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First Identification of *Trichinella* sp. in Golden Jackal (*Canis aureus*) in Romania

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ABSTRACT: Larvae of *Trichinella* sp. were identified in a golden jackal (*Canis aureus*) from Romania by both trichinelloscopy and artificial digestion. The larvae were identified as *Trichinella britovi* using a multiplex polymerase chain reaction biotyping method. This is the first report of *Trichinella* sp. in a jackal in Romania.

Key words: *Canis aureus*, jackal, Romania, trichinellosis, *Trichinella britovi*.

The golden jackal (*Canis aureus*) is widespread in north and northeastern Africa, occurring from Senegal on the west coast of Africa to Egypt in the east. It has expanded its range from the Arabian Peninsula into western Europe to Austria and Bulgaria (Genov and Wassiley, 1989), and eastward into Turkey, Syria, Iraq, Iran, central and Southeast Asia (Baillie and Groombridge, 1996). Several authors have previously identified the golden jackal in Romania (summarized by Angelescu, 2004), and here we report the first identification of *Trichinella* sp. from a golden jackal that was collected in July 2006 from the Babadag Forest (44°90'N, 28°71'E), Tulcea, located in southeastern Romania.

Samples were taken from the anterior tibial muscle and the diaphragm, which are considered favored sites for *Trichinella* larvae in other wildlife species (Nockler et al., 2000). A preliminary trichinelloscopic exam was done as follows: 28 small pieces of muscle tissue (20 mm²) weighting 0.5 g were removed from each sample. Trichinelloscopy analyses were conducted with three samples for each type of muscle, and they were examined individually for *Trichinella* larvae using a trichinoscope with

15× and 40× magnification. Within 24 hr after positive identification of encysted muscle larvae (ML) of *Trichinella* sp., 100 g of infected tibial muscle and diaphragm, respectively, were analyzed by artificial digestion. Each tissue sample was minced into 3-mm pieces in a grinder and digested separately at 45±2 C in 2 l of a digestion solution of 1% HCl and 1% pepsin (1:10,000 NF, Merck KGaA, Darmstadt, Germany) in a 3-l beaker on a heated magnetic stirrer. Complete digestion was achieved after 1.5 hr, having less than 0.2 g of tissue retained on the 200-µm sieve. Particulate matter in the filtrate was concentrated by several sediment-decant cycles. The final 20 ml of sediment was transferred to a petri dish and examined by microscope at 20–30× magnification. Larvae of *Trichinella* sp. were washed several times in cold water, and they were counted in triplicate under a microscope.

Crude DNA from five larvae was prepared for the polymerase chain reaction (PCR) molecular typing according to the protocol of Pozio and La Rosa (2003), with slight modification; single larvae were washed five times in phosphate-buffered saline (PBS), and then each larva was placed in 5 µl of PBS in a 0.5-ml tube. Then, Tris-HCl (2 µl at pH 7.6) was added, overlaid with a drop of mineral oil, heated to 90 C for 10 min, and then cooled on ice. Proteinase K (3 µl at a final concentration of 100 µg/ml) was added and incubated at 48 C overnight. Before using the crude DNA, the samples were heated up to 90 C for 10 min and then



FIGURE 1. Encysted larva of *Trichinella britovi* in the tibial muscle of golden jackal (*Canis aureus*) (trichineloscopy 40 \times).

cooled on ice. Crude DNA (5 μ l) from a single larva were used in a multiplex PCR using the primer set I and II according to a published protocol (Zarlenga et al., 1999), with slight modifications; amplifications were performed using a 94 C for 1-min denaturation step; a 55 C, 1-min annealing step; and a 72 C for 1-min extension step. Thirty-five cycles of this program were run with 1.5 units of Taq polymerase enzyme (Promega, Madison, Wisconsin, USA) per sample. PCR-amplified products were separated by electrophoresis in a 2% agarose (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) gel with Tris acetate-EDTA buffer, and they were stained with ethidium bromide. Muscle larvae of the three European encapsulate *Trichinella* strains were used as controls (*Trichinella spiralis* [ISS51], *Trichinella nativa* [ISS10], and *Trichinella britovi* [ISS100]).

Encysted ML of *Trichinella* were identified by trichinelloscopic exam in both the tibial muscle (Fig. 1) and the diaphragm. Using the artificial digestion, the intensity of the infection was estimated at 55 larvae/g (LPG) for the tibial muscle and at 36 LPG for the diaphragm; 80% of the larvae were motile with the round shape characteristic of free-living *Trichinella* sp. muscle larvae. Analysis of the PCR-amplified larval geno-

mic DNA showed a double band pattern, with a first band at 127 base pairs and a second band at 253 base pairs, which is indicative of *T. britovi*. The control DNA samples of the reference isolates, mentioned above, generated characteristic amplified fragments, confirming the identity of the jackal isolate as *T. britovi*.

Trichinella britovi is the etiologic agent of infection of the sylvatic carnivores living in temperate areas of the Palearctic region from the Iberian Peninsula to Kazakhstan, Iran, and Turkey (Pozio and Zarlenga, 2005). In Romania, the major reservoir of *Trichinella* spp. seems to be in wildlife, particularly carnivores, with prevalence as high as 39% for wolves (*Canis lupus*), 22% for red foxes (*Vulpes vulpes*), and 24% for wildcats (*Felis silvestris*; Lupascu et al., 1970). Therefore, not surprisingly, the first golden jackal for whom we had the chance to perform a trichinelloscopic exam was positive for *Trichinella* infection. Previous *Trichinella* infection cases in jackal have been reported in two neighboring countries of Romania, the former republic of Yugoslavia and Bulgaria (Rukavina and Brglez, 1970; Kurdova et al., 2004), countries presenting similar high prevalence rates of trichinellosis in wildlife (Kim, 1983). Improper hunting behavior (e.g., leaving the animal carcasses in the field after skinning) has been documented as the cause of such a high prevalence rate in Romania (Cironeanu, 1974). Similar results have been reported from other regions of the world: Kazakhstan (Batkaev and Vakker, 1992), Spain (Perez-Martin et al., 2000), Russia (Pozio et al., 2001), and France (Boireau, pers. comm.). In the USA, it was demonstrated that the prevalence rate of *Trichinella* infection cases in wild boar within a game park could be reduced from 76% to 4% by incinerating the viscera of field-dressed animals (Worley et al., 1994).

Because sylvatic *T. britovi* has the potential to be transmitted from a wild animal reservoir to domestic, free-ranging pigs (Pozio, 2000), the present finding empha-

sizes the need of an active surveillance program for *Trichinella* spp. in wildlife with special attention given to wild carnivores.

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