Basic and Applied Herpetology 35 (2021) 63-75

Prevalence and intensity of gastrointestinal parasites in the vulnerable spur-thighed tortoise (*Testudo graeca*) from the central-western of Morocco

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Received: 30 June 2020; returned for review: 02 December 2020; accepted 14 January 2021.

This study was undertaken to determine the prevalence and intensity of gastrointestinal parasites in wild *Testudo graeca* tortoises in Morocco. A total of 102 tortoises sampled in two arid areas: Central Jbilets Mountains (CJM) and Sidi Kaouki Forest (SKF) were macroscopically examined and subjected to qualitative (flotation and Baermann techniques) and quantitative (McMaster technique) microscopic examinations. The identified gastrointestinal parasites belong to two nematode families (Pharyngodonidae and Ascarididae) and three protozoa families (Balantidiidae, Eimeriidae and Entamoebidae). 92.1% of tortoises were determined to harbor one or more parasite types. Oxyurid adults were the most frequently encountered with a prevalence of 22.7% and 72.4% in CJM and SKF, respectively. Nematode eggs were found in almost all individuals of both populations studied. The prevalence of protozoa was 9.1% and 3.4% in CJM and SKF, respectively. Oxyurid adults showed the highest intensity in SKF (36.5 ± 30.2) whereas, ascarid adults are absent in CJM. Statistical analysis showed that the prevalence of infection by ascarids was significantly higher in adults than in juveniles. The intensity of infection by ascarid and oxyurid eggs differed significantly between sexes and localities, respectively. Our results showed a difference of infection between the two localities, which could be in relation with habitat quality. SKF is affected by both agriculture and overgrazing. In addition, the tortoises from this locality are active for a large part of the year, which increases their chance of encountering the parasites.

Key words: arid area; Ascarid; Balantidiidae; Eimeriidae; Entamoebidae; Overgrazing; Oxyurid.

The spur-thighed tortoise, *Testudo graeca* (L., 1758) is a Mediterranean species with a large-scale geographical distribution from Spain and Morocco, east to the semi-arid plains of Turkey, Turkmenistan and Iran (Znari *et al.*, 2005; Anadón *et al.*, 2012). In Morocco, the spur-thighed tortoise is frequently found in arid and semi-arid rocky and sandy areas, with bushy vegetation (Andreu *et al.*, 2004). It is a herbivorous species and consumes several wild plants (El Mouden *et al.*, 2006). In its natural habitat, the species is severely threatened by various factors like increas-

DOI: http://dx.doi.org/10.11160/bah.194

ing aridity due to climate change, overgrazing of their natural habitats and also by trade within Morocco with illegal exportation to many other countries, especially European (ZNARI *et al.*, 2005). Because it is submitted to all these negative pressures, several studies have been conducted to determine its population tendencies, as well as its physiological responses to environmental stresses (EL MOUDEN *et al.*, 2006, 2020; SEREAU *et al.*, 2010; MOULHERAT *et al.*, 2014; GRACIÁ *et al.*, 2017; CHERGUI *et al.*, 2019). However, information on tortoise-gastrointestinal parasite interactions is very scanty, while the parasitism is recognized as a fundamental factor naturally driving the dynamics of animal populations (FOURNIÉ *et al.*, 2015). In Spain, BENÍTEZ-MALVIDO *et al.*, (2019), highlighting the relationship between nematode parasites richness and abundance with growth rates of *T. graeca* across levels of habitat loss. In Morocco, the rare studies performed on gastrointestinal parasites of this vulnerable species concerned the systematic description (BOUAMER *et al.*, 2001a; 2001b; BOUAMER & MORAND, 2005). However, a previous study on salmonellas has found a wider spectrum of serotypes in an unprotected area compared to a protected one (HIDALGO-VILA *et al.*, 2008). This difference has been attributed to the negative effect of overgrazing by livestock and other domestic animals. These studies indicate a need for more research on parasites of this tortoise species to better understand its biotic interactions and conservation management.

Gastrointestinal parasites have the ability to regulate the size of host populations through their potential impact on fertility and host survival (HUDSON *et al.*, 1998; ALBON *et al.*, 2002). In tortoises, some nematodes can cause malabsorption or intestinal problems (MARTINEZ-SILVESTRE, 2011), and inflammatory lesions due to parasite migration (HIDALGO-VILA *et al.*, 2011). These health problems have prompted several researchers to carry out works on gastrointestinal parasite incidents (CHÁVARRI *et al.*, 2012; HEDLEY *et al.*, 2013; HALLINGER *et al.*, 2018; HUFFMAN *et al.*, 2018). In this context, many studies have been conducted on infections (BOUAMER *et al.*, 2003; GAGNO, 2005; TRAVERSA *et al.*, 2005; AL-BARWARI & SAEED, 2007; Fournié *et al.*, 2015;), but most of them on captive populations (DE CASTET-FLAMANT, 2002; CUTLER, 2004; PASMANS *et al.*, 2008; PELICHONE *et al.*, 2010; CHÁVARRI *et al.*, 2012; HALLINGER *et al.*, 2018). There have been few studies on tortoises living in a natural habitat where nematode infection dynamics may differ significantly from captivity (CHÁVARRI *et al.*, 2012). Therefore, the principal aim of the current study is to provide data on the prevalence and intensity of gastrointestinal parasites infecting free-living *T. graeca* tortoises, in two arid areas of Morocco. The comparison between the two populations from different environments will allow us to elucidate the impact of habitat factors on the parasite-host interaction and then on the health of animals.

MATERIAL AND METHODS

Study areas

The current work was carried out in spring 2018 in two areas from Morocco. The Central Jbilet Mountains (CJM) at 25 km north of Marrakech (31° 49'N, 7° 59'W,

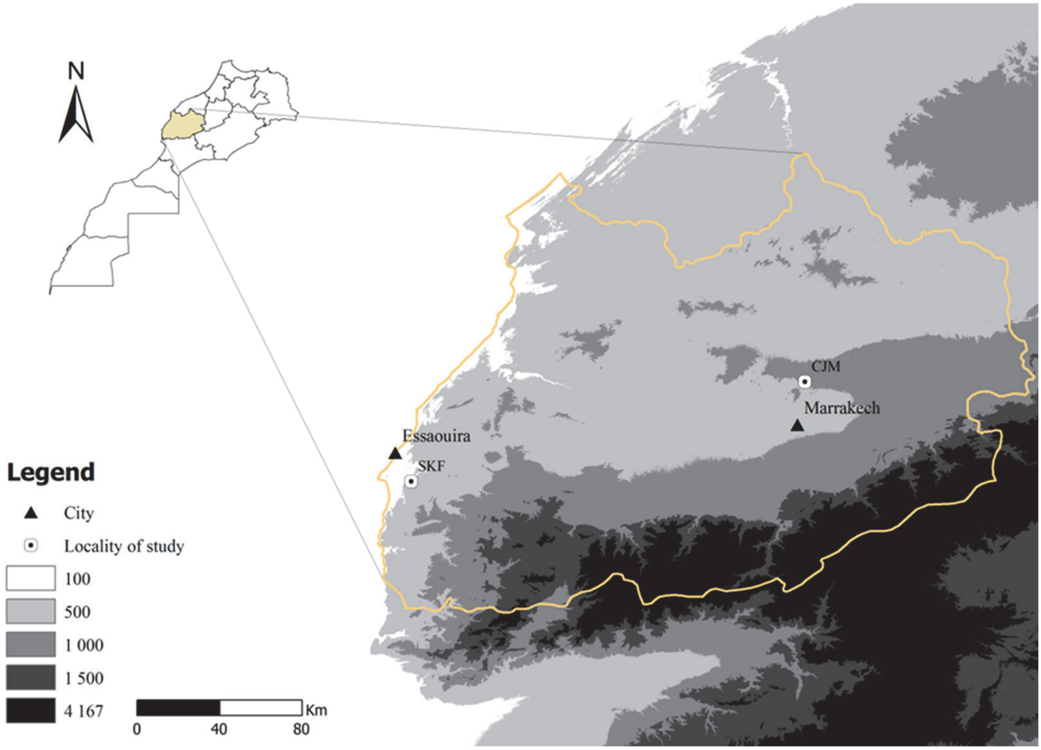

Figure 1: Location of the study areas where wild populations of *T. graeca* were collected. CJM: Central Jbilet Mountains, SKF: Sidi Kaouki forest.

580 m above sea level), and Sidi Kaouki forest (SKF) at 20 km south of Essaouira city (31° 23'N, 9° 42'W, 100 m above sea level) (Fig. 1). The CJM area is a stony area located at the foot of the Central Jbilet Mountains, with typical arid vegetation. The plant cover is mainly dominated by sparse tufts of jujube (*Ziziphus lotus*) and retames (*Retama monosperma*). In spring, approximately 100 herbaceous plant species develop mainly under the bushes. They are sheltered from overgrazing by sheep and goats affecting the study area (BEN KADDOUR *et al.*, 2005; EL MOUDEN *et al.*, 2006; LAGARDE *et al.*, 2012), which represent an important threat for tortoises through environment degradation and competition for food (MOULHERAT *et al.*, 2014). The climate of the region is an arid Mediterranean, with an average annual rainfall of about 240 mm and a rainy season extending from September to February (BEN KADDOUR *et al.*, 2008). The SKF extends over a limestone area covered with an Argan forest; *Argania spinosa* associated with cereal crops and locally with tufts of jujube and doum (*Chamaerops humilis*), partly degraded (BARJE, 2003). The average annual rainfall is 295 mm. The climate in this area is the coastal Mediterranean, with mild to cool temperatures (9.6°C – 22.2°C) because of the moderating influence of cold offshore ocean currents. This area is also subject to a severe over-

grazing by goats and camels (BARJE *et al.*, 2005; KARMAOUI, 2016). The two study areas host populations of *T. graeca* tortoises with estimated densities of 6 and 4 individuals / hectare, in CJM and SKF, respectively (BARJE, 2003; BEN KADDOUR *et al.*, 2006).

Sampling methods

During the period from May to June 2018, 102 wild *T. graeca* tortoises were sampled (44 from CJM and 58 from SKF). After capture, tortoises were weighted (± 1 g), measured (± 0.1 mm) and sexed according to their morphological characteristics (BEN KADDOUR *et al.*, 2008). For the study of gastrointestinal parasites, faecal samples freshly voided by individuals were collected. Animals were also placed individually in plastic boxes until they defecated. Each faecal sample was carefully collected and stored in a 50 ml plastic tube. All tortoises were immediately released in the site of capture.

Parasitological analysis

All coprological examinations were carried out within 24 h after sampling. Each individual faecal sample was macroscopically examined for the detection of adult parasites. They were washed several times in physiological saline (pH 7.3) and stored in absolute ethanol. Thereafter, they were ascribed to either oxyurid or ascarid bases upon their morphological characters. The voucher specimens were deposited at the Laboratory of Water, Biodiversity and Climatic Changes, Faculty of Sciences, Semlalia, Cadi Ayyad University, Marrakech, Morocco, in order to carry out future research concerning the co-evolution of these parasites and conduct genetic identification. The stool samples were processed with zinc sulphate using centrifugation-flotation and sedimentation techniques (33.3%), with a specific gravity, and with the Baermann technique, for identification of helminths eggs. The eggs were counted in a McMaster counting chamber. For the flotation procedure, approximately 3 g of fresh faecal sample (Homogenized sample) was added to 30 ml of flotation solution, centrifuged at 1500 × g for 3 min. The number of eggs per gram (EPG) of faeces was counted using a quantitative examination. An aliquot of 0.3 ml of the filtrate was removed using a Pasteur pipette and placed directly into two chambers of a McMaster slide. After 2 min, the eggs that had floated to the top were counted under a light microscope at 100 × magnification. In addition, the number of eggs counted for both chambers was multiplied by an appropriate factor to estimate the number per gram (EPG). For the protozoa, faeces were screened and identified under a light microscope at 400 × magnification. The identification of eggs was based on morphological and morphometric descriptions with the aid of bench aids for the Diagnosis of Intestinal Parasites (WHO, 1991, 1994).

Statistical analysis

Two parameters were calculated for each parasitological group: prevalence (100 × number of infected tortoises / total number of tortoises) and intensity of infection (number of parasites / number of infected tortoises). The prevalence of infection between age classes, were compared using Pearson Chi-square test. Multivari-

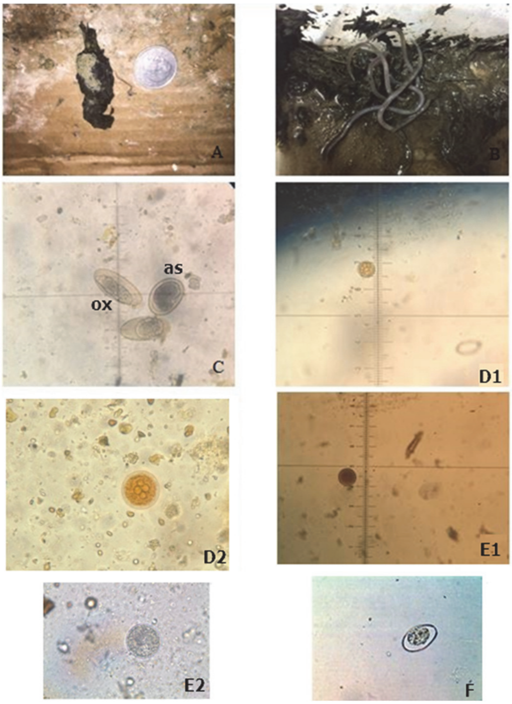

Figure 2: Different gastrointestinal parasite in *T. graeca* faecal samples. **A**: Oxyurids on tortoise faeces; **B**: Ascarid adults; **C**: Oxyurid egg (**ox**) and ascarid egg (**as**) (100x); **D1 and D2**: *Entamoeba* cyst. (stained with iodine); **E1 and E2**: *Balantidium* cysts; **F**: *Eimeria* oocyst (400x).

ate analysis of variance (MANOVA) and analysis of variance (ANOVA) were used to check for differences between sexes/age classes and localities correcting for the body size of tortoises with respect to oxyurids and ascarids load. For all statistical tests, a P-value <0.05 was considered statistically significant using the SPSS Statistics 23 software (SPSS, Chicago, IL, USA).

RESULTS

Among 102 tortoises examined during the study period, 92.1% were found to be infected by nematodes and/or protozoa parasites. Based on morphological characters the identified endoparasites belong to two nematode families (Pharyngodonidae and Ascarididae) and three protozoa families (Balantidiidae, Eimeriidae and Entamoebidae) (Fig. 2). Oxyurid nematodes were the most prevalent in faecal samples. The coprological analysis revealed the presence of oxyurid and ascarid nematodes in SKF population, while the population of CJM is marked only by the presence of oxyurid adults (Table 1). The latter were the most frequently encountered in tortoises with a prevalence of 72.4% and 22.7% in SKF and CJM, respectively. In the two localities, juveniles and females seem to be the most infected by adult oxyurids. Likewise, the females are the most infected (33.3%) by ascarid adults in SKF, followed by males (13.3%). The prevalence of infection with oxyurids was not significantly different among adults and juveniles (X^2 = 2.16, df = 1, P = 0.141). Whereas, the prevalence of infection with ascarids was significantly higher in adult tortoises compared to juveniles (X^2 = 5.43, df = 1, P = 0.019). Giving the results of intensity (Table 2), Oxyurid adults showed high values at SKF (36.5 ± 30.2) compared to ascarid adults (2 ± 1.9). In CJM, the intensity of oxyurid adults (28 ± 14.4) was significantly lower than that in SKF, where ascarid adults were absent.

Both oxyurid and ascarid eggs were present in all samples analyzed from the two localities, with a dominance of oxyurid eggs in all samples (Table 1). The prevalence of tortoises eliminating eggs was statistically significant between sexes (oxyurid eggs: X^2 = 14.42, df = 2, P = 0.010; ascarid eggs: X^2 = 9.31, df = 2, P = 0.010;

Table 1: Prevalence (%) of gastrointestinal parasites (nematodes and protozoa) in *T. graeca* tortoises from two areas (Central Jbilet Mountains and Sidi Kaouki forest) of Morocco.

	Central Jbilet Mountains (CJM)				**Sidi Kaouki Forest (SKF)**			
	Males	**Females**	**Juveniles**	**Total**	**Males**	**Females**	**Juveniles**	**Total**
Number of tortoises	24	12	8	44	30	24	4	58
Nematodes								
Oxyurid adults	8.3	33.3	50	22.7	53.3	91.7	100	72.4
Ascarid adults	0	0	0	0	13.3	33.3	0.07	20.7
Oxyurid eggs	83.3	100	100	90.9	66.7	100	100	82.8
Ascarid eggs	8.3	16.7	50	18.2	33.3	58.3	100	48.3
Protozoa (oo)cysts								
Balantidium cysts	8.3	16.7	0	9.1	0	8.3	0	3.4
Eimeria oocysts	0	16.7	0	4.5	0	0	0	0
Entamoeba cysts	33.3	66.7	25	41.0	13.3	25	0	17.2

localities: $X^2 = 165.48$, df = 6, P = 0.000).

The mean of egg intensity in faeces was 13 233 ± 14 070 EPG for oxyurids and 437 ± 949 EPG for ascarids in SKF. In CJM, it was 5511 ± 4652 EPG for oxyurids and 573 ± 1003 for ascarids (Table 2). Further, the estimated mean number of nematode eggs in tortoise faeces varied according to sex/age of the host, parasite species and locality, being between 17 379 and 173 eggs in SKF and 6889 and 4683 in CJM for oxyurids and between 797 andv 23 in SKF for ascarids. The intensity of infection by oxyurid eggs differed significantly between localities (MANOVA, Wilk'$^{\gamma}$ = 0.911, F = 4.86, P = 0.01) and between host sexes (MANOVA, Wilk'$^{\gamma}$ = 0.18, F = 65.48, P < 0.001). Concerning ascarid eggs, there was no significant difference of intensity between localities (MANOVA, Wilk'$^{\gamma}$ = 0.94, F = 2.96, P = 0.56). Whereas, it differed significantly between the sexes of hosts (MANOVA, Wilk'$^{\gamma}$ = 0.18, F = 66.04, P < 0.001).

With respect to protozoa (oo)cysts, *Balantidium* cysts were detected in stool samples of males and females in CJM with a prevalence of 8.3% and 16.7%, respectively (Table 1). In SKF, this parasite was discovered only in females with a prevalence of 8.3%. In the same way, *Eimeria* oocysts were identified solely in females belonging to CJM with a prevalence of 16.7%. For *Entamoeba* cysts, a total prevalence of 41% was recorded in tortoises from CJM, being higher for females (66.7%) compared to males (33.3%) and juveniles (25%). The similar tendency was noted in SKF but with low prevalence both in females (25%) and males (13.3%). The juvenile tortoises were not found to be parasitized by *Entamoeba* cysts in SKF. For protozoa, the mean values of intensity were low in all case with a maximum of 3 ± 00 OPG for *Eimeria* oocysts in CJM. For *Balantidium* and *Entamoeba*, the

Table 2: Intensity of adult nematodes, their eggs and protozoa (oo)cysts shed by *T. graeca* tortoises in Central Jbilet Mountains and Sidi Kaouki forest areas in Morocco. (EPG: eggs per gram, CPG: cysts per gram, OPG: oocysts per gam, SD: standard deviation).

			Central Jbilet Mountains (CJM)				**Sidi Kaouki Forest (SKF)**			
			Males	**Females**	**Juvenile**	**Total**	**Males**	**Females**	**Juvenile**	**Total**
No. Tortoises			24	12	8	44	30	24	4	58
Nematodes	Oxyurid adults	Mean ± SD	18 ± 1	43 ± 9.2	17 ± 3	28 ± 14.4	27 ± 22	49 ± 32.8	9 ± 2	36.5 ± 30.2
		Range	18 - 19	35 - 51	14 - 20	14 - 51	2 - 62	5 - 130	7 - 11	2 - 130
	Ascarid adults	Mean ± SD	0	0	0	0	3 ± 2.4	1.71 ± 1.25	1.25 ± 2.5	2 ± 1.9
		Range	0	0	0	0	1 - 7	1 - 4	5	1 - 7
	Oxyurids EPG	Mean ± SD	5017 ± 5309	6889 ± 4124	4683 ± 3549	5511 ± 4652	10 872 ± 15 185	17 379 ± 12 713	173 ± 92	13 233 ± 14 070
		Range	4000 - 19116	3250 – 12 717	250 - 9250	250 - 19116	383 - 42 833	1767 - 47 100	70 - 250	70 - 47 100
	Ascarid EPG	Mean ± SD	50 ± 00	2142 ± 695	50 ± 00	573 ± 1003	123 ± 146	797 ± 1284	38 ± 21	437 ± 949
		Range	50	1650 - 2633	50	50 - 2633	50 - 550	50 - 3733	19 - 61	19 - 3733
Protozoa (oo)cysts	*Balantidium* cysts CPG	Mean ± SD	1 ± 00	1 ± 00	0	1 ± 00	0	1 ± 00	0	1± 00
		Range	1	1	0	1	0	1	0	1
	Eimeria oocysts OPG	Mean ± SD	0	3 ± 00	0	3 ± 00	0	0	0	0
		Range	0	3	0	0 - 3	0	0	0	0
	Entamoeba cysts CPG	Mean ± SD	1.2 ± 0.4	1.4 ± 0.7	2 ± 00	1.4 ± 0.6	1 ± 00	1 ± 0.5	0	1.2 ± 0.4
		Range	1 - 2	1 - 3	2	1 - 3	1	1 - 2	0	1 - 2

intensities are either equal to one or absent (Table 2).

DISCUSSION

Our investigation allowed the detection of various digestive parasites, which is in accordance with previous studies in reptiles indicating a mix of gastrointestinal parasites with different life histories (digenetic and monoxenous), and that the reptile may serve as an intermediate or a definitive host (MITCHELL & DIAZ-FIGUEROA, 2005). Likewise, our results indicate that nematode infections are common in almost all tortoises examined, which is in line with parasitism reported in other tortoise populations (GAGNO, 2005, 2006; CHÁVARRI *et al.*, 2012; HEDLEY *et al.*, 2013). Further, the high prevalence and intensity of oxyurids recorded may be due to the species diversity within this group (GAGNO, 2006). In fact, a study of parasites at species level on *T. graeca* in Spain revealed the presence of 15 distinct species belonging to the oxyurid family Pharyngodonidae and only one species of ascarid (CHÁVARRI *et al.,* 2012). The most common oxyurid species were *Tachygonetria dentata* and *T. longicollis* and the rarest are *T. seurati* and *Thaparia thapari* (CHÁVARRI *et al.*, 2012). The prevalence of oxyurid adults determined in our study sites were nearly similar to those found in wild populations from Spain (70% to 94%) (CHÁVARRI *et al.*, 2012). The high prevalence of oxyurids could be explained by their commensal relationship with the host (ROCA, 1999; GAGNO, 2005). Oxyurid infections are generally suggested to have a beneficial effect in digestion and assimilation of plants, preventing constipation in herbivorous reptile hosts (RATAJ *et al.*, 2011; BENÍTEZ-MALVIDO *et al.*, 2019). Moreover, the oxyurid nematodes manifest low pathogenic effects on parasitized tortoises (TRAVERSA *et al.*, 2005; CHÁVARRI *et al.*, 2012). However, high oxyurid infection in herbivorous reptiles can cause severe digestive agglomeration that compromises the intestinal function and can cause chronic weight loss and even death (LOUKOPOULOS *et al.*, 2007). Likewise, the high number of oxyurids can cause anorexia (MARTINEZ- SILVESTRE, 2011). In our study, the number of parasites by tortoise were within the range of values estimated in other natural populations (ROCA, 1999; GAGNO, 2005; CHÁVARRI *et al.*, 2012) and the tortoises did not display any apparent signs of sickness.

The percentage of tortoises with oxyurid eggs in their faeces was between 66.7% for males and 100% for females and juveniles. These values are comparable to those mentioned by CHÁVARRI *et al.*, (2012) for a population of the same species from Spain (94%). The intestinal nematodes of chelonians have evolved life cycles which are either direct or need additional hosts to complete their life cycle (HEDLEY *et al.*, 2013). The consequence of such natural adaptability is that tortoises can be constantly exposed to the infectious stages of their parasites. Furthermore, the herbivorous diet of spur-thighed tortoise could favor infection by parasites, as suggested for other chelonians (HIDALGO-VILA *et al.*, 2009). The net result of these circumstances is an increase in the parasitic load (AL-BARWARI & SAEED, 2007).

Compared to oxyurids, the intensities of infection by ascarids are remarkably low. The difference between the two nematode groups seems to be associated with the pathology of ascarids in relation to their life cycle (CHÁVARRI *et al.*, 2012). The ascarid parasites migrate through various organs (HEDLEY *et al.*, 2013) and then can lead to inflammatory lesions in the lungs and other organs (RATAJ *et al.*, 2011). In the present study, the maximum prevalence by ascarids (adults and eggs) was determined in animals from SKF, whereas the tortoises from CJM were free of ascarid adults but eggs were found in faeces with a mean prevalence of 18.2% and an intensity of infection comprised between 50 and 2142 EPG/tortoise. The obtained prevalence in CJM is comparable to the value reported for free-living *T. graeca* in Spain (CHÁVARRI *et al.*, 2012), whereas, the prevalence in SKF is higher than this recorded in the same natural population but similar to the prevalence in captive tortoises (CHÁVARRI *et al.*, 2012). The ascarid infections in captive tortoises have been associated with carapace deformities and symptoms of upper respiratory tract disease (CHÁVARRI *et al.*, 2012). Based on this data, the tortoises in SKF seem to be more affected by parasites but they did not show any sign of health problems.

Prevalence and intensity of infections of protozoa are lower than that of nematodes. These parasites are frequently encountered in faecal examinations for domestic reptiles (WOLF *et al.*, 2014; CERVONE *et al.*, 2016) and are transmitted via the fecal-oral route (MITCHELL & DIAZ-FIGUEROA, 2005). The isolated protozoa in this study are the easiest protozoa to identify (WOLF *et al.*, 2014). In reptiles, mainly herbivores, ciliates contribute to intestinal digestion (SCULLION & SCULLION, 2009) and are considered as commensal organisms (RATAJ *et al.*, 2011). Nevertheless, *Balantidium* sp. is suspected as a potential pathogen and has been reported to cause colitis (HALLINGER *et al.*, 2018). It has been also described in the liver of heavily infected tortoises, associated with hepatic abscesses (SATBIGE *et al.*, 2017; HALLINGER *et al.*, 2018). They are also responsible for causing Balantidiasis with a range of mild to severe clinical appearances (SCHUSTER & RAMIREZ-AVILA, 2008; CERVONE *et al.*, 2016). Likewise, clinical signs such as anorexia and severe debilitation had been reported in a *Geochelone elegans* tortoise infected with *Balantidium* sp. in captivity (HALLINGER *et al.*, 2018). *Entamoeba* sp. is commonly found in chelonians represented by *E. invadens* as an agent of amoebic dysentery (PASMANS *et al.*, 2008). It should be considered pathogenic for herbivorous tortoises since they can cause anorexia, watery diarrhea and epizootic amebiasis (DE CASTET-FLAMANT, 2002; SCULLION & SCULLION, 2009). The genus *Eimeria* represents a frequent parasite in reptiles affecting the epithelium of the intestine and the biliary system (DE CASTET -FLAMANT, 2002; RAŚ-NORYŃSKA & SOKÓŁ, 2015). Generally, *Eimeria sp.* does not cause significant disease in their hosts, but due to a higher intensity of infection, the hosts may develop clinical diseases and *Eimeria sp.* can contribute to worsening the condition of ill or debilitated animals (MCGUIRE *et al.*, 2013; BARDI *et al.*, 2019).

Our results showed that the intensity of infection by oxyurid eggs differed significantly between localities, with higher intensity in SKF compared to CJM. This finding is in line with previous studies which indicated an association between habitat quality and infection of tortoise with oxyurids (BENÍTEZ-MALVIDO *et al.*, 2018, 2019; HUFFMAN *et al.*, 2018). SKF is a more fragmented habitat due to higher anthropogenic impact compared to the CJM locality. In addition to the agriculture which characterizes SKF, overgrazing by goats and camels (KARMAOUI, 2016) constitute pressure on the vegetation in the site, which leads to a scarcity of feeding resources (BARJE *et al.*, 2005). In this context, BENÍTEZ-MALVIDO *et al.*, (2019) suggested that in a low habitat loss, oxyurid infection was positively associated with growth rates, suggesting a mutualistic relationship. This association became negative at high habitat loss, suggesting a parasitic relationship. The present study indicates that tortoise females show the higher intensity of infection by oxyurids compared to males and juveniles. This is probably related with their larger size (BENÍTEZ-MALVIDO *et al.*, 2019). Previews studies indicated that overall female tortoises are at a higher risk of being infected by parasites than males (HEDLEY *et al.*, 2013; HUFFMAN *et al.*, 2018). But others found no sex differences in parasite distribution on the tortoise gastrointestinal parasites (TRAVERSA *et al.*, 2005).

Acknowledgement

We would like to thank "Haut Commissariat aux Eaux et Forêts et à la Lutte Contre la Désertication (HCEFLCD)" for the Permit to work on the field. This research was supported by Project PHC Maghreb 2019/19MAG50-41431WD.

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