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Survey of Contamination Rate Sheep to Sarcocystis in Slaughterhouse Boroujerd City and Studied by PCR

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ABSTRACT

Sarcocystis is a common disease in humans and animals, which cause is, the protozoa Sarcocyst Sheep, affected Sarcocyst four species of protozoa which include two species of pathogenic and non-pathogenic in both species. In this study, which is in the form of random sampling, in total, 322 samples from carcasses of slaughtered sheep were examined, the digestion method. The prevalence of Sarcocystis, and their relationship, according to sex and age of the animal, using the software SPSS-18, a statistical analysis was investigated. Incidence in this study was determined, equal to 82.91 percent. Prevalence of Sarcocystis, sex female, is 98.34 percent, and in males, it was found to be 37.03%, which was statistically significant difference was observed between the two sexes, male and female [$P \leq 0 / 05$]. Also, the prevalence of infection in different age groups, have no significant differences in sex, female [$P \geq 0 / 05$]. The prevalence of infection in different age groups, no significant differences were observed in male [$P \leq 0 / 05$]. Molecular results by PCR, showed that the amplified gene fragment of Sarcocyst [18srRNA] is created by the band, the 975 base pair [bp].

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INTRODUCTION

Sarcocystosis is a zoonotic disease in humans and animals, the protozoa Sarcocystis, its agent. The parasite is spread worldwide, and pollution, in many animals. It imposes severe losses in human and animal populations, health and economic terms, and it are one of the most common parasites in domestic ruminants. [1].

Sheep, who, in four species of Sarcocystis, two pathogenic species, which are transmitted by dogs as final hosts, and it is, contains microscopic cysts, and include:

- 1-Sarcocyst tenella
- 2-Sarcocyst arieticanis

Two non-pathogenic species, which is transmitted by cats as final host, and have macroscopic cysts, and they are [13].

- 1-Sarcocyst gigantea
- 2-Sarcocyst medusifomis

Natural and experimental disease in sheep and cattle, are accompanied by anorexia, fever, weight loss, anemia and abortion [3].

Man may be suffering from diarrhea, allergies, abdominal pain, eosinophilic myositis, peripheral blood eosinophilia, bloating, nausea, anorexia, vomiting, difficulty breathing, and pulse becomes severe, caused by eating undercooked meat and coddle, or sporocysts as the final and intermediate host. Meanwhile, individual reactions, are different, according to the bradyzoite or sporocysts in [12.14 8].

Few clinical signs are seen in the dirt, and recording the carcass, and the debasement of carcasses, and the economic losses caused by it, are the results of [4].

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Boroujerd city is a city in the North West Lorestan of the province, because of the mild weather, sheep and goats are common, in its suburban and rural pastures and mountain pastures, traditional, and the appropriate weather conditions, every year, thousands of head of cattle nomads, are entered into the pasture.

So far, studies of single cells Sarcocyst pollution, and its relation to age and sex of the animal, and confirm the presence of the parasite molecular analysis has been done, in the city of Boroujerd.

Sarcocyst protozoa, appearing in the body of the host interface, a muscle is, the shape and size of cysts, are different from each other [2]. Today, molecular methods can be detected directly, using genetic differences.

MATERIALS AND METHODS

This study was done randomly, in total, 322 carcasses, with a confidence level of 0.95 and an accuracy of 0.05, using the Cochran statistical formulas were calculated and evaluated. First, the study of gender sheep was determined, based on animal reproductive organs, then, old sheep [under one year, one to three years, and over three years], were determined using dental formula. 250 grams of various sections [esophagus, Cufflinks, diaphragm] was determined, and the labeling and coding, was transferred to the Department of Veterinary Laboratory, along with the ice and into the polystyrene. In the laboratory, using a digestion solution [11], the resulting emulsion, was transferred to a test tube, and the liquid was centrifuged for 10 min, with the upper 1500. After pouring the liquid, the resulting sediment supplies were spread on glass slides, and were fixed with methanol, stained with Giemsa, and it was painted. Then, with the optical microscope, the form of the parasite bradyzoite, was considered as a positive example. The remaining sediment, washed with sterile saline, and buffer TE, was used to extract DNA, according to the instruction Sambrook 2001 [18].

Primer designs are:

18srRNA gene nucleotide sequence of a *Sarcocystis gigantea* [NO.L24384], and *ariticani* [NO.L24382], and *tenela* [NO.L24383], were investigated, and after selection by *Sinagene*, and software, *Gene Runner* design F and R are carried out, which is open 21 to 60% of GC, is the following:

F5-GCTTCGACGGTAGTGTATTG-3

R5-CAAGAAAGAGCTATCAATCTG-3

Terms PCR, and optimization was performed according to the method *DelimiAsl et al.*, 2010. [2] In the end, DNA from the final product, on a 0.8% agarose gel containing ethidium bromide electrophoresis, was amplified. In the end, the detector device [Gel Daciomentashen], resulting bands were read, and were compared with a marker.

Results:

In the present study, the 322 samples analyzed, carcass, by digestion method, 267 head of sheep, respectively, Sarcocystis infection.

Table 1 and Figure 1

In this study, we also studied the two sexes, male and female, have obtained the following results, which are presented in Table 2.

Also, in the present study, the age of the sheep, using dental formula, then determine age, the animals were divided into three groups. The results are shown in Table 3.

The results showed that a high percentage of slaughtered sheep, in the city of Boroujerd, are infected, protozoa *Sarcocystis* [82.91 percent]. Also, contamination levels in both male and female were significantly [$P \leq 0.05$]. In terms of pollution, and its relation to age, differences were observed in the female, [$P \geq 0.05$]. In males, this difference was statistically significant [$P \leq 0.05$].

The molecular study, all positive samples were the same band, and amplified fragment was the same, with marker 975bp, Figure 2.

Discussion:

Despite the prevalence of *Sarcocystis* in animals, in so far, a comprehensive molecular diagnostic report, have been presented. In the present study, which was conducted by the method of digestion, the slaughter of Boroujerd, Lorestan Province, in total, 82.91% of the sheep, showed contamination.

In one study, the slaughter of Shiraz, 100%, *Sarcocystis* infection was shown, that in terms of pollution, high levels were reported, which is consistent with our studies [16]. Results of a study in the city of Karaj, the diagnosis *Sarcocystis* species, using PCR-RFLP, showed that, *Sarcocystis gigantea* is the predominant species [2].

Studies in Ardebil slaughter, indicated, the percentage of contaminated samples *Sarcocystis*, in sheep, 33.9%. Infection in sheep and female, were reported, more than males, which corresponded to the present study [10]. Studies *Arshad et al.*, Showed that there are significant differences in the sex of the animal study, which is consistent with our results [1]. Also, *et al* Fallah studies, the study of aging and the prevalence of *Sarcocystis*, so there was no significant statistical difference [$P \leq 0.05$]. In this study, the prevalence increased with increasing

age of sheep, cattle, so that the traps of 0.9 percent a year, and animals over a year, 6.4 percent of contamination have been reported [5]. In the present study, to investigate the sheep female, of all ages, it was observed that, with increasing age, the prevalence increased, whereas, in the 3-1 year old male sheep, less the percentage rate shown ratio sheep a year. Accordingly, the rams over three years, again showed a higher prevalence than the lower age groups. That there should be studied. However, statistically, there was no correlation between age and the prevalence of protozoan Sarcosyst, the sheep female [$P \geq 0.05$]. The reason, perhaps, the high rate of infection among different ages. Accordingly, the difference between the different ages, and the prevalence of male sheep, was significantly [$P \leq 0.05$].

Table 1: Prevalence of Sarcocystis infection of sheep, in Karaj city slaughterhouse.

Prevalence	Samples contaminated by digestion method.	Subjects
82.91 percent	267	322



Fig. 1: sarcocystisBradyzoit, after digestion, and stained with Giemsa's.

Table 2: Prevalence of Sarcocystis infection, based on animal sex.

Prevalence	Number of Sarcocystis infection by digestion method.	All samples were examined	Sex
98.34 percent	237	241	female
37.03 percent	30	81	male

Table 3: Prevalence of Sarcocystis infection in cattle by age.

Percent prevalence	Number of Sarcocystis infection by digestion method.	The number of male sheep	Prevalence	Number of Sarcocystis infection by digestion method.	Number of female sheep	Dental formula based on age [years]
11.1%	1	9	88%	22	25	$1 \geq$
8.33 %	2	24	98.61 %	71	72	3-1
56.25 %	27	48	100%	144	144	$3 \leq$

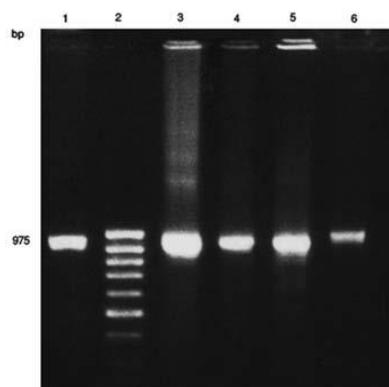


Fig. 2: gene amplification products 18SrRNA, by PCR.

2. Marker 975 bp, bands 1, 3, 4, 5, 6, studied samples of the digestion product sarcocystis, Boroujerdcity in sheep

Prevalence of Sarcocystis infection, animal sex, in Shahrekord, noted that pollution, the gender female, more male, which corresponded with our studies, in this case [6].

Also, other studies have found that, by age and sex, have an impact on the prevalence of Sarcocystis infection in cattle. In this study, the rate of infection in both sexes, and all ages, has been a hundred per cent [7]. These results are different from most studies.

Studies done in the world, with 70 to 100 percent of herbivores and humans showed Sarcocystis infection, which is consistent with our studies in this regard [12]. The study Sarcocystis infection in Portugal, it was observed that age correlates with the degree of contamination in cattle [9].

The West Australian study on 714 cows, it was observed that, with increasing age, the prevalence of cows, Sarcocystis, also increased [18].

Sequencing and molecular studies, it was shown that, Toxoplasma and Sarcocystis species have genetic similarities with the origin [21]. In this study, studies of other investigators have determined the reasons for the selection of the PCR fragment 18srRNA, [19] A study Nested-PCR, showed that pathogenic from non-pathogenic species of sheep, it is possible to use blood samples [15]. Also, in a study, RAPD-PCR, it was shown that there are two types of pathogenic and non-pathogenic in sheep [15]

In this study, we sought to identify Sarcocystis, molecular analysis after digestion method, a single band of 975bp was approved, and found that it is related to Sarcocystis species. Since there is little difference between the various species of parasites, we need to test Mdrym cutting enzyme molecule, to identify species. Tenter *et al* study, the method ssrRNA-PCR, confirmed our studies [20]. Also, studies Uggla, showed that there are common roots taxonomy, between the two of coccidia [Sarcocyst and Toxoplasma] [21].

Delimi *et al.*, In Tehran, the town of Shahriar, showed that a species of Sarcocystisgigantea [OviFelice], the dominant species, and the cat is the definitive host [2]. Macroscopic cysts are seen in older sheep, which may be even a centimeter. But small is not visible, and may make it difficult for the slaughterhouse, the diagnosis [12]. For this reason, in this paper, tissues, were first isolated by digestion method, and epidemiological studies, was used to detect biomolecular.

However, in most studies, the researchers, a high percentage of pollution, Sarcocyst single cells, has been shown, in the present study, the high prevalence of slaughtered sheep in the city of Boroujerd, was observed, which confirmed for the molecule.

There is the argument that, with increasing age, pregnancy and childbirth, the animal's immune system is female, is weak. Also, keep the sheep female, due to the economic value of lambing, and produce more, can lead to the possibility of more contact with sporocysts, during the life of the host interface female, and more pollution and incidence of protozoa Sarcocyst to. But why infection rates, at different ages, the female, the difference was not significant statistically, may have other reasons, which should be investigated by respected investigators.

Perhaps, because of significant differences in lambs, following a year over three years, a protozoan infection of Sarcocyst, for the production of meat quality, and the quality is such that, under one year old Male, use are of high quality forage as feed breast milk and more time, to the top male and three years, that this leads to a lack of access males under one year, the sporocystsSarcocyst, in this era. Finally, it led to differences in male.

Recommended, enzymatic methods for the detection of parasite species, done by researchers, the dominant species of the parasite, as specified in this area, because Tenella and articanisSarcocystiscanis, are two pathogenic species, which are not visible, with the naked eye.

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