of parasites in natural populations, that is most individuals harbour few parasites and a few harbour many. They relate the aggregation may be due to variation in the exposure or susceptibility to infection within the host population. Various factors influence susceptibility in the sheep such as host age, sex, previous exposure to infection and nutritional status. Variation in any of these factors, causing aggregation

both within and across parasite taxa, could therefore explain the positive associations observed between protozoan species. The second, or alternatively, to this main effect, it is possible that concurrent parasite infection exerts a synergistic effect. Although not easy to detect (Behnke *et al.*, 2005) such interactions have recently been documented for protozoa (Bajer *et al.*, 2002) in wild mammal populations. That is, infection with one species enhances proliferation of another species. For instance, Catchpole *et al.* (1976) found that pure inoculations of four species of *Eimeria* in lambs produced shorter patent infections than when all four species were administered at once. The immunogenicity of the different parasite species and the extent to which hosts develop cross-immunity will play a role in this phenomenon. The positive pair-wise interactions that found in this study may be result from synergistic effect of concurrent parasite infection.

In conclusion, the prevalence and parasite load are affected by host age and season. We need further research to study the interaction between multiple-species infections in natural host-parasite systems. These results also provide relevant “base-line” data for assessing the effectiveness of future control strategies against coccidiosis in sheep.

ACKNOWLEDGMENTS

We are grateful to Al-Baha University for financing this project (Grant No. 4/1433). The authors would like to thank the workers of the farms for their assistance in the collection of specimen and their help. We also thank the farms owners who allowed us to use animals for the study.

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ARABIC SUMMARY

الإصابة الطبيعية المشتركة والتركيب النوعي لطيفيل للأيميريا التي تصيب الأغنام في منطقة الباحة، المملكة العربية السعودية

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يهدف هذا العمل إلى (1) تعريف أنواع الأيميريا التي تصيب الأغنام، (2) فحص العوامل التي من المحتمل أن تؤثر على معدل الإصابة وكثافتها (3) دراسة الارتباطات بين أنواع الأيميريا المختلفة. تمت هذه الدراسة في منطقة الباحة بالمملكة العربية السعودية من شهر مارس 2012 م وحتى مايو 2013 م. تم فحص 487 عينة براز أغنام ووجد أن 227 (46.61%) عينة مصابة بأحد أنواع الأيميريا. تبين تركيب الأنواع لطيفيل الأيميريا يتكون من ثماني أنواع. وكانت هذه الأنواع هي أيميريا كرانداليس *E. crandallis* (29%)، أيميريا باكوينسيس *E. bakuensis* (27.9%)، أيميريا ويبريدجيسيس *E. weybridgensis* (23.4%)، وأيميريا أھساتا *E. ahsata* (12.3%)، أيميريا إنتركات *E. intricata* (9.9%)، أيميريا فوراي *E. faurei* (7.6%)، وأيميريا بارفا *E. parva* (4.7%)، وأيميريا باليدا *E. pallida* (2.9%). وأوضحت النتائج أن أيميريا كرانداليس *E. crandallis* (29%)، أيميريا باكوينسيس *E. bakuensis* (27.9%)، أيميريا ويبريدجيسيس *E. weybridgensis* (23.4%) كانت أعلى نسبة إصابة. تبين أن متوسط الثراء النوعي لكل عائل 1.27 ± 0.07 . أوضحت الدراسة أن عمر العائل والموسم والمكان تلعب دور مهم في الثراء النوعي لأيميريا لكل عائل. كانت أهم العوامل التي لها تأثير معنوي في نسبة وكثافة الإصابة هي عمر العائل والموسم والمكان. بينت النتائج أنه يوجد ارتباط سالب بين عمر العائل من جانب ونسبة وكثافة الإصابة من جانب آخر. وبينت الدراسة أن نسبة الإصابة بأكثر من نوع في نفس الوقت كانت 90.74% وكانت عدد الأنواع من تتراوح من نوعين إلى خمس أنواع. وبينت الدراسة أن بعض الأنواع للأيميريا توجد دائماً معاً كعدوى مشتركة ووجدت ارتباطات إيجابية بين أنواع مختلفة. خلصت هذه الدراسة إلى أن نسبة وكثافة الإصابة تتأثر بعوامل أهمها هي عمر العائل والموسم. نحتاج إلى مزيد من البحث لدراسة التفاعل بين الإصابة بأكثر من نوع من أنواع الأيميريا في حالة الإصابة في نظام الإصابة الطبيعية.