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Alaria alata mesocercariae in domestic pigs and wild boars in South Banat, northern Serbia

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ABSTRACT

Alaria alata (Diplostomidae, Trematoda), a potentially zoonotic pathogen, is the only *Alaria* species in Europe. In recent years, increasing reports of mesocercariae of *A. alata* in wild boars have been recorded in European countries; however there have been no described cases in domestic pigs over the past decades. Out of 272 diaphragm samples from wild boars (*Sus scrofa*) and domestic pigs (*Sus scrofa*), from Serbia, included in the present investigations, mesocercariae of *A. alata* were found in six (3%) samples from wild boars and in two (2.77%) samples from domestic pigs. Due to the lack of sensitivity of artificial digestion with a magnetic stirrer which was applied in the study, the true prevalence is estimated to be considerably higher in the area of investigation. Confirming the infection in domestic pigs and a wide distribution in wild boars in the area of investigation, the present paper should influence the growing awareness of alariosis as a potential human disease. The meat from free-range domestic pigs and game meat as a potential source of alariosis should always be adequately thermally treated before consumption.

1. Introduction

An increasing popularity of free-range animal farming has been recorded in Europe, including Serbia, in the past decades. Although the open production system has some advantages such as improved animal welfare, an increased food safety risk from infectious and parasitic diseases is a main disadvantage [1]. Beside commercial and family farms, pig production in Serbia also exists in backyard holdings and free-range holdings. In these open production systems, domestic pigs (*Sus scrofa*) may make contact with various sources of infection including some zoonotic agents such as *Alaria alata*.

A. alata (Diplostomidae, Trematoda) is the only known species of the genus *Alaria* in Europe and has a complex three-host life cycle. The adult flukes live in the intestine of the definitive hosts [2]. Beside earlier reported canids such as the red fox (*Vulpes vulpes*), wolf (*Canis lupus*), raccoon dog (*Nyctereutes procyonides*) and dog (*Canis lupus familiaris*), later investigations have confirmed that distinct species from felidae and mustelidae families can also have a role as the definitive host [3–7]. The life cycle of *A. alata* includes freshwater snails and amphibians as the first and the second intermediate hosts, respectively. In frogs and other amphibians, furcocercariae develop to mesocercariae representing an infective form for definitive and paratenic hosts. The mesocercarial stage allows the parasite to survive several host transitions unharmed and without losing its infectivity toward the definitive host [2,8]. Very distinct animal species from different classes of vertebrates have been reported as carriers of *Alaria* spp mesocercariae, indicating a nonspecific relationship of this developmental stage towards its host [2].

Humans may also be infected with mesocercariae of *Alaria* spp as the paratenic host. In the USA and Canada several cases, including one with a fatal outcome, have been reported to be caused by *Alaria americana*, a species closely related to *A. alata* [9]. Human alariosis manifests itself in various clinical signs depending on the localization of the larvae. In confirmed cases, they were found under the skin, in the eye and in the respiratory tract [10,11]. Although the infection caused by *A. alata* has not yet been confirmed in humans, such a possibility may be assumed by the reproduction of the disease in Rhesus monkeys (*Macaca mulatta*) following experimental infection [12].

The evidence of *A. alata* mesocercariae in wild boars (*Sus scrofa*) in Europe has recently been increasing, with reported investigations from Germany [13], Austria [14], the Czech Republic [15], France [16], Estonia, Lithuania [17], Latvia [18], and also from Serbia [19,20] and from three neighboring countries: Croatia [21], Hungary [22] and Bulgaria [23]. Over the past decades, there have been no reported cases

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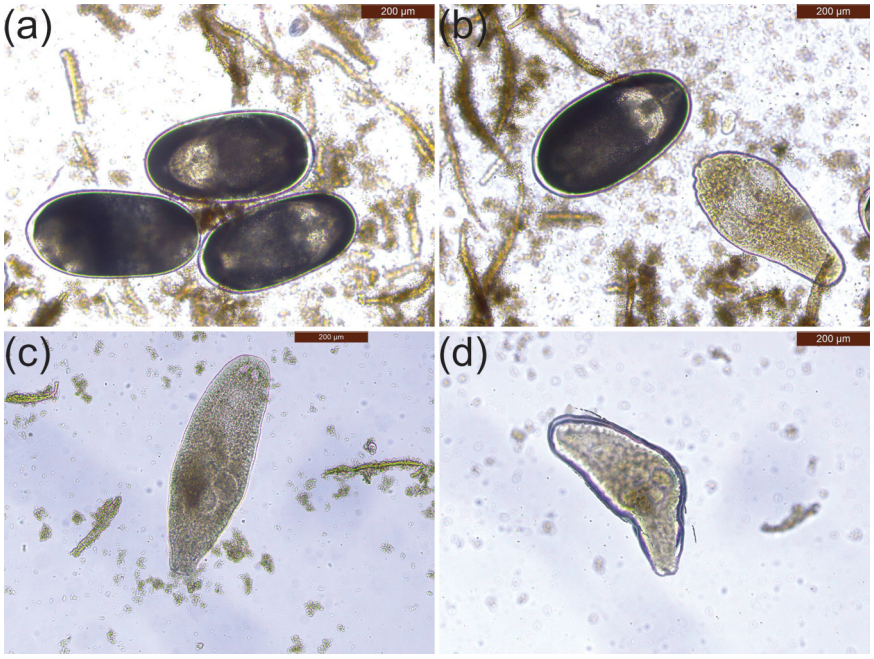

Fig. 1. *Alaraia alata* mesocercariae from meat of wild boars. (a) Encysted mesocercariae. (b) One encysted and one free mesocercaria. (c) Free, motile mesocercaria. (d) Mesocercaria from Fig. 1c in movement.

of mesocercariae of *A. alata* in domestic pigs in European countries. Consequently, the aim of the present investigations was to explore the presence of mesocercariae of *A. alata*, aside from in wild boars, in domestic pigs with outdoor access in the target area.

2. Material and methods

The investigations were conducted in South Banat, Vojvodina province, northern Serbia. Most of the South Banat territory is under the epizootiological surveillance of the Veterinary Specialized Institute Pančevo, whereas a very small part near the capital of Serbia is under the epizootiological surveillance of the Scientific Veterinary Institute of Serbia. The material for examination consisted of meat samples of wild boars and domestic pigs which were regularly delivered to the laboratories of these two institutes for *Trichinella* inspection. In total, 200 diaphragm samples of wild boars and 72 diaphragm samples of free-range and backyard domestic pigs were examined. From each animal at least 10 g of diaphragm was tested in either a pooled or individual sample.

All samples were examined by artificial digestion with a magnetic stirrer. Meat samples (100 g) were chopped by blender and transferred to a 3 l glass beaker containing the digest fluid prepared with 2 l of tap water, 16 ml of 25% HCl and 10 g of pepsin. The mixture was stirred by the magnetic stirrer for 30 min and subsequently poured through a sieve into a separatory funnel. After 30 min of sedimentation, approximately 40 ml of the digest fluid was dispensed into a 50 ml tube and sedimented for 10 min, after which 30 ml of the supernatant was withdrawn by aspiration from the top, leaving a volume of 10 ml. This secondary sediment was poured into a gridded Petri dish and examined by stereomicroscope (× 40 magnification).

The identification *of Alaria* mesocercariae was made based on characteristics such as piriform body with anterior oral sucker, acetabulum positioned in the center of the parasite, two pairs of granulated penetration glands limiting the anterior part of the acetabulum, and large double ceca placed posterior to the acetabulum [24]. Since *A. alata* is the only *Alaria* sp. in Europe [2] including Serbia [25], mesocercariae were interpreted as mesocercariae of *A. alata*.

3. Results

Out of 272 diaphragm samples of wild boars and domestic pigs, mesocercariae of *A. alata* were found in six (3%) samples of wild boars and in two (2.77%) samples of domestic pigs. In five samples mesocercariae were encysted (Fig. 1a), in one sample both encysted and free forms were present (Fig. 1b), whereas in two samples only free, motile forms were found (Fig. 1c, d). Wild boars whose samples tested positive originated from hunting grounds located in the area of Pančevo, Opovo and Kovačica municipalities while domestic pigs whose samples tested positive originated from one backyard holding in Kovilovo, a suburban settlement of Belgrade, the capital of Serbia.

4. Discussion

To the best of our knowledge, this is the only reported case of *A. alata* mesocercariae in domestic pigs over the past several decades in Europe. The pigs whose samples tested positive had been raised in a backyard and had access to pasture in the region in which alariosis was prevalent in red foxes [26]. Production systems that allow outdoor access for farm animals improving animal welfare, such as backyard holdings and free-range farming, may create new risks to animal and public health [1]. The present finding confirms that, among the health risks, backyard farming can pose a risk of infection with *A. alata*.

Very early consumption of raw meat products poses the highest risk of infection with *A. alata* for consumers [27]. In the area of investigation there is a long tradition of home-made pork production especially of different types of sausage which are commonly tasted and eaten when freshly made, soon after slaughter. Based on the specificity of this home-made production and data on the resistance of the mesocercarial stage of the parasite [27], it can be assumed that the consumption of raw sausages may pose the highest risk of potential infection with *Alaria* mesocercarae in Vojvodina province. González-Fuentes et al. [27] found vital *A. alata* mesocercariae in raw type sausages 24 h after production, while cryo-resistance tests have shown that larvae can survive for at least five days in frozen meat [16].

Alariosis is very widespread in wild carnivores as definitive hosts in Serbia with a prevalence of 49.41% in red foxes and 30% in golden jackals [25]. In three hunting seasons 2013, 2014, and 2015

mesocercariae of *A. alata* were found in 10.3% of mixed tissue samples of wild boars from seven hunting grounds in northern Serbia. The study included two regions of Vojvodina province: Srem and Bačka, while the Banat region remained uninvestigated [20]. The higher prevalence as compared with the present investigations conducted in the South Banat region is most likely a result of using the migration technique as a more sensitive method, and of including floodplains as known risk factors for the presence of the agent. Based on the literature dealing with comparison of artificial digestion with a magnetic stirrer with migration technique [28], the true prevalence of infection in the present investigation may be estimated to be 60% higher. A similar frequency of *A. alata* mesocercariae in wild boars has been reported from other European countries such as Austria (6.7% [14]), the Czech Republic (6.8% [15]), Latvia (7% [18]) and Hungary (1.6% [22]), while a very high prevalence (30.5–40.0%) has been recorded in Lithuania [17].

Despite the lack of sensitivity as compared with the newly developed migration technique [28], artificial digestion with a magnetic stirrer provided very important data about the presence of *A. alata* mesoceracariae in wild boars and domestic pigs in South Banat. A limitation in terms of the estimation of the true prevalence does not affect further implications. According to national legislation, the analysis for *Trichinella spiralis* is compulsory in domestic pigs and wild boars in Serbia, whereas *A. alata* has not been recognized as a zoonotic pathogen and no official procedure is in place to respond to the detection of an *Alaria* positive sample. An increasing number of accidental findings of *A. alata* mesocercariae during *Trichinella* inspection should serve as a reason for reconsideration of the appropriate regulations. Although human alariosis caused by *A. alata* has not yet been confirmed, based on scientific knowledge, some countries have predicted such a possibility. The Swiss Federal Office for the Environment, for instance, categorized *A. alata* as a zoonotic parasite in 2003 [2].

Confirming the infection in domestic pigs and its wide distribution in wild boars in South Banat, the present paper should influence the growing awareness of alariosis as a potential human disease. The meat from free-range domestic pigs and game meat as a potential source of alariosis should always be adequately thermally treated before consumption.

Declarations of interest

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