Research Article

Investigation of the Prevalence of *Cryptosporidium Spp.* in Lambs with Carbol-Fuchsin Dye and Immunochromatographic Rapid Test Kits in Kırıkkale

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Abstract

Cryptosporidiosis is an acute or chronic enteric disease caused by *Cryptosporidium* species and seen in neonatal or immunocompromised animals and humans. It causes a serious infection, especially of newborn calves and lambs that results in a serious economic loss. In this study, we aimed to diagnose *Cryptosporidium spp.* using carbolfuchsin staining and a ready-made diagnostic kit and subsequently to determine its prevalence in neonatal lamb in Kırıkkale province. The study also aimed to determine the etiologic frequency of *Cryptosporidium spp.* in lamb diarrhea. This further aimed to compared carbol-fuchsin staining and the rapid diagnostic kit by means of diagnostic rate. The fecal samples used in the study were collected from 0-30 days old newborn lambs in Kırıkkale province. Fecal samples were collected from a total of 110 lambs with and without symptoms of Cryptosporidiosis and stained with Carbol-fuchsin dye in accordance with the technique and examined with immersion oil at a magnification of 100x. As a diagnostic criterion, observation of a single agent is considered as enough evidence to consider the case positive. Positive samples were scored according to their density and photographs were taken. Samples were also examined with a second diagnostic procedure, the rapid diagnostic kit, following the vendor recommended protocol. *Cryptosporidium spp.* was detected with an incidence rate of %4,54 (5/110) in carbol-fuchsin staining while it was %47,2 (52/110) in the ready-made diagnostic kit method that indicates there was a significant difference between the two used methods by means of the diagnostic rate ($p<0.001$). There was no gender and age differences by means of diagnosed cases ($p=0.382$ and $p=0.397$, respectively). As result, there is a considerable *Cryptosporidium spp.* prevalence in the Kırıkkale province. Furthermore, compared to carbol-fuchsin staining the rapid diagnostic kit seems to be more sensitive to *Cryptosporidium spp.*

Keywords: *Crytosporidium spp*, Carbol-fuchsin staining, lamb, neonatal diarrhea, prevalence, rapid diagnostic kit

Introduction

Cryptosporidiosis has been described worldwide as a neonatal disease that causes yield loss in ruminant species such as sheep and goats (Viu, Quilez, Sanchez-Acedo, del Cacho and Lopez-Bernad, 2000). It was first reported in 1974 in lambs with diarrhea in Australia (Barker and Carbonell, 1974). The role of the causative agent in the etiology of lambs was defined by natural and experimental infections in 1980 (Ulutaş and Voyvoda, 2004). In Türkiye, it was first reported through a microscopic diagnosis in lambs with an incidence of 12% in Elazığ in 1989 (Sevinç, Uslu and Derinbay, 2004). With a development of molecular methods, many species and sub genotypes belonging to *Crytosporidium* genus have been described in sheep up to date. The most common species in sheep are *Cryptosporidium ubiquitim, C. parvum and C. xiaomi*. *Cryptosporidum spp.* infections are more common in neonatal animals than in adults, and observation of oocyst emergence in asymptomatic cases without clinical signs is important for early detection of infections (Danišová, Halánová, Valenčáková and Luptáková, 2018).

Crytosporidium spp. may vary by means of geographical distribution and prevalence *C. parvum* appears to be dominant in Europe while *C. xiaomi* in Australia, and *C. ubiquitum* in the North and South America and Asia. *C. bovis, C. hominis, C. andersoni, C. suis, and C. fayeri* are among the other *Crytosporidium* species also found in sheep.

Cryptosporidium species with monoxene development undergo through the stages of schizogony, gametogony and sporogony from asexual and sexual reproduction forms. The infection occurs following oral intake of oocysts (Sevinç and Dik, 2015).

Cryptosporidiosis is one of the main causes of diarrhea in lambs in the neonatal period (Paraud and Chartier, 2012). In infected animals, diarrhea is accompanied by dehydration, loss of appetite, abdominal tension and lethargy. Diarrhea occurs 3-7 days after intake of infected oocysts. During this period, when oocyst excretion is high, oocysts can be seen in the stool between 10^5 and 10^7 per gram. After relief of clinical signs, oocyst excretion may continue for several more days. In animals infected with *Cryptosporidium parvum* as well as other enteropathogenic species, the infection appears more severe and death occurs within 2-3 days after the onset of diarrheal symptoms (Coop and Wright 2003).

A large number of techniques have been used in the diagnosis of *Cryptosporidium spp*. infection in humans and animals. There are various staining techniques used to determine the presence of oocysts in feces, microscopy of histopathological sections to examine the stages of the parasite's life cycle, and detection of antigens and DNA of the causative agent (Mirhashemi et al., 2015; Robinson and Chalmers, 2020). Various traditional techniques such as Sheather's sugar/zinc sulfate solution flotation, formol ether concentration method, formal ethyl acetate sedimentation are also used in the diagnosis of oocysts (Rekha, 2016). However, there are difficulties in diagnosis because the clinical findings are not pathognomonic and the size of the oocysts is small, the excretion of oocysts is intermittent, and it is difficult to distinguish oocysts from other substances such as fungi and yeast in the stool. For this reason, various staining methods and modifications of these methods have been developed over time (Khurana and Chaudhary, 2018). Carbol-Fuchsin, a staining technique used for the diagnosis of oocysts, is a simple but become pale in a relatively shorter time period (Ahmed and Karanis, 2018). In the Carbol-funchsin staining method, oocysts are seen as strongly reactive, small round spheres of small round size, which do not take dye, unlike other factors in the feces. Sporozoites, on the other hand, are slightly visible as darker spots within the oocyst (Potters and Van Esbroeck, 2010).

In addition to the traditional methods used for the detection of oocysts in diagnosis, various diagnostic techniques based on the detection of antigens in feces have been developed. The need for experienced personnel for microscopic examination in the diagnosis of oocysts with traditional methods and the fact that it is time-consuming has revealed the need for a simple, fast and objective method for diagnosis over time. These methods have been developed to eliminate the need for special equipment and experienced laboratory staff and to

examine more than one sample at the same time (Gabr, Abdellatif, Abd El-Hafeez and Abd Rabou, 2014; O' Leary, Sleator, & Lucey, 2021). One of them in an immunochromogenic commercial kit, which has a sensitivity and specificity range of 58-95% from microscopic methods. The test allows the detection of *C. parvum* antigens in non-concentrated feces within a few minutes and can be used in the field (Kaya et al., 2014; Papini, Bonelli, Montagnani, & Sgobini, 2018; Gerace, Lo Presti ve Biondo, 2019). This technique is easy enough to be applied by anyone without the need for experts, as it has a long shelf life, the analysis method is easy to apply and does not require special training, and the result is visible (Kaya et al., 2014). However, it only provides quantitative results, which are cysts sufficient for the detection of the presence of pathogens in enterprises (Danišová, Halánová, Valenčáková and Luptáková, 2018).

Materials and Methods

Collection of stool samples

The fecal samples were collected from 110 lambs of 1-30 days old with and without diarrhea symptoms in the Kırıkkale Province between December 2023 and April 2024. The lambs were either located in various regions of the Kırıkkale Province or submitted to Kırıkkale University Faculty of Veterinary Medicine Animal Hospital.

Samples were collected from different districts to find the regional distribution of this infection, which is a seasonal disease, in Kırıkkale Province. The ear number, age, gender, and information about the mother of each animal whose feces were taken were noted. Stool samples were taken from their rectum with the help of swabs. The materials taken were brought to Kırıkkale University Parasitology Department Routine and Research Laboratory on the same day and examined on the same day.

Examination of Fecal Samples

Carbol-fuchsin staining method and the Rapid diagnostic kit method (Bovine CRYPTO Ag Combo test (VET Diagnostix)) were applied to each stool sample and the results were noted. A drop of carbol-fuchsin staining with the help of a pipette on some feces taken on a clean slide was performed according to the method used by Heine, J. (1982). Immersion oil was dripped on these prepared preparations and examined under a microscope at x100 objective. The rapid diagnostic kit, which was a ready-made diagnostic kit, is an immunochromatographic testing method. The kit applied to the samples according to the vendor recommended procedure to examine the fecal samples for *Cryptosporidium spp., Giardia spp., Escheria coli, Rota virus* and *Corona virus* agents, each appearing in a different sites of the kit.

Statistical Analysis

The samples collected in the study were divided into two separate groups according to the examination method (Carbol-fuchsin and rapid diagnostic kit) and grouped according to the result values (negative and positive). These values were evaluated according to gender and age groups (<7 and 7+). In the study, according to the method, in the evaluation of gender and age data, from Chi-square analysis; McNemar test was used to compare the results of the methods regardless of age and gender. In the Khi square analysis, Yates correction was applied to the positive results of Carbol-fuchsin. In the study, the ROC Curve model was used to determine the test sensitive to the presence of symptoms in the sampled individuals and AUC values were calculated. Analyses with IBM SPSS 27 package program; graphics are completed with Graphpad Prism 9.5.1. $p<0.001$ values were considered positive.

Results

The incidence of *Cryptosporidium spp.* was determined as 4.54% (5/110) by Carbol-fuchsin staining in fecal samples taken from lambs. In the stool samples examined with the Rapid diagnostic kit method, the positivity rate for Cryptosporidium spp. was found to be 47.2% (52/110). 47 of the samples that were found negative by Carbol-fuchsin staining method were evaluated as positive with a ready-made diagnostic kit. All samples that were detected as positive by staining method were also found positive in the ready-made diagnostic kit (Table 1.). There was no gender and age differences by means of diagnosed cases ($p=0.382$ and $p=0.397$, respectively).

The proportion of animals with diarrhea symptoms was 32.6% (17/52) and 67.3% (35/52) in those without diarrhea symptoms in the samples detected positive with the Rapid diagnostic kit. In the

samples examined with the Rapid diagnostic kit, the incidence rates of *Giardia spp., Escheria coli, Rota virus* and *Cryptosporidium spp.* alone were found to be 21.8%, 8.1%, 0.9%. Again, the rate of infection with a mixture of *Cryptosporidium spp., Giardia spp., E. coli* and *Rota virus* agents was found to be 18.18% (20/110) in the stool samples examined with a ready-made diagnostic kit. *Cryptosporidum spp.+ Giardia spp.* 10% (11/110), *Cryptosporidum spp.+ Escheria coli* 7.2% (8/110), *Cryptosporidum spp.+Rota virus* 0.9% (1/110), *Cryptosporidum spp.+ Giardia spp.+ E. coli* 1.8% (2/110).

Table 1. Number and percentages of animals examined with Carbol-fuchsin and Rapid diagnostic kit

Metod	Number of pozitive animale	Average of Serum Antibody (ng/mL)	Percent
	(+ hs)	(- hs)	%
Carbol-fuchsin pozitive (C+)	5	105	4.54
Rapid diagnostic kit pozitive (H+)	52	58	47.2
C+ ve H+	5	105	4.54
C- ve H+	47	63	42.72
C+ ve H-	0	110	0
C- ve H-	105	5	4.54

Total number of animals 110, CF= Carbol-fuchsin, H= Rapid diagnostic kit

Discussion

There are many studies in the world and in Türkiye using microscopic diagnostic methods, molecular methods such as ELISA and PCR for the diagnosis of Cryptosporidium spp. in lambs (Papanikolopoulou et al., 2018; Majeed et al., 2018; Dessi et al., 2020; Kabir et al., 2020; Khan et al., 2022; Aslan-Celik et al., 2023; Sarı, Balkaya, Taşçı, Arslan and Küçükler, 2023). In recent years, coproantigen commercial test kits such as enzyme immunoassays or immunochromatographic tests have also been used for rapid diagnosis. The reported positivity rate in fecal samples examined by Carbol-fuchsin staining method in lambs in the world has been reported as 1.3% (Bordes et al., 2020). In the studies conducted in Türkiye, the reported positivity rate was stated as 4.55% and 46.53% (Ulutaş and Voyvoda, 2004; Arslan et al., 2016). In the study, the rate of oocyst presence in stool samples stained with Carbol-fuchsin was found to be 4.54%. In the study conducted by Bordes et al. (2020) with the carbol-fuchsin staining technique, the fact that the rate is less can be explained by the collection of samples from asymptomatic lambs, while it can be explained by the fact that it is not seen due to the low density in fecal staining or the host cannot always assign oocysts. Arslan et al. (2016) found the ratio with the carbol-fuchsin staining technique is very close to each other. In the study conducted by Ulutaş and Voyvoda (2004), oocyst presence was detected more by staining method. This situation may suggest that the positivity rate is high because the samples taken from 144 lambs in only one farm in Aydın Province.

Cryptosporidium spp. was detected at a rate of 0%, 9.2%, and 10% in the stool samples examined in studies conducted with the rapid diagnostic kit (Abd-Al-Aal, Al-Kabbany, and Tehrani 2016; Danišová, Halánová, Valenčáková and Luptáková, 2018; Ozcelik, 2018). This rate was determined as 47.2% in the stool samples examined in the Kırıkkale region. Danisova et al. (2018) compared four different techniques, including PCR, and concluded that the result found with the rapid diagnostic kit in lambs was not reliable. Özçelik (2018), on the other hand, looked at C. parvum, Clostridium perfringes, Rotavirus, E. coli F5, and epsilon toxins that will cause diarrhea in lambs in his study in Elazığ Province, and found that the rate of Cryptosporidium spp. was low compared to our study. This may suggest that Cryptosporidium, which is the factor we are investigating, will be caused by the time the samples were taken, since it is a seasonal disease. Although Abd-Al-Aal et al. (2016) found the rate of Cryptosporidium spp. to be 9.2% in their study, they suggested that it would be low because the study was conducted with samples taken from sheep. In our study, a statistically significant difference was obtained between the rapid diagnostic kit method and carbol-fuchsin staining by means of diagnosis of Cryptosporidiosis ($p<0.001$).

Cryptosporidum spp. infections are more common in neonatal animals than in adults, and observation of oocyst emergence in asymptomatic cases without clinical signs is important for early detection of infections (Danišová, Halánová, Valenčáková and Luptáková, 2018). Bordes et al.

(2020) found the rate of Cryptosporidium spp. to be 1.3% in the stool samples of 79 lambs without clinical symptoms examined by Carbol-fuchsin staining method. In our study, diarrhea symptoms were observed in only 17 (32.7%) of the animals examined with a diagnostic kit. This may suggest that it is proportionally high because it is a suitable period for the occurrence of the disease seasonally. In the master's thesis conducted by Evgin (2022) in Kırıkkale and surrounding provinces, the rate of Cryptosporidium spp. in lambs with diarrhea was determined as 13% by carbol-fuchsin staining method. This ratio actually gives an idea about the spread of the agent in the region. Arslan et al. (2016) found that this rate was 9.09% in dead lambs with all signs of diarrhea. Ulutaş et al. (2004) found Cryptosporidium spp. in lambs with diarrhea (79.1%, 53/67%) and lambs without diarrhea in 144 feces sampled from lambs with and without diarrhea (18.2%, 14/77%). Kaminjolo et al. (1993) stated that the incidence of Cryptosporidium oocysts was higher in animals with diarrhea than in those without diarrhea, but this was not statistically significant. Danisova et al. (2018) examined the rate of Cryptospridium spp. in lambs with diarrhea symptoms with a rapid diagnostic kit was 0.0%, while Özçelik (2018) examined it was 10% in lambs with diarrhea symptoms. Mild and high-severity diarrhea symptoms were observed in 4.54% of the 5 samples evaluated positively in the fecal samples collected in the Kırıkkale region and stained with carbol-fuchsin, while 14 of the samples with diarrhea symptoms evaluated negatively by staining method were positive with the rapid diagnosis kit. In our study, although it was determined that the positivity rate was high in diarrheal samples, it was revealed that clinical symptoms were not important according to the ROC curve model statistically performed in the two methods compared.

In the literature reviewed, there was no data on the effect of Cryptosporidium spp. on sex and a certain age range in lambs. In our study, no significant gender and age differences by means of diagnosis with either method (p=0.382 and p=0.397, respectively). All samples taken in our study were sampled from lambs aged 0-30 days.

Climatic factors such as temperature, precipitation, humidity and extreme weather conditions can affect the spread or incidence of *Cryptosporidium spp.*The fact that countries around the world are located at different latitudes and longitudes and the differences in precipitation regimes seen throughout the year depending on climate types have an impact on the spread of infection (Wang, Wang & Cao, 2023). Green et al. (2004) linked the spread of infection to precipitation. While 55.4% was found in the fecal sample taken from the lambs during the period when the rainfall was highest, 17.3% was found in the samples taken during the low rainfall period. In our study, according to the 2023-2024 water year precipitation report of the General Directorate of Meteorology, it was reported that precipitation was below normal (5%) in the Central Anatolia Region (General Directorate of Meterology, 2024). This situation, which was encountered during the study period, may have reduced the results obtained in the rapid diagnostic kit with the carbol-fuchsin staining method.

The need for experienced personnel in the direct diagnosis of the agent in the stool microscopically, the low density of oocysts excreted in the feces or the damage of the excreted oocysts by mechanical and enzymatic factors adversely affect the diagnosis microscopically. Again, due to the high costs of equipment used for molecular methods in diagnosis and the need for experienced personnel, practical diagnostic methods such as ready-made diagnostic kits are needed (Cheun et al., 2013; Danišová, Halánová, Valenčáková and Luptáková, 2018) On the other hand, the sensitivities and specificities of these kits, which are based on qualitative antigen detection in feces, vary. In a study conducted to investigate the sensitivity of four different immunochromatographic test kits, a rate of 47.2-70.6% was detected. The use of these diagnostic methods alone also shows that some cases may be overlooked. Therefore, it has been stated that these tests can only be useful in the absence of experienced personnel (Agnamey et al., 2011). It has also been stated that the positive or negative results obtained by a single diagnostic method in the stool of these tests are not reliable and insufficient, especially in those in the life-threatening risk group (weak immune system). Danisova et al. (2018) stated that the use of C. parvum and C. hominis antibodies in commercially produced kits in commercially produced kits does not respond to the antigens of some genetically distant species or only responds weakly, and underlines that there will be

various copro antigens that cannot be detected by immunological tests.

Conclusions

In this study, the presence of infection was detected accurately and quickly with the rapid diagnosis kit and carbol-fuchsin staining, the latter is one of the classical methods. However, the high number of differences in results between the two methods suggested that the staining technique, which is important for definitive diagnosis, requires a second confirmation due to reasons such as low agent density. Although the 47% positivity obtained with the rapid diagnostic kit in the study proves to be more reliable than the carbol-fuchsin staining. It has been concluded that it is important to reduce animal losses by starting treatment early with early diagnosis for rapid diagnosis without a laboratory. As a result, the rapid diagnostic kit method is more sensitive in Cryptosporidiosis compared to carbol-fuchsin staining. There is a considerable *Cryptosporidium spp.* prevalence in the Kırıkkale Province.

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References

1. A Özçelik,. (2018). Elazığ yöresinde İshalli Neonatal Kuzu Ve Oğlakların Bazı İshal Etkenlerinin Hızlı test kitleri ile Teşhisi. Yüksek Lisans Tezi, Fırat Üniversitesi Sağlık Bilimleri Enstitüsü, Elazığ.
2. Abd-Al-Aal, Z., El-Kabbany, A., Tahrani, L. (2016). Comparison between two diagnostic methods for detection of Cryptosporidium spp. infecting farm animals in Kuwait. Bulletin of Faculty of Science, Zagazig University, 38, 31075.
3. Agnamey, P., Sarfati, C., Pinel, C., Rabodoniriina, M., Kapel, N., Dutoit, E., Garnaud, C., Diouf, M., Garin, J. F., Totet, A., Derouin, F. (2011). Evaluation of four commercial rapid immunochromatographic assays for detection of Cryptosporidium antigens in stool samples: a blind multicenter trial. Journal of Clinical Microbiology, 49, 1605-7.
4. Ahmed, S. A., Karanis, P. (2018). Comparison of current methods used to detect Cryptosporidium oocysts in stools. The International Journal of Hygiene and Environmental Health, 221, 743-63.
5. Aslan-Çelik, B., Çelik, Ö. Y., Ayan, A., Orunç-Kılınç, Ö., Akyıldız, G., İrak, K., Selçuk, M. A., Ercan, K., Baldaz, V. & Oktay-Ayan Ö. (2023). Occurence and genotype distributionof Cryptosporidium spp. and Giardia duodenalis in sheep in Siirt, Turkey. The Polish Journal of Veterinary Sciences, 26, 359-66.
6. Arslan, S., Öncel, T., Malal, M. E., Satir, E., Sait, A., Baca-Ünsal, A., Aydoğan-Yaman, D. (2016). Bacteriological, virological and parasitological etiology in diarrhea cases in determined in post-mortem lambs and kids in Marmara Region. Van Veterinary Journal, 27(3).
7. Barker, I. K., Carbonel, P. L. (1974). Cryptosporidium agni sp. N. from lambs, and Cryptosporidium bovis sp. N. from a calf, with observations on the oocyst. Zeitschrift fur Parasitenkunde, 44, 289-98.
8. Bordes, L., Houert, P., Costa, D., Favennec, L., Vial-Novella, C., Fidelle, F., Grisez, C., Prévot, F., Jacquiet, P., Razakandrainibe, R. (2020). Asymptomatic Cryptosporidium infections in ewes and lambs are a source of environmental contamination with zoonotic genotypes of Cryptosporidium parvum. Parasite, 27, 57.
9. Cheun, H. I., Chung, B. S., Ma, D. W., Goo, B. L., Cho, S. H., Ji M. J., Lee W. J. (2013). Development of a Diagnostic Kit to Detect Cryptosporidium parvum and Giardia lamblia. Osong Public Health and Research Perspectives, 4, 146-51.
10. Coop, R. L. & Wright, S. E. (2003). Cryptosporidiosis and Coccidiosis. İçinde W.B. Martin I.D. Aitken (Edt.), Diseases of Sheep. (ss.153-155). USA: Blackwell Science.
11. Danišová, O., Halánová, M., Valenčáková, A., Luptáková, L. (2018). Sensitivity, specificity and comparison of three commercially available immunological tests in the diagnosis of Cryptosporidium species in animals. The Brazilian Journal of Microbiology, 49, 177-83.
12. Dessi, G., Tamponi, C., Varcasia, A., Sanna, G., Pipia, A. P., Carta, S., Salis, F., Díaz, P., Scala, A. (2020). Cryptosporidium infections in sheep farms from Italy. Parasitology Research, 119, 4211-18.
13. E Evgin., (2022). Kırıkkale Bölgesinde Neonatal Kuzu İshallerinin Parazitolojik Yönden Değerlendirilmesi. Yüksek Lisans Tezi, Kırıkkale Üniversitesi Sağlık Bilimleri Enstitüsü, Kırıkkale.
14. Gabr, N.S., Abdellatif, M. Z., Abd El-Hafeez, E., Abd Rabou, R. A. (2014). Comparison between ELISA and various stains techniques in laboratory diagnosis of cryptosporidiosis. Journal of the Egyptian Society of Parasitology, 44, 509-16.
15. Gerace, E., Lo Presti, V. D. M., Biondo, C. (2019). Cryptosporidium infection: epidemiology, pathogenesis, and differential diagnosis. European Journal of Immunology, 9, 119-23.
16. Green, R. E., Amarante, A. F. T. & Mascarini, L. M. (2004). The seasonal distribution of Cryptosporidium oocysts in sheep raised in the State of São Paulo. Revista Brasileira de Parasitologia Veterinária, 13, 125-27.
17. Heine, J. (1982). An easy technique for the demonstration of cryptosporidia in faeces. Zentralblatt fur

Veterinarmedizin, 29, 324-27

18. Kabir, M. H. B., Ceylan, O., Ceylan, C., Shehata, A. A., Bando, H., Essa, M. I., Xuan, X., Sevinc, F., Kato, K. (2020). Molecular detection of genotypes and subtypes of Cryptosporidium infection in diarrheic calves, lambs, and goat kids from Turkey. Parasitology International, 79, 102163.
19. Kaminjolo, J. S., Adesiyun, A. A. (1994). Rotavirus infection in calves, piglets, lambs and goat kids in Trinidad. British Veterinary Journal, 15, 293-99.
20. Kaya, E., Akata, I., Bakırcı, S., Dereli, D., Küçükgüven, E., Yılmaz, İ. (2014). Immünokromatografik kart testlerin çalışma prensibi ve üretim teknikleri. Duzce Medical Journal, 16 (3).
21. Khan, N. U., Usman, T., Sarwar, M. S., Ali, H., Gohar, A., Asif, M., Rabbani, F., Khan, R. U., Sultana, N., Khan, N. A., Mobashar, M., Shah, A. A., Wanapat, M. (2022). The prevalence, risk factors analysis and evaluation of two diagnostic techniques for the detection of Cryptosporidium infection in diarrheic sheep from Pakistan. PLoS One, 17(7).
22. Khurana, S., Chaudhary, P. (2018). Laboratory diagnosis of cryptosporidiosis. Tropical parasitology, 8, 2-7.
23. Majeed, Q. A. H., El-Azazy, O. M. E., Abdou, N. M. I., Al-Aal Z. A., El-Kabbany, A. I., Tahrani, L. M. A., AlAzemi, M. S., Wang, Y., Feng, Y., Xiao, L. (2018). Epidemiological observations on cryptosporidiosis and molecular characterization of Cryptosporidium spp. in sheep and goats in Kuwait. Parasitol Resarch, 117, 1631-36.
24. Meteroloji Genel Müdürlüğü. https://www.mgm.gov.tr/veridegerlendirme/yagis-raporu. (Erişim. 8 haziran 2024).
25. Mi, R., Wang, X., Huang, Y., Mu, G., Zhang, Y., Jia, H., Zhang, X., Yang, H., Wang, X., Han, X., Chen, Z. (2018). Sheep as a potential source of zoonotic Cryptosporidiosis in China. Applied and Environmental Microbiology, 31, 84(18) e00868–e00818.
26. Mirhashemi M. E., Zintl, A., Grant, T., Lucy, F. E., Mulcahy, G., De Waal, T. (2015). Comparison of diagnostic techniques for the detection of Cryptosporidium oocysts in animal samples. Experimental Parasitology, 151-152, 14-20.
27. O'Leary J.K., Sleator, R. D., Lucey, B. (2021). Cryptosporidium spp. diagnosis and research in the 21st century. Food and Waterborne Parasitology, 24, e00131.
28. Papanikolopoulou, V., Baroudi, D., Guo, Y., Wang, Y., Papadopoulos, E., Lafi, S.Q., Abd El-Tawab, M. M., Diakou, A, Giadinis, N. D., Feng, Y., Xiao, L. (2018). Genotypes and subtypes of Cryptosporidium spp. in diarrheic lambs and goat kids in northern Greece. Parasitology International, 67, 472-75.
29. Papini, R., Bonelli, F., Montagnani, M., Sgorbini, M. (2018). Evaluation of three commercial rapid kits to detect Cryptosporidium parvum in diarrhoeic calf stool. Italian Journal of Animal Science, 1-6.
30. Paraud, C., Chartier, C. (2012). Cryptosporidiosis in small ruminants. Small Ruminant Research, 103, 93-97.
31. Potters, I., Van Esbroeck, M. (2010). Negative staining technique of Heine for the detection of Cryptosporidium spp.: a fast and simple screening technique. The Open Parasitology Journal, 4, 1-4.
32. Rekha, H. K. M., Puttalakshmamma, G. C., D'Souza, P. E. (2016). Comparison of different diagnostic techniques for the detection of cryptosporidiosis in bovines. Veterinary World, 9,211-15.
33. Robinson, G., Chalmers, R. M. (2015). Cryptosporidium detection in water: From microscope to genomics. In Cryptosporidium: Parasite and Disease (ss. 59-83). Springer.
34. Ryan, U., Hijjawi, N. (2015). New developments in Cryptosporidium research. The International Journal for Parasitology, 45, 367-73.
35. Ryan, U., Fayer, R., Xiao, L. (2014). Cryptosporidium species in humans and animals: current understanding and research needs. Parasitology, 141, 1667-85.
36. Sarı, B., Balkaya, İ., Tascı, G. T., Arslan, M. Ö., Kucukler, S. B. (2023). Prevalence of Cryptosporidium, Eimeria Species and Gastrointestinal Helminths in Lambs and Sheeps in Erzurum Region. Türk Doğave Fen Dergisi, 105-11.
37. Sevinc, F., Uslu, U., Derinbay, O. (2005). The Prevalence of Cryptosporidium parvum in lambs around Konya. Turkish Journal of Veterinary and Animal Science, 29, 1191-94.
38. Sevinç, F, Dik, B. (2015). Cryptosporidium. İçinde (Edt.), N. Dumanlı K. Karaer Veteriner Protozooloji. (ss. 127). Ankara: Medisan Yayıncılık.
39. Ulutas, B., Voyvoda, H. (2004). Cryptosporidiosis in diarrhoeic lambs on a sheep farm. Turkiye Parazitoloji Dergisi, 28, 15-17.
40. Viu M, Quilez J, Sanchez-Acedo C., del Cacho, E., Lopez-Bernad, F. (2000). Field trial on the therapeutic efficacy of paromomycin on natural Cryptosporidium parvum infections in lambs. Veterinary parasitology. ; 90: 163-70.
41. Wang, X., Wang, X., Cao, J. (2023). Environmental Factors Associated with Cryptosporidium and Giardia. Pathogens, 12, 420.