

PREVALENCE OF *CRYPTOSPORIDIUM* SPP. (EUCCIDIORIDA: CRYPTOSPORIIDAE) IN SEVEN SPECIES OF FARM ANIMALS IN TUNISIA

SOLTANE R.*, GUYOT K.**, DEI-CAS E.** & AYADI A.*

Summary:

1,001 faecal samples were obtained from 89 sheep (lambs and adult), 184 goats, 190 horses, 178 rabbits, 110 camels, 200 broiler chicken and 50 turkeys housed in farms from different localities in Tunisia. All samples were analysed for *Cryptosporidium* oocysts by microscopic examination of smears stained by modified Ziehl Neelsen technique. The parasite was detected in ten lambs and adult sheep (11.2 %) and nine broiler chicken (4.5 %). Molecular characterization, performed in four animals, identified *C. bovis* in three lambs and *C. meleagridis* in one broiler chicken. This work is the first report on *Cryptosporidium* in farm animals in Tunisia.

KEY WORDS : *Cryptosporidium*, Apicomplexa, farm animals, prevalence, genotyping, Tunisia.

Résumé : PRÉVALENCE DE *CRYPTOSPORIDIUM* SPP. (EUCCIDIORIDA : CRYPTOSPORIIDAE) CHEZ SEPT ESPÈCES D'ANIMAUX DE FERME EN TUNISIE
1 001 prélèvements fécaux ont été obtenus à partir de 89 moutons, 184 chèvres, 190 chevaux, 178 lapins, 110 chameaux, 200 poulets et 50 dindes élevés dans des fermes de différentes localités en Tunisie. Tous les prélèvements ont été analysés pour la recherche de *Cryptosporidium* par examen microscopique des frottis colorés au Ziehl Neelsen modifié. Le parasite a été détecté chez dix ovins (11,2 %) et neuf poulets (4,5 %). La caractérisation moléculaire réalisée pour quatre isolats a identifié *C. bovis* chez trois agneaux et *C. meleagridis* chez un poulet. Ce travail est le premier rapport sur *Cryptosporidium* chez des animaux de ferme en Tunisie.

MOTS CLÉS : *Cryptosporidium*, Apicomplexa, animaux de ferme, prévalence, génotypage, Tunisie.

Cryptosporidium (Apicomplexa: Eucoccidiorida: Cryptosporiidae) is a ubiquitous enteropathogen and has been reported from many geographical regions of the world. The parasite is recognized as one of the most common causes of diarrhoea in humans and farm animals (Caccio, 2005; de Graaf *et al.*, 1999; Joachim, 2004). Cryptosporidial infection of livestock may have an important economic impact on farmers because of high morbidity and sometimes high mortality rates among animals (Sunotel *et al.*, 2006). Despite its wide distribution and obvious relevance to animal health, no data are available about the *Cryptosporidium* prevalence in animals in Tunisia. The present study was undertaken to assess the relevance of *Cryptosporidium* in poultry and mammals in Tunisian farms in different geographical locations.

MATERIAL AND METHODS

FAECAL SPECIMENS AND SAMPLE SITES

A total of 1,001 faecal specimens was collected in 2003 and 2004 from animals located in privately owned farms or State farms located in different provinces of Tunisia (Table I, Fig. 1). 89 were collected from ovine (30 lambs and 59 adults) housed in three different farms (Fig. 1). Other faecal specimens were sampled from 184 goats, 190 horses, 178 rabbits, 110 camels, 200 broiler chickens and 50 turkeys (Table I, Fig. 1). All these samples were stored at 4° C in a 2.5 % aqueous potassium dichromate solution until examination.

MICROSCOPIC EXAMINATION AND MOLECULAR ANALYSIS FOR GENOTYPING

For the detection of *Cryptosporidium* by microscopy, we proceeded as previously described in another of our work also published in this issue (Soltane *et al.*, 2007). Parasite genotyping could have been done only for four isolates including three from lambs and one from broiler chicken. DNA extraction and PCR at 18S rDNA and Laxer loci were performed as described in Soltane *et al.* (2007). The nucleotide sequences gene-

* Laboratoire de Parasitologie-Mycologie, Faculté de Médecine, Sfax, Tunisie.

** Institut Pasteur de Lille, Laboratoire Écologie du Parasitisme (EA3609, IFR142) et Service de Parasitologie-Mycologie, Département de Microbiologie, Faculté de Médecine, Centre Hospitalier Régional Universitaire, Lille, France.

Correspondence: Raya Soltane.

Tel. : 00 216 21 248 198 – Fax : 00 216 71 399 789.

E-mail: soltane_raya@yahoo.fr

| Animals (age) | Farm number-geographical location | No. of examined animals | No. of positive for <i>Cryptosporidium</i> (%) |
|-----------------------------|-----------------------------------|-------------------------|--|
| Lambs (< 3 months) | 2 - Tunis * | 30 | 5 (16.7 %) |
| | 2 - Tunis | 4 | 0 |
| Sheep (> 1 year) | 3 - Sidi Bouzid | 25 | 3 (12 %) |
| | 4 - Médenine | 30 | 2 (6.7 %) |
| | Total | 59 | 5 (8.5 %) |
| | 5 - Tataouine | 40 | 0 |
| Goat (1-7 years) | 6 - Médenine | 35 | 0 |
| | 7 - Le Kef | 30 | 0 |
| | 8 - Siliana | 25 | 0 |
| | 9 - Tataouine | 54 | 0 |
| | 10 - Tunis | 50 | 0 |
| Horse (1-3 years) | 11 - Nabeul | 35 | 0 |
| | 12 - Kairouan | 25 | 0 |
| | 13 - Kairouan | 30 | 0 |
| | 14 - Nabeul | 50 | 0 |
| Rabbit (1-2 months) | 15 - Tunis | 178 | 0 |
| Camel (3-8 months) | 16 - Ben Guerdan | 110 | 0 |
| | 17 - Tunis | 90 | 3 (3.3 %) |
| Broiler chicken (0-56 days) | 18 - Le Kef* | 110 | 6 (5.5 %) |
| | Total | 200 | 9 (4.5 %) |
| Turkey | 19 - Siliana | 50 | 0 |

* Farms from which the samples for genotyping were derived.

Table I. – Prevalence of *Cryptosporidium* in farm animals.

rated in this study have been deposited in GenBank under accession numbers EF158460 to EF158461.

RESULTS

A total of 1,001 faecal specimens were collected from 89 ovine, 184 goats, 190 horses, 178 rabbits, 110 camels, 200 broiler chickens and 50 turkeys housed in 18 farms in different localities in Tunisia from 2003 to 2004 (Fig. 1). *Cryptosporidium* was found in lambs or adult sheep from all three included farms (Table I), with an overall prevalence of 11.2 %. Lambs were found more often infected (5 out of 30, 16.7 %) than adult sheep (5 out of 59, 8.5 %) but the difference was not statistically significant. Diarrhoea was not present in any of these animals. In chicken, *Cryptosporidium* was only identified in broiler chicken aged up to 56 days with a prevalence of 4.5 % (Table I). Chicken seem to be more susceptible to infection at the age of 22 to 28 days. In this study, *Cryptosporidium* was not found in horses, rabbits, camels, and turkeys.

The molecular-based analysis concerned four *Cryptosporidium* isolates: three from lambs and one from chicken. DNA was amplified by nested PCR and the resulting fragment of the 18s rRNA gene was sequenced. Sequences obtained from the three lamb-derived isolates were 100 % homologous to each other. A Blast analysis in the GenBank database did not find any



Fig. 1. – Map of Tunisia showing the geographical location of the different farms from where sampled animals were originated.

exact match with an existing sequence but clustered these sequences with the *C. bovis* sequence (identity of 99.5 % with EF514234). The sequence of the chicken-derived isolate was identified as *C. meleagridis*. The *Cryptosporidium* DNA diagnostic fragment characterized by Laxer *et al* (Guyot *et al.*, 2002; Laxer *et al.*, 1991) was amplified by PCR for the broiler chicken-derived isolate only. In agreement with the genotyping of the 18S rRNA gene, *C. meleagridis* was identified by RFLP (data not shown). The three *Cryptosporidium* DNA samples from lamb-derived isolates (identified as *C. bovis* at the 18S rRNA gene) failed to be amplified at this same locus, in spite of repeated attempts.

DISCUSSION

Cryptosporidium infections in sheep and poultry have been reported in many countries throughout the world. The results that we report in the present work have shown that *Cryptosporidium* infection occurred also in these animals in Tunisia. This study with that one we concomitantly published in this issue (Soltane *et al.*, 2007) are thus the first reports about *Cryptosporidium* prevalence in different farm animals in a country of North Africa. In the present study, *Cryptosporidium* was identified in ovine with a prevalence of 16.7 % in lambs and 8.5 % in adult sheep. None of these *Cryptosporidium*-infected animal showed disease symptoms. This is coherent with the fact that *C. bovis* was identified in three lambs (Table D). Indeed, *C. bovis*, a species recently described (Fayer *et al.*, 2005), is reported to be not associated with signs of disease (Santin *et al.*, 2004). *C. bovis* has been reported as preferentially infect post-weaned calves (> 3 months of age) but has been also found in sheep (Fayer *et al.*, 2006; Santin *et al.*, 2007). Very recently, Santin *et al.* (2007) identified also *C. bovis* in lambs. This study has shown that the molecular diagnostic tool based on the Laxer sequence, that is already known to failed in the detection of *C. felis*, *C. canis*, *C. suis* and *C. muris* (Jiang & Xiao, 2003), also failed to amplify *C. bovis*. The variability in the primer sequences is the probable explanation for the failure in amplifying DNA.

Though, in different regions of the world, *Cryptosporidium* infections have been reported in goats, rabbits (Marlier *et al.*, 2003; Peeters, 1988; Shiibashi *et al.*, 2006), horses (Browning *et al.*, 1991; Darabus *et al.*, 2001; Grinberg *et al.*, 2003; Majewska *et al.*, 1999; Majewsk *et al.*, 2004; Olson *et al.*, 1997), all examined animals in these species in Tunisia were not found to be infected with *Cryptosporidium*. The age of sampled animals as well as animal management practices or environmental conditions could explain such results.

The present work is the first report on *Cryptosporidium* occurrence in different host species in Tunisia asso-

ciating molecular characterization of few parasite isolates. However, more comprehensive epidemiological studies are needed to elucidate accurately the circulation of *Cryptosporidium* in farms in this country. Moreover, further subtyping of parasite isolates using highly polymorphic markers will be very useful for the study of the worldwide distribution of *Cryptosporidium* species subtypes in livestock (Feng *et al.*, 2007; Thompson *et al.*, 2007; Trotz-Williams *et al.*, 2006; Xiao *et al.*, 2007).

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