Prevalence and epidemiological report of *cryptosporidium* spp. in horses and donkeys from northeastern region of Algeria.

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Abstract

Cryptosporidium spp. is known to be the majore cause of diarrhea in animals. This study aims to determine the prevalence of *cryptosporidium* infections in horses and donkeys from Souk Ahras province, and assessthe risk factors related to this infection. For this, 93 stool samples (41 horses and 52 donkeys) were collected in three districts of Souk-Ahras province (Sedrata, Taoura, Zarouria) from January to May 2024. Laboratory analysis were performed using microscopy tool, after modified Ziehl Neilsen staining, The total rate of *cryptosporidium* spp. infections prevalence were 19,35% (CI 95%: 8.99-29.72). Only the age influences equines infections by *Cryptosporidium* spp. ($p<0.05$), with a high prevalence rate in animals which have ≤ 1 year (CI 95%: 10.50%-53.14%) compared to those which have > 1 year (CI 95%: 3.63%-27.36%). Neither the gender, nor the animal species or the location have shown significant effect in this study ($p>0.05$).

Keywords: *Cryptosporidium* Spp., equine, microscopy, prevalence, Souk-Ahras

Introduction

Cryptosporidium, is a coccidian protozoan, affects the gastrointestinal system of various animals, including mammals, birds, fish, reptiles, and amphibians (Ryan et al., 2014). This protozoan is transmitted following a direct life cycle, after sheding of *Cryptosporidium* sporulated oocysts, into the environment by infected hosts, thus spreading directly through oral ingestion to other hosts (Valigurova et al., 2008; Fayer, 2010). Infected animals, could present asevere diarrhea caused by intestinal diseases, which conduct to significant economic losses and sometimes mortality in ruminants (Morin, 2002). Currently, *Cryptosporidium* spp. presents a well-established zoonotic risk and is recognized as an emerging gastrointestinal pathogen (Xiao, 2010; Šlapeta, 2013).

The epidemiology of cryptosporidiosis, including prevalence and risk factors, have been extensively studied in some animal species such as ruminants, birds, and pigs over the world (Ryan et al., 2014). However, the epidemiological situation of this disease remains poorly understood in some countries and in some animal species especially in equines species (Benhadji et al., 2020; Baroudi et al., 2018).Despite that equines especially horses and donkeys,are used in daily human activities such as transport, work and others, and live closely with human in some areas worldwide, but the role of this animal species in *cryptosporidium* spp. transmission still not sufficiently clear (Wang et al., 2020). Indeed, several studies have been conducted in Brazil, Algeria, Iraq , Egypt,and Chileto more understand the epidemiology of this infection in equine species(Laatamna et al., 2013; Laatamna et al., 2015; Inacio et al., 2017;Jihad et al., 2020;Tuemmers et al., 2022**;** Salem et al., 2023). The high observed prevalences of *cryptosporidium* spp. in equines using either molecular or microscopy tools reached up 67.0% and 69.00% in horses and donkeys respectively (Jihad et al., 2020; Tuemmers et al., 2022).Moreover, the most authors reportedthat age and season affect the infections of equines by *cryptosporidium* spp. (Inacio et al., 2012 ; Jihad et al., 2020 ;Tummers et al 2022). However, the effect of other factors is still not determine such asgender, breeding system and farming practices and needed to be confirmed by more studies in different area (Li et al., 2022).In Algeria, animal cryptosporidiosis is documented in a many report, in cows, sheep, goat, camel, birds, fish and rabbits (Maxamhud et al., 2023;Reghaissia et al., 2021; Bouragba et al., 2020; Sahraoui et al., 2019; Dadda et al., 2021; Ouakli et al., 2018;Laatamna et al., 2018; Baroudi et al., 2018; Benhouda et al., 2017; Baroudi et al., 2013). Data on equine cryptosporidiosis is still scare, and only two studies have been conducted on the epidemiology of this parasite infections, where very low prevalence was observed (2.37%-2.89%) (Laatamna et al., 2013; Laatamna et al., 2015). In addition, these two studies cover only four provinces of Algeria (Algiers, Tiaret, Setif and Bordj Bou Arriridje). In our knowledge, no study was conducted on *cryptosporidium* spp. infections in horses and equines from Souk-Ahras province. This study aims to identify the prevalence rates and risk factors of *cryptosporidium* spp. in equines from northeastern of Algeria, particularly in Souk-Ahras province using microscopy analysis.

Materials and methods

Samples and study area

Overall**,** 93stool samples belonged to 52 donkey and 41 horse have been collected from 03 localities from Soukahras province (Sedrata, Zaarouria, Taoura) from January to May 2024. The studied province is situated in the extreme eastern of Algeria (Fig 01). Ithas a Mediterranean climate in the northern and continental in the far southern. The temperature is varied according to the season, (down to 10°C in January and 45°C in August). Souk Ahras characterized by cold, wet winter and hot and dry summer (Abid, 2016).

Out of 59 stables were visited to perform this study. gender, age and breeding system of

studied animals were recorded and all included animals were clinically healthy. The stool were collected directly from the rectum and preserved immediately in potassium dichromate (2.5%). Then transported to the pedagogical laboratory of parasitology in Institute of Agronomic and Veterinary Sciences, University of Souk-Ahras to perform the microscopic analysis.

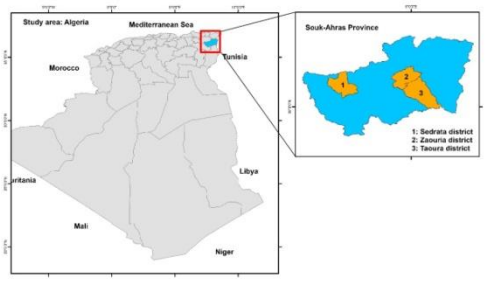

Figure 01: Location of study area.

Microscopic Analysis

All stool samples were examined for looking for *cryptosporidium* spp. oocysts, using the formalin-ether concentration procedure and the modified Ziehl Neelsen staining designed specifically for *cryptosporidia* (Henriksen and Pohlenz 1981).The oocysts of *cryptosporidium* were identified as described by (Deluol 1999).

Statistical analysis

The statistical analysis was performed by the SciStat® software. The variable univariate logistic regression analysis was performed primarily to achieve “chi2” test. Then the differences were considered significant when P was <0.05 and the incidence rate (95% CI), was calculated by the https://iremi.univ-reunion.fr/spip.php?article664 website.

Results

Cryptosporidium spp. oocysts appear bright red or pink on a blue background (fig 2).Following microscopic examination of 93 samples, 18 cases were positive, corresponding to an infection rate of 19.35% (18/93) (CI95%:8.99-29.72).The infection rates were higher in animal lived in Taoura26 % (11/43) (CI95%:10.33%-40.83%) district compared to those lived in Zaarouria16 % (4/21)(CI95%:0.00%-40.87%) and Sedrata10% (3/29) districts (CI 95%:0.00%-28.91%). Univariate logistic regression was calculated after considering, the donkeys and horses as same individuals because they belonged to the same genus (*Equus*) and family (*Equidae*). Statistical analysis shows a significant difference between age groups, for *Cryptosporidium* spp. infections ($p<0.05$). The rate of infection among age groups, were high at younger animals (≤ 1 year) (31,81% (7/22) (CI95%: 10.50%-53.14%)) compared to older ones (>1 year) (15, 49% (11/71) (CI95%: 3.63%-27.36%)). Regarding to the gender, there is no significant difference between equine males and females ($p > 0.05$). Our findings have shown that the prevalence rate was substantially greater in males ((23, 52 %) (4/17) (CI 95%: 1.00%-47.78%))Than in females (18, 42 %) (14/79) (CI95%:6.95%-29.89%). The species factor was also studied for all individuals and found not to have a significant influence (>0.05) on *Cryptosporidium* spp. Infections (in donkeys (19,23%) (10/52) (CI 95%: 5.36%-33.10%)) and horses ((19,51%) (8/41) (CI 95 %: 3.89%-35.13%)). Table 01 summarizes all obtained results in this work.

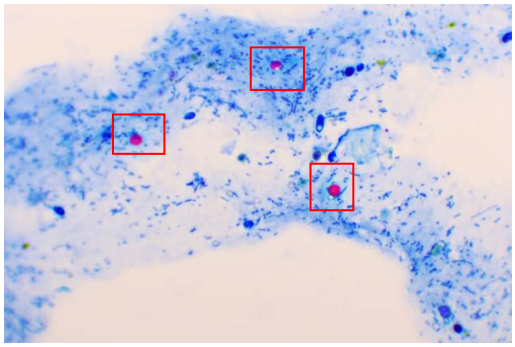

Figure 02: microscopic examination of cryptosporidium spp. oocysts using in equines (objective *100).

Discussion

This study reported for the first time the prevalence of *cryptosporidium* spp. in equines (Horses and donkeys) from the Algerian northeastern region using microscopic analysis. Our findings have indicated that the

Table 01: Prevalence of *cryptosporidium* spp. in equines according to gender, age and equine species.

Risk factor		Positive % (N° positive/Total)	N° Negative	CI95%	P[b]	Signifiance
Gender	Male	23,52 (4/17)	13	[1.00 ; 47.78]	3.1680	NS
	Female	18,42 (14/76)	62	[6.95 ; 29.89]		
Age	> 1 year	15,49 (11/71)	60	[3.63 ; 27.36]	0.0001	S
	≤ 1 year	31,81 (7/22)	15	[10.50 ; 53.14]		
Species	Horse	19,51 (8/41)	33	[3.89 ; 35.13]	0.0650	NS
	Donkey	19,23 (10/52)	42	[5.36 ; 33.10]		
Total	-	18.55 (18/93)	75	[8.99 ; 29.72]	-	-

NS : no significate , **S** : Significate, **CI** : Confidence Interval, **P**: P value, **b**: Pearson Chi-Square test

horses and donkeys in Souk Ahras province could be infected by *cryptosporidium* spp. by an overall prevalence rate of 19.35% (18/93) (donkeys: 19,23% and horses: 19,51%) distributed in Taoura 26 % (11/43), Zaarouria16 % (4/21) and Sedrata10 % (3/29) districts. Few studies were performed on *cryptosporidium* spp. in equines using microscopy and the most of them were conducted using PCR. Similar prevalences were observed in Canada in horses (17.4%), China in horses and donkeys (17.05%), Germany in horses (19.04%), Italy in horses (19.17%), Brazil in horses (21.73%) and New Zeland in horses (17.96%)(Olson et al., 1997 ; Grinberg et al., 2009 ; Jian et al., 2012; Galuppi et al., 2015; Inacio et al., 2017; Raue et al., 2017). Other reports in Algeria, UK, Poland, Brazil, China, USA, and Algeria have shown very low prevalence rates of *cryptosporidium* spp. infections in equines,which ranged from 0% to 12.65% (Majewska et al., 1999 ; De Souza et al., 2009; Burton et al., 2010; Laatamna et al., 2013;Laatamna et al., 2017; Zhang et al., 2019;Khan et al., 2020 ; Wei et al., 2020; Zhang et al., 2021; Xu et al., 2023). The highest recorded prevalence rate was 69% in Iraq in horses (Jihad et al., 2020). Diagnosis tools, weather conditions of area, abundance of other animals and oocysts in environment and type of breeding are factors, which conduct to record different rates of *cryptosporidium* infections (Alruhaili et al., 2024).

In the present work only the age had a significance statistical difference on *cryptosporidium* spp. infections in equines with a high prevalence rate in younger equines comparing with olderones. The same observation was saved in the previous reports (Langkjaer et al., 2007; Wang, 2020).This finding could be explained by the lose of the protective effects of the maternal antibodies in age of 2-6 months, or to the changes of immune functions responding to the weaning stress, which provide an immune system suppression resulting in a decreased resistance of the young animals (Lu et al., 2008; Ren et al., 2018).Gender, months of the year and animal species had no significant statistical difference on *cryptosporidium* spp. infections in this work, which is in contrary with which reported by other studies. The most studies have shown that prevalence of *Cryptosporidium* in female *Equus* was identified to be higher than that in male *Equus* (Li et al., 2022).Weaker body resistance of female *Equus* after giving birth could makes them more susceptible to *Cryptosporidium* infection than males (Chen et al., 2013).Regarding to the animal species factor, our results have reported that either horses or donkeys share a similar prevalence rates, which is in disagreement with which have observed previously (li et al., 2022). The poor harness, lack of veterinary care, and improper nutrition make them more susceptible to be infected than horses (Davis, 2019).

Conclusion

The present work provided data on global prevalence and risk factors of *cryptosporidium* spp. infections in equines. This study investigated for the first time, the presence of *cryptosporidium* species in 93 equines (41horses and 52 donkeys) from Sedrata, Taoura, and Zaarouria districts of Souk-Ahras province, using modified Ziehl Neilsen staining. Equine infections by *cryptosporidium* species reached to 19, 35% (18/93) in the present study, which considered as a high prevalence rate observed in this animal genus in Algeria. Equines live in Taoura district (26%) presented a high frequency of *cryptosporidium* spp. infections regarding to thosewhich live in Sedrata and Zaarouria districts (10% and 16% respectively). This work showed that young animals are more susceptible to be infected by *cryptosporidium* spp. than older ones, with 31% and 15%respectively. Gender and animal species didn't affect the frequency of infections by *cryptosporidium* spp. This study allowed glimpsing a series of perspectives, which could clear more the state of cryptosporidiosis infections in equines, also the role of equines in the transmission of *cryptosporidium* species to human. In fact, other epidemiological and molecular studies which investigate the presence of this parasite in equines should be conducted on a higher number of samples and from different regions in the province and all th ecountry. Moreover, correct prevention and control measures should be taken in time for specific age groups to minimize this kind of infections in equines.

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Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflicts ofinterest.

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