# Parasite prevalence in *Mycobacterium* spp. infected dairy goats in the region of Murcia (south-east Spain)

T. Carrau <sup>(1)\*</sup>, M.M. Garijo <sup>(2)</sup>, C. Martínez-Carrasco <sup>(1)</sup>, D. Pérez <sup>(1)</sup>, L.M.R. Silva <sup>(3)</sup>, A. Taubert <sup>(3)</sup>, C. Hermosilla <sup>(3)</sup> & R. Ruiz de Ybáñez <sup>(1)</sup>

 Parasitología, Departamento de Sanidad Animal, Facultad de Veterinaria, Campus de Excelencia Internacional Regional 'Campus Mare Nostrum', Universidad de Murcia, 30100 Espinardo, Murcia, Spain
 Departamento de Producción y Sanidad Animal, Salud Pública Veterinaria y Ciencia y Tecnología de los Alimentos, Facultad de Veterinaria, Universidad Cardenal Herrera-CEU, C/ Tirant lo Blanc, 7, 46115 Alfara del Patriarca, Valencia, Spain

(3) Institute of Parasitology, Justus Liebig University Giessen, BFS, Schubertstr. 81, 35392 Giessen, Germany \*Corresponding author: tessacarrau@gmail.com; Present address: Fraunhofer Institute for Molecular Biology and Applied Ecology, Winchester Strasse 2, D-35394 Giessen, Germany

### Summary

Eighty-four Murciano–Granadina dairy goats slaughtered in the region of Murcia (South-East Spain) were necropsied to evaluate parasitic infections. The majority of the animals (94.0%) were parasitised and multiple infections were present. Twenty-one parasite species were found, including 18 nematode species (Muellerius capillaris, Dictyocaulus filaria, Neostrongylus linearis, Cystocaulus ocreatus, Teladorsagia circumcincta, T. occidentalis, T. trifurcata, Marshallagia marshalli, Camelostrongylus mentulatus, Trichostrongylus capricola, Nematodirus abnormalis, N. filicollis, N. spathiger, T. vitrinus, T. colubriformis, Trichuris spp., Chabertia ovina and Skrjabinema ovis); one trematode species (Dicrocoelium dendriticum); one arthropod species (*Oestrus ovis*); and one protozoa genus (Eimeria spp.). Additionally, 17.85% of the animals were Mycobacterium spp. positive. Therefore, comparison between parasite prevalence, intensity and abundance in tuberculosis-positive and -negative animals was performed. Statistically significant differences between the prevalence of lungworms and gastrointestinal nematodes in Mycobacterium spp. infected and free goats were found. The paper discusses this co-infection between Mycobacteriaceae and endoparasites.

### **Keywords**

Co-infection – Dairy goat – Endoparasite – Gastrointestinal nematodes – Goat – *Mycobacterium* spp. – Prevalence – Tuberculosis.

### Introduction

Caprine tuberculosis (cTB) is caused by *Mycobacterium bovis* and *M. caprae* (1, 2). A wide range of species are susceptible to tuberculosis (TB) and some are spillover hosts in which infection is not self-maintaining (3). Tuberculosis is a chronic disease in which post-mortem examination frequently reveals yellowish-white caseous or caseocalcareous lesions of different sizes, often encapsulated, and frequently located in the lungs and in the mediastinal or mesenteric lymph nodes (2, 4). Furthermore, TB remains an important

zoonotic disease with implications for public health, as well as being the cause of an economic impact on the caprine industry due to decreased goat production, increased mortality rates and costs of diagnosis (3). TB is found in goats worldwide (4, 5, 6), and Spain, which rears approximately 2.6 million head of goats (7), is not considered officially tuberculosis-free (OTF) (8). Consequently, there is a risk of cTB spreading between small ruminants and other domestic animals, particularly when they share pastures. In order to control this zoonotic bacterial disease, many Spanish regions follow cTB through control programmes using the intradermal tuberculin test (IDT), the same diagnostic assay

that is used for cattle (3). In fact, according to European Union Regulation (EC) 853/2004 (9), the surveillance of TB in goats in non-OTF countries is highly important, as goats are still used for raw milk production. In the case of mixed cattle-goat herds, milk must be tested for the presence of TB due to its zoonotic potential (6). The Murciano-Granadina goat breed is one of the most important dairy goat varieties in the Murcia region and its milk is primarily used for cheese production. Moreover, the region contains 14.0% of Spain's national goat population (391,000 heads) (7), which cohabit in traditional systems of meat and meatmilk production with intensive milk production systems (10). Despite the recent increase in permanent confined production system herds in southern Spain (10), the goatgrazing system is also used in Murcia as an alternative way of reducing production costs and preserving the environment. However, grazing is associated with a higher risk of parasitic infections, such as gastrointestinal helminths, that usually result in lower productivity due to stunted growth, poor weight gain and low feed conversion (11). Most economic losses are therefore associated with gastrointestinal parasitic infections, although lungworms can also have a serious impact on small ruminant productivity in many temperate areas (11, 12). Therefore, the existence of co-infected animals with Mycobacterium spp. and endoparasites might result in severe economic losses in affected goat herds.

There is a growing interest in the scientific community in co-infections, not only because most of the hosts in question are usually infected with multiple invasive pathogen species, but also because immunological host mechanisms are derived from macroparasite-bacterial interactions that have previously been documented (13). As such, the mammalian adaptive immune response mobilises distinct pathways to control intracellular pathogens (e.g. viruses and most bacteria) versus extracellular macroparasites (e.g. helminths). Thus, the immune response to intracellular parasites involves T helper 1 (Th1) cells, while immune defences against extracellular parasites are primarily mediated by T helper 2 (Th2) cells. These two T-cell-dependent pathways are cross-regulated by the cytokines and chemokines produced by each T helper cell type - those produced by Th1 cells suppressing Th2 cell immune functions and vice versa (14, 15, 16, 17). Therefore, laboratory-based studies have shown that it may be difficult for a host to mount effective Th1 and Th2 immune responses simultaneously (17).

The aim of this study was to evaluate the prevalence, intensity and abundance of infection of different endoparasites in natural infected goats from the Murcia region. Very little information on parasites and *Mycobacterium* spp. coinfection in goats in Mediterranean countries, including Spain, is yet available. Identifying co-infected animals and focusing disease control efforts on co-infected herds with cTB and parasites may help to improve goat husbandry systems in Spain and to decrease the spread of diseases.

# Materials and methods

### Animals and area of study

The geographical area studied has a hot sub-tropical semi-arid climate with Mediterranean influences. It has mild winters and hot summers with an average annual precipitation of less than 300 mm and an average annual temperature of 18°C (18). The Murcia region contains six livestock regions: Altiplano, Valle de Guadalentin, Campo de Cartagena, Río Mula, North-East and Vega del Segura. Sampled goat herds were selected from these different regions according to their participation in the whole goat production process in the region. The examined animals had not been treated with any anthelmintic drugs for at least ten weeks prior to examination. Furthermore, 15 of the 84 goats were diagnosed as being infected with cTB based on the single intradermal comparative cervical tuberculin test (SICCT) and this was confirmed at the local slaughterhouse, where active pulmonary cTB was found.

Carcasses from a total of 84 Murciano–Granadina goats were recovered from one of the region's slaughterhouses located in Cartagena. All animals were: less than four years of age; female; and raised in a semi-intensive rearing system. Each carcasse was carefully identified and stored in plastic bags, submitted to the Laboratory of Parasitology and Parasitic Diseases of the University of Murcia, and frozen at –20°C for further analysis.

### Laboratory procedures

The faeces, heads and viscera of all goats were examined and processed in order to collect and identify parasites. Faeces (20 g) recovered from the rectum were firstly analysed using a qualitative concentration flotation technique with Sheather's solution as the flotation fluid  $(\beta=1.27)$  to detect helminth eggs and protozoan/coccidian oocysts and/or cysts. Positive samples were quantitatively analysed and eggs per gram (EPG) and oocysts per gram (OPG) of faeces were calculated using the McMaster technique. In addition, the concentration by sedimentation technique was performed to identify trematode eggs. Lastly, in order to identify lungworm larval stages, 5 g of faeces were processed using the Baermann's method for active larval migration. All coprological analyses were performed according to the United Kingdom Ministry of Agriculture, Fisheries and Food's Manual of Veterinary Parasitological Laboratory Techniques (19).

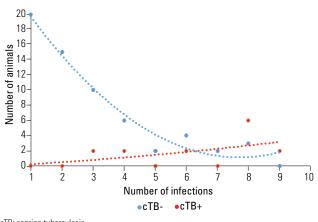
Heads were evaluated following removal through a midsagittal cut. Frontal and horn sinuses were examined for dipterous larvae, as were the mouth and palatal regions. Collected larvae (maggots) were placed in a tube with 70.0% ethanol and stored until further morphological identification could take place. The gastrointestinal tract was removed and processed separately (abomasum, and small and large intestines). After a longitudinal cut, mucosae were scraped and carefully examined for large parasites. The content of the digestive tract was washed and sieved through a series of mesh screens (final mesh pore size: 0.3 mm). Nematodes were separated by taxa and stored in 70.0% ethanol; for species identification, they were fixed and cleared with lactophenol.

The liver was also examined for hepatic parasitism. Specifically, bile ducts were cut open, and parenchyma was longitudinally sliced and examined for the presence of flukes. Additionally, gall bladders were removed, cut open and their contents examined under a stereomicroscope. Each fluke found was recovered, fixed in 10.0% ethanol and, subsequently, stained with Semichon's carmine for further morphological identification.

Finally, the lungs were carefully examined for lesions compatible with bronchopulmonary lungworms. Trachea and bronchi were examined, and all nematodes collected were fixed with lactophenol for further identification.

### **Epidemiological parameters**

Prevalence, intensity and abundance of infection for each parasitic species were defined according to Margolis *et al.* (20) and Bush *et al.* (21). Briefly, prevalence is defined as the number of hosts infected with a particular parasitic species (or taxonomic group) within the number of hosts examined, expressed as a percentage; intensity is defined as the number of individuals of a particular parasitic species in a single infected host, expressed as the number of specimens per infected animal (spia); and abundance is defined as the



cTB: caprine tuberculosis

### Fig. 1

Comparison of the intensity of infection for caprine tuberculosispositive and -negative animals, where 'number of infections' refers to the number of different parasites infecting the host (p < 0.05) number of individuals of a particular parasitic species per host examined. In addition, the sensitivity (proportion of true positives divided by the sum of true positives and false negatives) of the different coprological techniques was calculated against the necropsy findings, which were treated as the gold standard. Statistical analysis was performed using Microsoft<sup>®</sup> Excel 2010 and Graphpad (22). A Mann– Whitney test was used to compare the values between the groups. Differences were considered statistically significant at the level of p < 0.05.

### Results

From the 84 studied animals, 84 heads, 44 respiratory tracts, 84 livers and 83 gastrointestinal tracts were analysed. The majority of the animals (94.0%, n = 79) harboured parasites in, at least, one location. All cTB-positive goats (n = 15) and 92.8% (n = 64) of cTB-negative goats were parasitised. In addition, the comparison of the intensity of infection in cTB infected and non-infected animals was calculated and showed a negative correlation ( $\mathbf{g} = -0.193$ ) (Fig. 1).

Coprological analyses showed a high overall prevalence of parasite excretion of 86.9% (n = 73). Oocysts of protozoan infections, *Eimeria* spp., were identified in the majority of the samples (84.5%; n = 71), while undifferentiated strongyle-type eggs were found in 31.0% (n = 27) of samples: 7.1% (n = 6) contained *Trichuris* spp. eggs, 5.9% (n = 5) contained *Dicrocoelium dendriticum* eggs, and 2.4% (n = 2) contained *Nematodirus* spp. eggs. Finally, three lungworm larval species were also found using the modified Baermann method: *Muellerius capillaris* (9.5%; n = 8), *Dictyocaulus filaria* (4.7%; n = 4) and *Cystocaulus ocreatus* (1.2%; n = 1) (Table I).

In the coprological examination, significant differences between the prevalence of all gastrointestinal nematode eggs and *Eimeria* spp. infections were found between cTB-positive and cTB-negative animals. However, no significant differences were obtained between cTB-positive and cTB-negative animals for the excretion of *D. dendriticum* eggs and lungworm larvae (Table I). Moreover, no statistical differences were observed when the variable 'geographical region' was evaluated for coprological findings.

Necropsy showed that few animals (n=8) presented *Oestrus ovis* infestation, with an overall mean prevalence of 9.5%. This value was similar in cTB-negative goats (10.1%; n=7) and in cTB-positive ones (6.7%; n=1), and no significant differences were found between these two groups (Table II).

The mean prevalence of hepatic infections with *D. dendriticum* was 20.2% (n = 17), and both cTB-negative (18.8%; n = 13) and cTB-positive (26.7%; n = 5) dairy goats showed similar prevalence values. However, contrarily, neither

EPG/OPG	Prev. (%)	Intensity range	Median	CI		itive			CI		ative				
				95 –	95 +	Prev. (%) cTB-positive	Intensity range	Median	95 –	95 +	Prev. (%) cTB-negative	Intensity range	Median	95 —	95 +
<i>Eimeria</i> spp.	84.5	20–98,000	1,730	3,985	10,017	100	20-10,560	1,080	589.5	4,349	81.2	20-98,000	1,880	3,985	10,017
<i>Strongyle</i> eggs	31.0	20-12,400	0	-19.6	658.1	80	20–220	60	37.3	125.3	20.3	20-12,400	0	-19.6	685
<i>Nematodirus</i> spp. eggs	2.4	20–400	0	-4.6	14.8	6.7	20	0	-1.5	4.2	1.4	400	0	-4.5	14.83
<i>Trichuris</i> spp. eggs	7.1	20–60	0	0.2	4.1	26.7	20	0	0.3	10.4	2.9	40–60	0	0.24	4.15
<i>Dicrocoelium dendriticum</i> eggs	5.9	20—40	0	0.1	2.8	13.3	20—40	0	-0.1	1.9	4.3	20	0	0	0.09
<i>Cystocaulus ocreatus</i> larvae	1.2	11–68	-0.3	3.8	0	0	0	0	0	5.8	11–68	0	-0.3	-0.284	3.76
<i>Muellerius capillaris</i> larvae	9.5	2–57	-0.2	3.1	6.7	2	0	-0.2	0.4	5.8	8–57	0	-0.2	-0.17	3.14
<i>Dictyocaulus filaria</i> larvae	4.7	4	-0.1	0.1	6.7	4	0	-0.3	0.8	0	0	0	0	-0.04	0.14

Table I Faecal findings and comparison between results in caprine tuberculosis negative and positive goats

cTB: caprine tuberculosis

OPG: oocysts per gram

*Fasciola hepatica* eggs nor adult flukes were recovered during the study (Table II).

Only 23.8% (n=20) of the analysed goats were free of nematodes in all gastrointestinal sections. Almost half of the examined abomasums (45.0%; n = 40) and small intestines (48.0%; n = 39) were parasitised, while only a third of the large intestines (37.0%; n=31) harboured nematodes. The total number of nematodes recovered in this study from the gastrointestinal tract was 18,963 specimens, 40.8% males (n=7,741) and 59.2% females (n=11,222)(sex ratio = 1.45). Eighteen species of nematodes were collected from these sections, and 64.4% of the parasitised animals showed multiple infections, hosting 3 to 11 nematode species. All of the helminth species that were identified are referred to in Table II. Teladorsagia circumcincta was the most prevalent gastrointestinal species (63.1%; n = 53), followed by Trichostrongylus vitrinus (23.8%; n=20), T. trifurcata (15.5%; n=13) and T. colubriformis (15.5%; n = 13). Intensity ranged from 1–1,300 spia for T. circumcincta, to 1–60 spia for T. vitrinus, 3–150 spia for T. trifurcata and 1–80 spia for T. colubriformis.

When prevalence and abundance of gastrointestinal nematodes (Figs 2 and 3) between cTB-positive and

cTB-negative goats were compared, significantly higher values for both variables (p < 0.05) were observed for cTB-positive animals. Moreover, *Camelostrongylus mentulatus, T. capricola* and *Chabertia ovina* were present exclusively in cTB-positive animals. The intensity found for those nematodes was 3 spia for *C. mentulatus,* 1–60 spia for *T. capricola* and 2–20 spia for *C. ovina*, respectively (see Table II).

Finally, during lung necropsy, three different lungworm species were isolated: *D. filaria*, *M. capillaris* and *Neostrongylus linearis*, each showing the same overall prevalence (2.4%, n = 2). Intensity ranged from 4–9 spia for *N. linearis*, to 10–25 spia for *M. capillaris*, and 3–26 spia for *D. filaria*. All three lungworms were always found in cTB-positive animals (Table II). Statistical analysis showed significantly higher differences for lungworm prevalence in cTB-positive animals (p < 0.05).

Moreover, when all parasites in post-mortem findings were compared, applying the 'region of origin' variable, no significant differences were found.

The sensitivity of coprological results compared with post-mortem findings was also calculated. The flotation concentration procedure showed 92.0% sensitivity; specifically,

#### Table II

Post-mortem species found and comparison between caprine tuberculosis positive and negative animals

				CI		ats			CI		oats			CI	
Parasite species	Prev. (%)	Intensity range	Median	95 —	95 +	Prev. (%) positive cTB goats	Intensity range	Median	95 —	95 +	Prev. (%) negative cTB goats	Intensity range	Median	95 —	95 +
Oestrus ovis	9.5	_	_	-	_	6.7	-	_	-	-	10.1	-	-	_	_
Dicrocoelium dendriticum	20.2	_	_	_	_	26.7	-	_	_	_	18.8	-	_	_	_
Muellerius capillaris	2.4	10–25	0	-0.22	1.5	13.3	10–25	0	-1.5	6.5	0	0	0	0	0
Dictyocaulus filaria	2.4	3–26	0	-0.27	0.96	13.3	3–26	0	-1.9	6.1	0	0	0	0	0
Neostrongylus linearis	2.4	4—9	0	-0.08	0.39	13.3	4—9	0	-0.5	2.4	0	0	0	0	0
Teladorsagia circumcincta	63.1	1-1,300	0	27.8	113	87.0	3–173	50	22.3	122.3	28.9	1-1,300	0	20.3	123.5
Teladorsagia trifurcata	15.5	3-150	0	0.3	8.2	26.7	3–7	0	-0.1	3.4	13.0	3–150	0	0.1	9.8
Marshallagia marshalli	5.9	3-170	0	-1.6	6.4	20.0	3–14	0	-0.6	2.6	2.9	7-170	0	-2.4	7.5
Ostertagia occidentalis	1.2	30	0	-0.4	1.1	0	0	0	0	0	1.4	30	0	-0.4	1.3
Camelostrongylus mentulatus	1.2	3	0	-0.03	0.1	6.7	3	0	-0.4	1.1	0	0	0	0	0
Trichostrongylus capricola	7.1	1-60	0	0.2	4.9	40.0	1–60	13	-2.6	26.1	0	0	0	0	0
Nematodirus spathiger	9.5	3–20	0	0.2	1.7	13.3	3–20	3	-2.2	6.8	8.7	3–13	0	0.1	1.5
Nematodirus filicollis	9.5	1-20	0	0.1	1.0	26.7	1-20	0	-1.8	7.1	5.8	3–7	0	-0.1	0.5
Trichostrongylus vitrinus	23.8	1-60	0	1.8	1.5	66.7	1–60	13	-2.8	50.5	14.5	10–203	0	-0.5	11.8
Trichostrongylus colubriformis	15.5	1—80	0	0.2	4.9	53.3	1—80	3	-3.2	34.3	7.2	3–37	0	-0.3	1.8
Nematodirus abnormalis	15.5	3-120	0	-1.1	4.7	6.7	3	0	-0.4	1.1	11.2	3–120	0	-1.3	5.6
<i>Trichuris</i> spp.	11.9	1-120	0	0.8	2.6	33.3	1-120	0	-1.4	5.0	7.2	0	1–30	-0.1	0.3
Chabertia ovina	1.2	2–20	0	-0.2	0.7	6.7	9—590	0	-1.5	4.2	0	0	0	0	0
Skrjabinema ovis	10.7	9—590	0	1.7	35.0	26.6	15–150	0	-23.6	152.3	7.2	0	15–203	-0.7	17.4

CI: confidence interval

cTB: caprine tuberculosis

Prev.: prevalence

90.0% for strongyle eggs, 75.0% for *Trichuris* spp. eggs and 95.1% for *Nematodirus* spp. eggs. Furthermore, the sedimentation technique for the concentration of *D. dendriticum* eggs in faeces showed 66.7% sensitivity. Additionally, the sensitivity of the Baermann technique was 100.0% for *M. capilaria*, 75.0% for *D. filaria* and 50.0% for *N. linearis*.

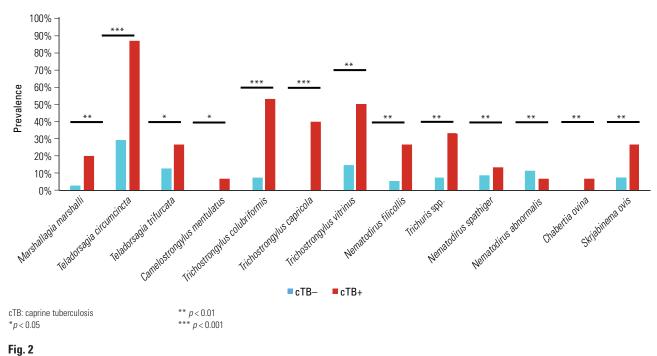
## Discussion

Gaining a better understanding of the development of naturally occurring parasitism in goats and its interaction, if any, with bacterial pathogens, such as *Mycobacterium* spp., is increasingly important when aiming to define patterns of disease spread. The absence of previous data on goat parasitic diseases and simultaneous cTB infection in Mediterranean countries increases the importance of this study even more.

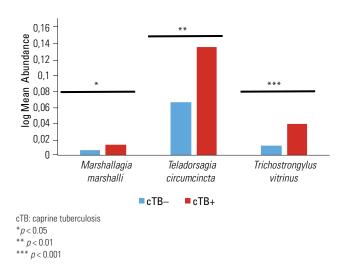
The authors' results suggest that the parasite fauna of the goats from the Murcia region are similar to those described previously in the Iberian Peninsula for other closely related ruminant species (23, 24, 25, 26, 27).

In this study, the prevalence of *Eimeria* spp. infections (84.5%) was similar to that previously recorded in goats worldwide (28, 29) and in adult sheep herds from the Murcia region (30).

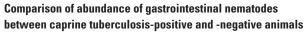
Furthermore, in the studied area the climatic conditions for dipteran life-cycle completion are optimal. However, the



Comparison between prevalence of gastrointestinal nematode species in caprine tuberculosis-positive and -negative animals



#### Fig. 3



authors' results showed a low prevalence of the dipteran parasite (9.5%) (Table II), which is in contrast with the rather high prevalence of *O. ovis* previously reported in caprine flocks in the south of France (43.4%) (31) and Greece (17.9%) (32). Moreover, higher *O. ovis* prevalence was also reported in Murcia's ovine livestock (38.1%) (25) and in the ovine population reared in other Mediterranean regions such as Sardinia (91.0%) (33), Turkey (40.3%) (34) and Sicily (55.8%) (35). It is worth noting that these

previous data were mostly recovered from sheep flocks, where prevalence is usually higher than in goat herds (31, 32, 36).

The prevalence of *D. dendriticum* infection (20.2%) (Table II) was similar to that of previous studies on ovine flocks in Murcia (26) as well as cattle herds in Spain (23, 37). *Dicrocoelium dendriticum* is known to be associated with a rather dry and hot climate, showing a higher prevalence in arid rather than humid regions, with such dry conditions being more favourable for the intermediate hosts' development (38, 39, 40). Therefore, the absence of *E hepatica* from this study is remarkable since it uses the amphibian snail as its intermediate host, and thus these conditions are also favourable for the fluke's survival.

All gastrointestinal nematode species described in this study have been previously reported in goats from Spain (41, 42). In these previous studies, *Teladorsagia circumcincta* (63.1%) was the most prevalent nematode parasitising the goat abomasum, followed by *T. trifurcata* (15.5%) and *M. marshalli* (5.9%), while other species were less prevalent, as reported both in other European studies on goats (43, 44) and in studies carried out on caprine herds in southern Spain (42). As observed in the abomasum, nematode prevalence appeared quite grouped in the small intestine; *T. vitrinus* (23.8%) was the most common nematode, followed by *T. capricola* (7.1%). Finally, *Trichuris* spp. (11.9%) and *S. ovis* (10.7%) showed similar

prevalences in the large intestine as documented for other small ruminants elsewhere (27).

Regarding respiratory nematode infections, three lungworm species were identified during necropsy: *D. filaria, M. capillaris* and *N. linearis*. All of them had previously been reported in wild ruminants in the Iberian Peninsula (45). In the current study, these species' prevalences were lower than those reported previously in caprine flocks (46, 47, 48); this may be due to the dryness of the study area since infection is more probable in areas with moist soils (49). Using the Baermann method, *D. filaria, M. capillaris* and *C. ocreatus* larvae were also found here, confirming the contamination of goat pastures.

Additionally, both necropsy and faecal flotation examination were performed on all dairy goats, and the sensitivity of coprological analysis was calculated based on the positive findings, showing a discrepancy between coprological and post-mortem section results. Consequently, the authors' results confirm that the coprological technique could underestimate the real prevalence and intensity of parasitic infections. Evidence for the lower sensitivity of coprology in estimating the real prevalence of gastrointestinal parasitism in cross-sectional studies has been previously documented (50, 51). For instance, during pre-patent or post-patent periods, it is extremely difficult to identify nematode infections by coprological methods and, when possible, a larger sample size, more precise diagnostic tools (52), and repeated sampling of the same individuals (53) should be used.

Unfortunately, very little is known regarding the coinfection of cTB and parasites in goats, but interactions between parasites and other bacterial pathogens have been reported to occur in other ruminant species (15), including an association between TB positive animals and helminth infections (54, 55, 56). In this regard, goats appear to show a nematode-induced immune suppression that seems to compromise host immune resistance against TB (56), facilitating the host invasion of *Mycobacterium* spp. as previously postulated (57). To prove this, more detailed studies would be necessary that focus on the cellular immune mechanisms of co-infection between parasites and cTB-positive goats in Spain and other Mediterranean countries, as well as on the role of co-infected animals in the dispersion of these pathogens. However, the authors' results show that the association between parasites and TB in goats could play an important epidemiological role, and the design of control measures for lowering the number of infections through these grazing-transmitted parasites, may diminish the probability of being infected by other bacterial infections such as cTB.

# Conclusions

To conclude, the results of the current study reveal the following associations between cTB-positive and cTB-negative animals:

– determined parasites are only present in cTB-positive animals (i.e. *M. capillaris*, *D. filaria*, *N. linearis*, *C. mentulatus*, *T. capricola* and *C. ovina*);

higher prevalences exist for co-infected cTB goats with certain nematode species (i.e. *M. marshalli*, *T. circumcincta*, *T. trifurcate*, *C. mentulatus*, *T. colubriformis*, *T. capricola*, *T. vitrinus*, *N. filicollis*, *Trichuris* spp., *N. spathiger*, *N. abnormalis* and *S. ovis*);

– a higher occurrence of infection (*M. marshalli*, *T. circumcincta* and *T. vitrinus*) exists in cTB-positive animals. In contrast, the authors' findings for trematode (*D. dendriticum*), arthropod (*O. ovis*) and protozoan (*Eimeria* spp.) infections showed no statistical associations between co-infection of cTB and these parasites.

Further investigation and more detailed information on the spectrum of pro-inflammatory and immunoregulatory cytokines/chemokines of caprine T cells in co-infected animals are needed in order to confirm the results of the current study.

### Acknowledgements

The authors would like to acknowledge the slaughterhouse in Cartagena for providing all the goat samples used in this study, and also the students of the Department of Animal Health, University of Murcia, for their assistance in processing these samples.

### Prévalence des parasites chez des chèvres infectées par *Mycobacterium* spp. dans la région de Murcia (sud-est de l'Espagne)

T. Carrau, M.M. Garijo, C. Martínez-Carrasco, D. Pérez, L.M.R. Silva, A. Taubert, C. Hermosilla & R. Ruiz de Ybáñez

### Résumé

Une étude a été conduite sur 84 chèvres laitières de race Murciano-Granadina abattues dans la région de Murcia (sud-est de l'Espagne) afin d'évaluer la présence d'infections parasitaires. Dans leur grande majorité (94,0 %), les chèvres étaient infestées de parasites et le nombre de pluri-infections était important. Au total, 21 espèces de parasites ont été observées, dont 18 espèces de nématodes (Muellerius capillaris, Dictyocaulus filaria, Neostrongylus linearis, Cystocaulus ocreatus, Teladorsagia circumcincta, T. occidentalis, T. trifurcata, Marshallagia marshalli, Camelostrongylus mentulatus, Trichostrongylus capricola, Nematodirus abnormalis, N. filicollis, N. spathiger, T. vitrinus, T. colubriformis, Trichuris spp., Chabertia ovina et Skriabinema ovis), une espèce de trématode (*Dicrocoelium dendriticum*), une espèce d'arthropode (*Oestrus ovis*) et des membres d'un genre de protozoaire (*Eimeria* spp.). En outre, la présence de Mycobacterium spp. a été détectée chez 17,85 % des chèvres examinées. Par conséquent, une étude comparative a été réalisée pour déterminer la prévalence, l'intensité et l'abondance des parasites chez les animaux ayant donné des résultats respectivement positifs et négatifs à la détection de la tuberculose. Des différences significatives au plan statistique ont été constatées entre les chèvres infectées et indemnes de Mycobacterium spp., respectivement, pour ce qui concerne la prévalence des vers pulmonaires et des nématodes gastrointestinaux. Les auteurs font le point sur cette co-infection par des mycobactéries et des endoparasites.

### Mots-clés

Chèvre – Chèvres laitières – Co-infection – Endoparasite – *Mycobacterium* spp. – Nématode gastro-intestinal – Prévalence – Tuberculose.

Prevalencia de parásitos en cabras lecheras infectadas por *Mycobacterium* spp. de la región de Murcia (sudeste de España)

T. Carrau, M.M. Garijo, C. Martínez-Carrasco, D. Pérez, L.M.R. Silva, A. Taubert, C. Hermosilla & R. Ruiz de Ybáñez

### Resumen

Tras ser sacrificadas, ochenta y cuatro cabras lecheras murciano–granadinas de la región de Murcia (sudeste de España) fueron sometidas a necropsia para determinar la presencia de eventuales parasitosis. La mayoría de los animales (un 94,0%) presentaban parásitos y múltiples infecciones. Se descubrieron veintiuna especies de parásito: 18 especies de nemátodo (*Muellerius capillaris, Dictyocaulus*)

filaria, Neostrongylus linearis, Cystocaulus ocreatus, Teladorsagia circumcincta, T. occidentalis, T. trifurcata, Marshallagia marshalli, Camelostrongylus mentulatus, Trichostrongylus capricola, Nematodirus abnormalis, N. filicollis, N. spathiger, T. vitrinus, T. colubriformis, Trichuris spp., Chabertia ovina y Skrjabinema ovis); una especie de tremátodo (Dicrocoelium dendriticum); una especie de artrópodo (Oestrus ovis) y un género de protozoo (Eimeria spp.). Además, el 17,85% de los ejemplares eran positivos para Mycobacterium spp. A partir de ahí se procedió a comparar la prevalencia, intensidad y abundancia de parásitos en los animales positivos para la tuberculosis con respecto a los negativos. Se observaron diferencias estadísticamente significativas entre las cabras que estaban infectadas por Mycobacterium spp. y las que no lo estaban en cuanto a la prevalencia de nemátodos pulmonares y gastrointestinales. Los autores examinan esta infección concomitante entre micobacteriáceas y endoparásitos.

#### **Palabras clave**

Cabra – Cabra lechera – Endoparásito – Infección concomitante – *Mycobacterium* spp. – Nemátodos gastrointestinales – Prevalencia – Tuberculosis.

# References

- Álvarez J., de Juan L., Bezos J., Romero B., Sáez J.L., Reviriego Gordejo F.J., Briones V., Moreno M.Á., Mateos A., Domínguez L. & Aranaz A. (2008). – Interference of paratuberculosis with the diagnosis of tuberculosis in a goat flock with a natural mixed infection. *Vet. Microbiol.*, **128** (1–2), 72–80. doi:10.1016/j.vetmic.2007.08.034.
- Sharpe A.E., Brady C.P., Johnson A.J., Byrne W., Kenny K. & Costello E. (2010). – Concurrent outbreak of tuberculosis and caseous lymphadenitis in a goat herd. *Vet. Rec.*, 166 (19), 591–592. doi:10.1136/vr.b4825.
- Bezos J., Álvarez J., Romero B., de Juan L. & Domínguez L. (2014). – Bovine tuberculosis: historical perspective. *Res. Vet. Sci.*, 97 (Suppl.), S3–S4. doi:10.1016/j.rvsc.2014.09.003.
- Quintas H., Reis J., Pires I. & Alegria N. (2010). Tuberculosis in goats. Vet. Rec., 166 (14), 437–438. doi:10.1136/vr.c1678.
- Shanahan A., Good M., Duignan A., Curtin T. & More S.J. (2011). – Tuberculosis in goats on a farm in Ireland: epidemiological investigation and control. *Vet. Rec.*, 168 (18), 485. doi:10.1136/vr.c6880.
- 6. Pesciaroli M., Alvarez J., Boniotti M.B., Cagiola M., Di Marco V., Marianelli C., Pacciarini M. & Pasquali P. (2014).
  – Tuberculosis in domestic animal species. *Res. Vet. Sci.*, 97 (Suppl.), S78-S85. doi:10.1016/j.rvsc.2014.05.015.
- Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente (MAPAMA) (2014). – Anuario de Estadística. MAPAMA, Madrid. Available at: www.magrama.gob.es/ es/estadistica/temas/publicaciones/anuario-de-estadistica/ (accessed on 18 July 2017).

- European Commission (EC) (2014). Bovine and swine diseases: 2014 annual report. Available at: https://ec.europa. eu/food/sites/food/files/animals/docs/la\_bovine\_final\_ report\_2014.pdf (accessed on 18 July 2017).
- European Commission (EC) (2004). Commission Regulation of 30 April 2004 concerning laying down specific hygiene rules on the hygiene of foodstuffs, Regulation (EC) No. 853/2204. Off. J. Eur. Union, L 139, 151 pp. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ. do?uri=OJ:L:2004:139:0055:0205:en:PDF (accessed on 13 November 2017).
- Castel J.M., Ruiz F.A., Mena Y. & Sánchez-Rodríguez M. (2010). – Present situation and future perspectives for goat production systems in Spain. *Small Rum. Res.*, **89** (2–3), 207–210. doi:10.1016/j.smallrumres.2009.12.045.
- Coop R.L. & Holmes P.H. (1996). Nutrition and parasite interaction. Int. J. Parasitol., 26 (8–9), 951–962. doi:10.1016/ S0020-7519(96)80070-1.
- Parkins J.J. & Holmes P.H. (1989). Effects of gastrointestinal helminth parasites on ruminant nutrition. *Nutr. Res. Rev.*, 2 (1), 227–246. doi:10.1079/NRR19890016.
- Fenton A. (2008). Worms and germs: the population dynamic consequences of microparasite-macroparasite co-infection. *Parasitology*, **135** (13), 1545–1560. doi:10.1017/ S003118200700025X.
- 14. Yazdanbakhsh M., Kremsner P.G. & van Ree R. (2002).
   Allergy, parasites, and the hygiene hypothesis. *Science*, 296 (5567), 490–494. doi:10.1126/science.296.5567.490.

- 15. Jolles A.E., Ezenwa V.O., Etienne R.S., Turner W.C. & Olff H. (2008). Interactions between macroparasites and microparasites drive infection patterns in free-ranging African buffalo. *Ecology*, **89** (8), 2239–2250. Available at: http://wendyturner.org/pdf/Jolles\_2008.pdf (accessed on 18 July 2017).
- Abbas A.K., Murphy K.M. & Sher A. (1996). Functional diversity of helper T lymphocytes. *Nature*, **383** (6603), 787– 793. doi:10.1038/383787a0.
- Morel P.A. & Oriss T.B. (1998). Cross regulation between Th1 and Th2 cells. *Crit. Rev. Immunol.*, 18 (4), 275–303. doi:10.1615/CritRevImmunol.v18.i4.10.
- Agencia Estatal de Meteorología (AEMET) (2010). Datos climatológicos. AEMET, Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente, Madrid. Available at: www. aemet.es/es/serviciosclimaticos/datosclimatologicos (accessed on 18 July 2017).
- United Kingdom Ministry of Agriculture, Fisheries and Food (1986). – Manual of veterinary parasitological laboratory techniques. Her Majesty's Stationery Office, London, 7–15.
- Margolis L., Esch G.W., Holmes J.C., Kuris A.M. & Schad G.A. (1982). – The use of ecological terms in parasitology (report of an *ad hoc* committee of the American Society of Parasitologists). *J. Parasitol.*, 68 (1), 131–133. doi:10.2307/3281335.
- Bush A.O., Lafferty K.D., Lotz J.M. & Shostak A.W. (1997).
   Parasitology meets ecology on its own terms: Margolis *et al.* revisited. J. Parasitol., 83 (4), 575–583. doi:10.2307/3284227.
- 22. GraphPad Software (2007). Version 5.00 for Windows. GraphPad Software, San Diego, California. Available at: www. graphpad.com (accessed on 26 October 2017).
- González-Lanza C., Manga-González M.Y. & Del-Pozo-Carnero P. (1993). – Coprological study of the Dicrocoelium dendriticum (Digenea) egg elimination by cattle in highland areas in León Province, Northwest Spain. Parasitol. Res., 79 (6), 488–491. doi:10.1007/BF00931589.
- Meana A., Luzón-Peña M., Santiago-Moreno J., De Bulnes A. & Gómez-Bautista M. (1996). – Natural infection by gastrointestinal and bronchopulmonary nematodes in mouflons (*Ovis musimon*) and their response to netobimin treatment. J. Wildl. Dis., **32** (1), 39–43. doi:10.7589/0090-3558-32.1.39.
- 25. Garijo M.M., Alonso F.D., Martínez-Carrasco C. & Ruiz de Ybáñez M.R. (2004). Presencia de Oestrus ovis (Linnaeus 1761) en el ganado ovino de la región de Murcia (sureste de España). Rev. Iber. Parasitol., 65 (1–4), 3–9. Available at: http://bibliotecavirtual.ranf.com/i18n/catalogo\_imagenes/grupo.cmd?path=1001720 (accessed on 18 July 2017).

- 26. Garijo M.M., Alonso F.D., Martínez-Carrasco C. & Ruiz de Ibáñez M.R. (2005). – Trematodosis y cestodosis en el ganado ovino de la región de Murcia (sureste de España). *Rev. Iber. Parasitol.*, **65** (1–4), 55–61. Available at: http:// bibliotecavirtual.ranf.com/i18n/catalogo\_imagenes/grupo. cmd?path=1001727 (accessed on 18 July 2017).
- 27. Mayo E., Ortiz J., Martínez-Carrasco C., Garijo M.M., Espeso G., Hervías S. & Ruiz de Ybáñez M.R. (2013). – First description of gastrointestinal nematodes of Barbary sheep (*Ammotragus lervia*): the case of *Camelostrongylus mentulatus* as a paradigm of phylogenic and specific relationship between the parasite and its ancient host. *Vet. Res. Commun.*, **37** (3), 209–215. doi:10.1007/s11259-013-9563-0.
- Norton C.C. (1986). Coccidia of the domestic goat *Capra hircus*, with notes on *Eimeria ovinoidalis* and *E. bakuensis* (syn. *E. ovina*) from the sheep Ovis aries. Parasitology, 92 (2), 279–289. doi:10.1017/S0031182000064052.
- De la Fuente C. & Alunda J.M. (1992). A quantitative study of *Eimeria* infections of goats from central Spain. *Vet. Parasitol.*, 41 (1–2), 7–15. doi:10.1016/0304-4017(92)90003-r.
- 30. Carrau T., Pérez D., Silva L.M., Macías J., Martínez-Carrasco C., Taubert A., Hermosilla C. & Ruiz de Ybáñez R. (2016).
  Postparturient rise in the excretion of *Eimeria* spp. in Manchega dairy sheep. J. Vet. Med. Res., 3 (2), 1047, 6 pp. Available at: https://www.researchgate.net/profile/Carlos\_ Hermosilla/publication/304624886\_Postparturient\_Rise\_in\_ the\_Excretion\_of\_Eimeria\_Spp\_in\_Manchega\_Dairy\_Sheep/ links/5776931e08aead7ba071ce07/Postparturient-Rise-inthe-Excretion-of-Eimeria-Spp-in-Manchega-Dairy-Sheep. pdf?origin=publication\_detail (accessed on 18 July 2017).
- Dorchies Ph., Bergeaud J.P., Tabouret G., Duranton C., Prevot F. & Jacquiet Ph. (2000). – Prevalence and larval burden of *Oestrus ovis* (Linné 1761) in sheep and goats in northern Mediterranean region of France. *Vet. Parasitol.*, 88 (3–4), 269–273. doi:10.1016/S0304-4017(99)00215-0.
- Papadopoulos E., Prevot F., Diakou A. & Dorchies P. (2006).
   Comparison of infection rates of *Oestrus ovis* between sheep and goats kept in mixed flocks. *Vet. Parasitol.*, **138** (3–4), 382– 385. doi:10.1016/j.vetpar.2006.02.023.
- 33. Scala A., Solinas G., Citterio C.V., Kramer L.H. & Genchi C. (2001). – Sheep oestrosis (*Oestrus ovis* Linné 1761, Diptera: Oestridae) in Sardinia, Italy. *Vet. Parasitol.*, **102** (1–2), 133–141. doi:10.1016/S0304-4017(01)00515-5.
- Arslan M.O., Kara M. & Gicik Y. (2009). Epidemiology of Oestrus ovis infestations in sheep in Kars province of northeastern Turkey. Trop. Anim. Hlth Prod., 41 (3), 299–305. doi:10.1007/s11250-008-9190-x.
- Caracappa S., Rilli S., Zanghi P., Di Marco V. & Dorchies P. (2000). – Epidemiology of ovine oestrosis (*Œstrus ovis* Linné 1761, Diptera: Oestridæ) in Sicily. *Vet. Parasitol.*, **92** (3), 233– 237. doi:10.1016/S0304-4017(00)00317-4.

- Duranton C. & Dorchies P.H. (1997). In vitro culture of Oestrus ovis (Linné 1761) first instar larvae: its application to antiparasitic drug screening. Int. J. Parasitol., 27 (1), 125–128. doi:10.1016/S0020-7519(96)00166-X.
- 37. Reina D., Navarrete I., Hernández S. & Habela M. (1987). Contribución al conocimiento de la parasitofauna de Cáceres. Primera relación. II. Helmintos. *Rev. Iber. Parasitol.*, Volumen Extraordinario, 85–90. Available at: www.researchgate.net/ publication/312950581\_Contribucion\_al\_conocimiento\_de\_ la\_parasitofauna\_de\_Caceres\_Primera\_relacion\_II\_Helmintos (accessed on 18 July 2017).
- Chartier C. & Reche B. (1992). Gastrointestinal helminths and lungworms of French dairy goats: prevalence and geographical distribution in Poitou-Charentes. *Vet. Res. Commun.*, 16 (5), 327–335. doi:10.1007/BF01839182.
- Manga-González M.Y., González-Lanza C., Cabanas E. & Campo R. (2001). – Contributions to and review of dicrocoeliosis, with special reference to the intermediate hosts of *Dicrocoelium dendriticum*. *Parasitology*, **123** (7), 91–114. doi:10.1017/S0031182001008204.
- Otranto D. & Traversa D. (2003). Dicrocoeliosis of ruminants: a little known fluke disease. *Trends Parasitol.*, 19 (1), 12–15. doi:10.1016/S1471-4922(02)00009-0.
- Cordero del Campillo M., Castañón Ordóñez L. & Reguera Feo A. (1994). – Index-catalogue of Iberian parasites of animals, 2nd Ed. Universidad de León, Secretariado de Publicaciones, León, Spain, 650 pp.
- 42. Valcárcel F. & Romero C.G. (1999). Prevalence and seasonal pattern of caprine trichostrongyles in a dry area of central Spain. J. Vet. Med. B, Infect. Dis. Vet Public Hlth, 46 (10), 673– 681. doi:10.1046/j.1439-0450.1999.00297.x.
- Cabaret J. & Gasnier N. (1994). Farm history and breeding management influences on the intensity and specific diversity of nematode infection of dairy goats. *Vet. Parasitol.*, **53** (3–4), 219–232. doi:10.1016/0304-4017(94)90185-6.
- Borgsteede F.H.M. & Dercksen D.P. (1996). Coccidial and helminth infections in goats kept indoors in the Netherlands. *Vet. Parasitol.*, **61** (3–4), 321–326. doi:10.1016/0304-4017(95)00830-6.
- 45. Luzón M., Santiago-Moreno J., Meana A., Toledano-Díaz A., Pulido-Pastor A., Gómez-Brunet A. & López-Sebastián A. (2008). – Parasitism and horn quality in male Spanish ibex (*Capra pyrenaica hispanica*) from Andalucía based on coprological analysis and muscle biopsy. *Span. J. Agric. Res.*, 6 (3), 353–361. doi:10.5424/sjar/2008063-328.
- 46. Berrag B. & Urquhart G.M. (1996). Epidemiological aspects of lungworm infections of goats in Morocco. *Vet. Parasitol.*, **61** (1–2), 81–95. doi:10.1016/0304-4017(95)00803-9.

- Fentahun T., Seifu Y., Chanie M. & Moges N. (2012). Prevalence of lungworm infection in small ruminants in and around Jimma Town, Southwest Ethiopia. *Glob. Vet.*, 9 (5), 580–585. doi:10.5829/idosi.gv.2012.9.5.6643.
- 48. Béjar P., Díaz P., Pérez A., Prieto A., Panadero R., Fernández G., López C., Díez-Baños P. & Morrondo P. (2014).
  Infecciones por nematodos broncopulmonares en ganado caprino en la comunidad gallega. *In* Proc. XV Congreso Internacional de la Sociedad Española de Ovinotecnia y Caprinotecnia (M.J. Alcalde Aldea, L.F. de la Fuente Crespo & S. Adán Belmonte, eds), 17–19 de septiembre de 2014, Ourense, Spain, 406–411. Available at: www.researchgate. net/profile/Gonzalo\_Fernandez3/publication/265964625\_ Infecciones\_por\_nematodos\_broncopulmonares\_ en\_ganado\_caprino\_en\_la\_comunidad\_gallega/ links/5421b4eb0cf26120b79f2f36/Infecciones-pornematodos-broncopulmonares-en-ganado-caprino-en-lacomunidad-gallega.pdf (accessed on 18 July 2017).
- 49. Martínez-Carrasco C., Ruiz de Ybáñez M.R., Sagarminaga J.L., Garijo M.M., Moreno F., Acosta I., Hernández S. & Alonso F.D. (2007). – Parasites of the red fox (*Vulpes vulpes Linnaeus*, 1758) in Murcia, southeast Spain. *Rev. Méd. Vét.*, **158** (7), 331–335. Available at: www.revmedvet.com/2007/ RMV158\_331\_335.pdf (accessed on 12 July 2017).
- Morgan U.M. (2000). Detection and characterisation of parasites causing emerging zoonoses. *Int. J. Parasitol.*, **30** (12–13), 1407–1421. doi:10.1016/S0020-7519(00) 00129-6.
- Traub R.J., Monis P.T. & Robertson I.D. (2005). Molecular epidemiology: a multidisciplinary approach to understanding parasitic zoonoses. *Int. J. Parasitol.*, **35** (11–12), 1295–1307. doi:10.1016/j.ijpara.2005.06.008.
- 52. Gasser R.B. (2006). Molecular tools: advances, opportunities and prospects. *Vet. Parasitol.*, **136** (2), 69–89. doi:10.1016/j. vetpar.2005.12.002.
- 53. Martínez-Carrasco C., Berriatua E., Garijo M., Martínez J., Alonso F.D. & Ruiz de Ybáñez R. (2007). – Epidemiological study of non-systemic parasitism in dogs in southeast Mediterranean Spain assessed by coprological and postmortem examination. *Zoonoses Public Hlth*, **54** (5), 195–203. doi:10.1111/j.1863-2378.2007.01047.x.
- 54. Elias D., Wolday D., Akuffo H., Petros B., Bronner U. & Britton S. (2001). – Effect of deworming on human T cell responses to mycobacterial antigens in helminth-exposed individuals before and after bacille Calmette–Guérin (BCG) vaccination. *Clin. Experim. Immunol.*, **123** (2), 219–225. doi:10.1046/j.1365-2249.2001.01446.x.
- 55. Resende Co T., Hirsch C.S., Toossi Z., Dietze R. & Ribeiro-Rodrigues R. (2007). Intestinal helminth co-infection has a negative impact on both anti-*Mycobacterium* tuberculosis immunity and clinical response to tuberculosis therapy. *Clin. Experim. Immunol.*, **147** (1), 45–52. doi:10.1111/j.1365-2249.2006.03247.x.

- Potian J.A., Rafi W., Bhatt K., McBride A., Gause W.C. & Salgame P. (2011). – Preexisting helminth infection induces inhibition of innate pulmonary anti-tuberculosis defense by engaging the IL-4 receptor pathway. J. Experim. Med., 208 (9), 1863–1874. doi:10.1084/jem.20091473.
- 57. Ezenwa V.O., Etienne R.S., Luikart G., Beja-Pereira A. & Jolles A.E. (2010). Hidden consequences of living in a wormy world: nematode-induced immune suppression facilitates tuberculosis invasion in African buffalo. *Am. Naturalist*, **176** (5), 613–624. doi:10.1086/656496.