Research Note

Pearsonema plica and Eucoleus böhmi infections and associated lesions in wolves (Canis lupus) from Italy

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Summary

Reports of Pearsonema plica and Eucoleus böhmi infections in wolves (Canis lupus) in Europe are limited and data on associated lesions are lacking. In the present study urinary bladders, nasal turbinates and faecal samples from 8 necropsied wolves were examined for P. plica and E. böhmi infections and associated lesions. P. plica was identified in the bladder of four wolves. At histological examination, follicular chronic cystitis and eosinophilic cystitis were found. E. böhmi nematodes and eggs were identified from the nasal turbinates and rectal faecal samples of three wolves. Worms and eggs were found embedded in the mucosa among the nasal bone laminae. Two wolves were found coinfected by P. plica and E. böhmi. This is the first report of P. plica and E. böhmi infections in wolves from Italy and the first description of pathological lesions associated with P. plica infection in wolves.

Keywords: Pearsonema plica; Eucoleus böhmi; wolf (Canis lupus); cystitis; Italy

Introduction

In Europe, the wolf (Canis lupus) is globally classified as “Least Concern” by the International Union for Conservation of Nature, but several regional populations are considered at a higher risk of decline. Despite a recent increase in population range and numbers, the Italian wolf population is classified as “Vulnerable” (Large Carnivore Initiative for Europe, 2007). Indeed, carnivore-livestock conflicts determine a high human pressure on wolf populations and illegal killings, by poaching and poisoning are considered a main threat to wolf conservation (Large Carnivore Initiative for Europe, 2007). Since the wolf is a highly protected species and a host of conservation concern, studies aimed to investigate diseases and helminthoses are of relevant interest. Furthermore, similarly to what observed after the increase of red fox (Vulpes vulpes) populations in Europe (Srétet et al., 2003; Saeed et al., 2006; Magi et al., 2014), also the recovery of wolf populations may potentially be responsible for an increased risk of helminthic diseases transmission from wolves to humans and to domestic carnivores (Guberti et al., 1993; Eleni et al., 2014). Among helminthic infections of wolves from Italy, several cestode (Dipylidium caninum, Echinococcus granulosus, Mesocestoides lineatus, Taenia hydatigena, Taenia multiceps, Taenia pisiformis, Taenia ovis) and nematode (Ancylostoma caninum, Dirofilaria immitis, Toxascaris leonina, Toxocara canis, Trichinella britovi, Trichurus vulpis and Uncinaria stenocephala) species have been identified (Guberti et al., 1993; Pozio et al., 1996; Guberti et al., 2004; Pascucci et al., 2007). The nematode species Thelazia callipaeda and Angiostrongylus vasorum have also been recently reported (Otranto et al., 2009; Eleni et al., 2014). Among extraintestinal nematodes of carnivores, Pearsonema plica and Eucoleus böhmi have been reported in wolf populations from Europe (Zamoswski & Patik, 1960; Bagrade et al., 2009). P. plica infects the urinary tract of domestic and wild carnivores worldwide (Basso et al., 2013). The adult worms are thread-like, whitish nematodes that live superficially attached to or are buried within the bladder mucosa whereas the ureters and renal pelvis are seldom affected (Callegari et al., 2010). P. plica is considered to be of low pathogenic significance and in most cases parasite establishes only asymptomatic infections (Fernández-Aguilar et al., 2010). However, in heavy parasite loads P. plica has been found to be responsible for urinary signs and lesions in both carnivore pets (Callegari et al., 2010; Rossi et al., 2011; Basso et al., 2013) and wild canids (Fernández-Aguilar et al., 2010; Bork-Mimm & Rinder, 2010). Since the wolf is a highly protected species and a host of conservation concern, studies aimed to investigate diseases and helminthoses are of relevant interest. Furthermore, similarly to what observed after the increase of red fox (Vulpes vulpes) populations in Europe (Srétet et al., 2003; Saeed et al., 2006; Magi et al., 2014), also the recovery of wolf populations may potentially be responsible for an increased risk of helminthic diseases transmission from wolves to humans and to domestic carnivores (Guberti et al., 1993; Eleni et al., 2014). Among helminthic infections of wolves from Italy, several cestode (Dipylidium caninum, Echinococcus granulosus, Mesocestoides lineatus, Taenia hydatigena, Taenia multiceps, Taenia pisiformis, Taenia ovis) and nematode (Ancylostoma caninum, Dirofilaria immitis, Toxascaris leonina, Toxocara canis, Trichinella britovi, Trichurus vulpis and Uncinaria stenocephala) species have been identified (Guberti et al., 1993; Pozio et al., 1996; Guberti et al., 2004; Pascucci et al., 2007). The nematode species Thelazia callipaeda and Angiostrongylus vasorum have also been recently reported (Otranto et al., 2009; Eleni et al., 2014). Among extraintestinal nematodes of carnivores, Pearsonema plica and Eucoleus böhmi have been reported in wolf populations from Europe (Zamoswski & Patik, 1960; Bagrade et al., 2009). P. plica infects the urinary tract of domestic and wild carnivores worldwide (Basso et al., 2013). The adult worms are thread-like, whitish nematodes that live superficially attached to or are buried within the bladder mucosa whereas the ureters and renal pelvis are seldom affected (Callegari et al., 2010). P. plica is considered to be of low pathogenic significance and in most cases parasite establishes only asymptomatic infections (Fernández-Aguilar et al., 2010). However, in heavy parasite loads P. plica has been found to be responsible for urinary signs and lesions in both carnivore pets (Callegari et al., 2010; Rossi et al., 2011; Basso et al., 2013) and wild canids (Fernández-Aguilar et al., 2010; Bork-Mimm & Rinder, 2010).
In wolves, *P. plica* infection has been previously reported in Belarus (Shimalov & Shimalov, 2000), Spain (Segovia et al., 2001) and Latvia (Bagrade et al., 2009). However, the associated pathological lesions were never evaluated before. *E. böhmi* is a neglected nematode infecting domestic and wild canids (Perrucci et al., 2014). Adults of *E. böhmi* live threaded through the mucosa of nasal turbinates, frontal and paranasal sinuses (Piperisova et al., 2010). *E. böhmi* eggs may be found in nasal discharge and in faeces of infected animals, and are passed in a partial stage of embryonation with the developing larva often appearing square or rectangular (Supperer, 1953; Campbell & Little, 1991). In dogs, *E. böhmi* has been recently reported as a cause of respiratory signs with varying degrees of severity, associated with nasal discharge and chronic rhinitis (Baan et al., 2010; Piperisova et al., 2010; Veronesi et al., 2013). Convulsive seizures and meningoencephalitis following intracranial migration of *E. böhmi* eggs have been also observed in an infected dog (Clark et al., 2013). In the wolf, *E. böhmi* has been previously reported only in a single study in Poland (Zarnoswski & Patik, 1960).

Considering the lack of data in Italian wolf populations, the main aim of the present study was to evaluate *P. plica* and *E. böhmi* infections and associated lesions in wolves from Italy.

**Material and Methods**

Between March 2013 and October 2014, 8 (W1-W8) traumatically or unlawfully killed wolves from Central Italy were submitted by the local authorities for post mortem examination to ascertain the cause of death. Animals were frozen (-20°C) and allowed to thaw overnight before necroscopic examination. For each wolf the sex was recorded and animals were assigned to an age class (adult or juvenile) according to tooth eruption and tooth wear (Ballard et al., 1995). From all animals the bladder was removed, opened, examined for gross lesions and washed with 70 % ethanol. The bladders and lavage fluids were first observed under a stereomicroscope for presence and collection of nematodes. Then adult nematodes were counted, while the lavage fluids were centrifuged at 2000g for 2 minutes and the sediment microscopically examined for capillariid eggs. Under light microscope the isolated adults and eggs were identified at the species level on the basis of their morphometrical features (Levine, 1968). From 6 wolves (W3 – W8), the opened urinary bladder was fixed in 10 % neutral buffered formalin, embedded in paraffin wax, sectioned at 4 μm, stained with haematoxylin-eosin and examined for histopathological lesions. From all wolves rectal faecal samples were also collected and examined for the presence and number of *E. böhmi* eggs. Flotation test and a modified McMaster test with sensitivity of 20 eggs per gram of faeces (EPG) with low density solution (SG 1.2) according to Perrucci et al. (2014) were used. Based on morphology of egg content and wall surface the *E. böhmi* eggs were distinguished from eggs of the other trichuroid nematodes (Di Cesare et al., 2011). From four wolves (W4, W5, W6, W8), the skull was sawn and nasal cavities and sinuses were examined under a stereomicroscope to assess the presence of adult nematodes. Isolated parasites were collected and adult stages were counted and microscopically identified at the species level on the basis of their morphometrical features (Supperer, 1953). Samples of nasal turbinates were formalin-fixed, decalcified with specific solution (K-EDTA Decalcifier, Kaltek, Italy) and processed for histopathological examination as previously described.

![Fig. 1. Urinary bladder (wolf W4). Hyperplastic lymphocytic nodule (a) in the submucosa found associated to Pearsonema plica infection. (Hematoxylin-Eosin), bar 100 μm](image-url)
Results

Animals were 4 adult males (W1, W2, W7, W8), 3 adult females (W3, W4, W6), and a juvenile male (W5). At microscopic examination, the urinary bladder mucosa and the bladder lavage fluids of wolves W1, W4, W7 and W8 revealed the presence of adult worms and of oval, colourless capillariid eggs showing a thick wall and bipolar plugs. More precisely, 15, 12, 2 and 4 adult stages were isolated from wolves W1, W4, W7 and W8, respectively. While eggs measured 58.5 – 62.4 μm X 26 – 31.2 μm and showed a thick wall and bipolar plugs, adult female parasites (30 – 60 mm X about 60 μm) showed a cylindrical appendage near the end of the oesophagus. Adult male parasites (15 – 30 mm X about 48 μm) showed a long spicule with an unarmed and punctate spicule sheath. On the basis of their morphological features isolated worms and eggs were identified with *P. plica*. Gross lesions characterized by 1 – 2 mm scattered foci of mucosal bluish pigmentation were observed only in the bladder mucosa of one infected wolf (W4). At histological examination, follicular chronic cystitis with scattered hyperplastic lymphocytic nodules in the sub-mucosa (Fig. 1) was found in two wolves (W4 and W7). Nematodes cross-sections and eggs were also observed within the lumen or embedded in the mucosa epithelium of ureter in wolf W4. A mild diffuse eosinophilic cystitis was found in one wolf (W8). At histopathology, no lesions were observed in the remaining three wolves found negative at parasitological examination.

Capillarid nematodes threaded through the mucosa of the nasal turbinates were isolated after microscopical examination from W4, W5 and W8. In particular, 15, 8 and 3 adult nematodes were isolated from W4, W5 and W8, respectively. Adult females (37 – 40 mm X about 170 μm) showed a finely striated cuticle. The vulva did not protrude beyond the surface of the body and was located directly behind the passage of the oesophagus in the intestine and the pitted eggs measured 61 μm X 30.42 μm in average. Adult males (about 21 mm X 110 μm) showed a delicate weakly chitinised spiculum. The everted portion of the sheath of the spiculum was about 500 μm long and completely covered with long spines. At coprological examination capillariid eggs, measuring 56 – 60.2 μm X 29 – 31 μm, with polar plugs and containing a developing larva were found in faecal samples from W4 (900 EPG), W5 (540 EPG) and W8 (280 EPG). Eggs had a thimble-like surface showing a large number of small depressions. Morphological and metric features of adults and eggs were in accordance with the original description of *E. böhmi* by Supperer (1953). Gross lesions of nasal cavities were not assessable in W4 due to putrefactive changes and presence of abundant necrophagous entomofauna. Only small amounts of catarrhal exudate were observed in W5 and W8. At histological examination cross-sections of adult worms and eggs were seen among the nasal bone laminae, embedded in the turbinates mucosa (Fig. 2). Wolves W4 and W8 were found coinfectected by both *P. plica* and *E. böhmi*.

At faecal analysis wolves W4 and W8 were found also positive for *Sarcocystis* sp. sporocysts and *Euceles aerophilus* and *Ancylostoma/Uncinaria* eggs. W4 was found also infected with *Aonchotoca putorii*, while W5 was found coinfectected only by *Sarcocystis* sp. Microscopic examination of faecal samples from all the remaining wolves was negative for *E. böhmi*, but *Sarcocystis* sp. sporocysts were identified in faecal samples from W6 and W7. From the latter wolf, *Ancylostoma/Uncinaria* and *Taenia* sp. eggs were also isolated. No positive results were obtained at coprological examination of faecal samples from W1, W2 and W3 (summarised in Table 1).

Fig. 2. Nasal turbinates (wolf W4). Cross-sections of *Eucoleus böhmi* (a) among the nasal bone laminae. (Hematoxylin-Eosin), bar 10 μm
The Italian wolf population is estimated to be around 500 – 800 individuals distributed along the Apennines (Large Carnivore Initiative for Europe, 2007) and it is protected by EU and national laws. The International Union for Conservation of Nature classifies the Italian population as “Vulnerable”, stressing out that it may easily reverse from its current favourable status due to high human pressure (Large Carnivore Initiative for Europe, 2007). Limited data are available on *P. plica* and *E. böhmi* infections of wolves in Europe and they are completely lacking for the Italian wolf population. In the present study eight wolves were examined for *P. plica* and *E. böhmi* infections and associated lesions. Four out of the eight examined wolves were infected with *P. plica* and in three of them (W4, W7, W8) histopathology revealed follicular or eosinophilic cystitis, despite gross lesions being observed only in one of these wolves. Lesions of the urinary bladder associated with *P. plica* infection previously observed in dogs (Senior et al., 1980) and in an arctic fox (*Alopex lagopus*) (Fernández-Aguilar et al., 2010) were characterized by predominant eosinophilic inflammation along with infiltration of lymphocytes or occasional plasma cells. Chronic follicular cystitis has been recently described in infected red foxes, but it has been considered a less common finding compared to the eosinophilic cystitis (Alić et al., 2015). Data from this study are the first report of lesions associated with *P. plica* infection in the wolf.

A correlation between adult parasite load and the presence of gross lesions in the urinary bladder mucosa was observed in red foxes by Bork-Mimm and Rinder (2011). In the present study, 

<table>
<thead>
<tr>
<th>Wolf</th>
<th>Gender</th>
<th>Age</th>
<th>Collected samples</th>
<th>Parasite</th>
<th>Parasite Intensity</th>
<th>Anatomo-Histopathological Examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1</td>
<td>M</td>
<td>A</td>
<td>bif, f</td>
<td><em>P. plica</em> (bifm)</td>
<td>15</td>
<td>ubg: 1-2 mm scattered foci of mucosal pigmentation; ubh: follicular chronic cystitis and nematode cross-sections embedded in bladder and ureter mucosa</td>
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<td></td>
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<td>ncg and nch: not assessable due to putrefactive changes and presence of abundant necrophagous entomofauna</td>
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<tr>
<td>W2</td>
<td>M</td>
<td>A</td>
<td>bif, f</td>
<td></td>
<td></td>
<td>ncg: catarrhal exudate nch: worms and eggs embedded in the turbinate mucosa</td>
</tr>
<tr>
<td>W3</td>
<td>F</td>
<td>A</td>
<td>bif, f, ub</td>
<td></td>
<td></td>
<td>ubh: follicular chronic cystitis</td>
</tr>
<tr>
<td>W4</td>
<td>F</td>
<td>A</td>
<td>bif, f, nc, ub</td>
<td><em>P. plica</em> (bifm, ubm) <em>E. böhmi</em> (ft, mm) <em>Eucoleus aerophilus</em> (ft) <em>Sarcocystis</em> sp. (ft) <em>Aonchoteca putorii</em> (ft) <em>Ancylostoma/Uncinaria</em> (ft)</td>
<td>12 (900 EPG)</td>
<td></td>
</tr>
<tr>
<td>W5</td>
<td>M</td>
<td>J</td>
<td>bif, f, nc, ub</td>
<td><em>E. böhmi</em> (ft, mm, nc) <em>Sarcocystis</em> sp. (ft)</td>
<td>8 (540 EPG)</td>
<td>ncg: catarrhal exudate nch: worms and eggs embedded in the turbinate mucosa</td>
</tr>
<tr>
<td>W6</td>
<td>F</td>
<td>A</td>
<td>bif, f, nc, ub</td>
<td><em>Sarcocystis</em> sp. (ft)</td>
<td></td>
<td>nch: worms and eggs embedded in the turbinate mucosa</td>
</tr>
<tr>
<td>W7</td>
<td>M</td>
<td>A</td>
<td>bif, f, ub</td>
<td><em>P. plica</em> (bifm, ubm) <em>Sarcocystis</em> sp. (ft) <em>Ancylostoma/Uncinaria</em> (ft) <em>Taenia</em> sp. (ft)</td>
<td>2</td>
<td>ubh: follicular chronic cystitis</td>
</tr>
<tr>
<td>W8</td>
<td>M</td>
<td>A</td>
<td>bif, f, nc, ub</td>
<td><em>P. plica</em> (bifm, ubm) <em>E. böhmi</em> (ft, mm, nc) <em>Eucoleus aerophilus</em> (ft) <em>Sarcocystis</em> sp. (ft) <em>Ancylostoma/Uncinaria</em> (ft)</td>
<td>4 (280 EPG)</td>
<td>ubh: mild eosinophilic cystitis</td>
</tr>
</tbody>
</table>

Table 1. Parasites isolated from 8 deceased wolves (W1-W8) from Italy examined for *Pearsonema plica* and *Eucoleus böhmi* occurrence and intensity (number of isolated *P. plica* and *E. böhmi* adult stages and *E. böhmi* eggs/gram of faeces) and associated lesions. A= adult; bif= bladder lavage fluid; bifm= microscopical examination of bladder lavage fluid; EPG = eggs per gram of faeces; F= female; f= faeces; ft= faecal flotation test; J = juvenile; M= male; mm= McMaster test; nc= nasal cavities; ncg= gross lesions of nasal cavities; nch= histopathology of nasal cavities; ub= urinary bladder; ubg= gross lesions of urinary bladder; ubh= histopathology of urinary bladder; ubm= microscopical examination of urinary bladder.

Discussion

The Italian wolf population is estimated to be around 500 – 800 individuals distributed along the Apennines (Large Carnivore Initiative for Europe, 2007) and it is protected by EU and national laws. The International Union for Conservation of Nature classifies the Italian population as “Vulnerable”, stressing out that it may easily reverse from its current favourable status due to high human pressure (Large Carnivore Initiative for Europe, 2007). Limited data are available on *P. plica* and *E. böhmi* infections of wolves in Europe and they are completely lacking for the Italian wolf population. In the present study eight wolves were examined for *P. plica* and *E. böhmi* infections and associated lesions. Four out of the eight examined wolves were infected with *P. plica* and in three of them (W4, W7, W8) histopathology revealed follicular or eosinophilic cystitis, despite gross lesions being observed only in one of these wolves. Lesions of the urinary bladder associated with *P. plica* infection previously observed in dogs (Senior et al., 1980) and in an arctic fox (*Alopex lagopus*) (Fernández-Aguilar et al., 2010) were characterized by predominant eosinophilic inflammation along with infiltration of lymphocytes or occasional plasma cells. Chronic follicular cystitis has been recently described in infected red foxes, but it has been considered a less common finding compared to the eosinophilic cystitis (Alić et al., 2015). Data from this study are the first report of lesions associated with *P. plica* infection in the wolf. A correlation between adult parasite load and the presence of gross lesions in the urinary bladder mucosa was observed in red foxes by Bork-Mimm and Rinder (2011). In the present study,
Gross lesions were only observed in the bladder mucosa of one wolf (W4). Despite the difficulties in the determination of the exact number of worms embedded in the bladder and ureter mucosa, the number of P. plica adults isolated from this subject was higher (12) than that found in the other infected wolves. Furthermore, in this same animal cross-sections of nematodes were histologically observed not only embedded in the bladder mucosa, but also in the ureter mucosa.

In this study, three out of the eight wolves were found positive for E. böhmi adults and eggs. E. böhmi has been recently reported as a cause of respiratory signs in dogs where nasal chronic inflammation was observed (Baan et al., 2010; Piperisova et al., 2010; Veronesi et al., 2013). In the present study, in one (W4) of the infected wolves histopathological lesions were not assessable whilst in the remaining two positive wolves (W5 and W8) the inflammatory lesions were not observed. We hypothesise that in these latter wolves infection by E. böhmi could be recently established and therefore chronic inflammatory lesions reported were not present yet.

In the red fox, prevalence and pathogenic potential of P. plica and E. böhmi are quite high and this canid species is considered a reservoir of P. plica and E. böhmi infections for companion and hunting dogs in Europe (Davidson et al., 2006; Bork-Mimm & Rinder, 2011; Magi et al., 2014). Prevalence of P. plica infection in red foxes in Italy have been reported to be 56.8 % (Magi et al., 2014), but it is difficult to obtain similar data concerning prevalence of infection in the wolf population, since Canis lupus is a protected species for which there are no culling plans as for red foxes. Routine necroscopic procedures do not usually include opening of nasal cavities, mucosal scrapings and acquired from red foxes. Routine necroscopic procedures do not usually include opening of nasal cavities, mucosal scrapings and microscopic examination of isolated organs. This may contribute to the underestimation of P. plica and E. böhmi infections in wolves. Although the evaluation of Eucosmus aerophilus and Aonchotoca putorii infections was not included in the aims of the present study, data here reported provide the first record of these helminthic species in wolves from Italy.

In conclusion, previous data and findings from the present study stimulate a reflection on pathology and actual frequency of P. plica and E. böhmi infections among European wolves and on the potential role of this carnivores being a reservoir of these infections along with red foxes.

References


