Lungworm in the Domestic Mountain Horse and the Balkan Donkey in the Special Nature Reserve in Serbia

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Abstract
The domestic mountain horse and Balkan donkey are considered autochthonous species whose numbers are declining, so they are classified as endangered species. Both populations live in small herds in nature reserves Stara planina Nature Park (a mountainous region corresponding to the original habitat where this species was once bred); Zasavica Special Nature Reserve (a plain area at the mouth of the Zasavica in the Sava River), and Krčedinska ada (a marsh island on the Danube River near Novi Sad). Parasitic infections are a constant health problem in free-ranging animals. In our work, we present research on the presence of lung parasites in both populations of animals that are kept in free breeding in nature reserves. The research included 157 Domestic Mountain horses and 60 Balkan donkeys. For faecal examination we use modified Baermann's technique and detection of larvae was based on their morphological characteristics. During examinations of the domestic mountain horse which are autochthonous breed, kept in a semi-free system together with donkey at Star Planina Mountain infection with *Dictyocaulus arnfieldi* we established at 83, 33% horses and 59% donkeys. Despite the high degree of infection, the intensity of the infections was low, so that milder clinical symptoms were present in only a few cases. In Zasavica dictyocaulosis was established at 97% of horses and 100% at donkeys. At population breed in Krčedinska ada dictyocaulosis was established in all examined animals from both population (100%).

Keywords: Balkan donkey, *Dictyocaulus arnfieldi*, domestic mountain horse, lungworm

1. Introduction

The domestic mountain horse is one of the autochthonous horse breeds in Serbia and the Balkans. It was created by crossing the Tarpan (*Equus ferus*) and the Przewalskii (*Equus Przewalskii*) with the Arabian horse, resulting in numerous variations in coat color and body structure. The Balkan donkey is descended from the Somali donkey, which is a subspecies of the African wild donkey (*Equus africanus*). The term "autochthonous race" refers to animals that originated in certain biological areas with limited geographic units and are adapted to the living conditions of that area. In the Balkans, it has long been kept in hilly and mountainous areas. Both species are considered autochthonous species whose numbers are declining, so they are classified as endangered species [1]. From these reasons has been included in the National Animal Genetic Resources Conservation Program in the Republic of Serbia. Both populations live in small herds in nature reserves Stara planina Nature Park (a mountainous region corresponding to the original habitat where this species was once bred); Zasavica Special Nature Reserve (a plain area at the mouth of the Zasavica in the Sava River), and

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Krčedinska ada (a marsh island on the Danube River near Novi Sad) [2].
Parasitic infections are a constant health problem in free-ranging animals. The pasture-based diet enabled them to be in constant contact with eggs, larvae and transitional hosts of many types of parasites. For these reasons, the return to the traditional way of breeding, keeping autochthonous species of animals on pasture, opened up the question of the types of parasites with which they are infected and the suppression of helminths including lungworms [3,4,5,6,7].
In our work, we present research on the presence of lung parasites in both populations of animals that are kept in free breeding in nature reserves.

2. Materials and methods

The research included 157 Domestic Mountain horses and 60 Balkan donkeys from nature reserves Stara planina, Zasavica and Krčedinska ada. All the animals were kept in a semi-free system on pastures - during the day they were in pastures, moving freely in a space of several kilometres around the farm, only to be closed during the night Horses and donkeys were in good condition and health.
Fresh faeces of were sampled regardless of the sex or age of the animals that were on the investigated field. From herds bed at Stara planina we collected 124 samples of horses and 39 from donkeys. In Zasavica we collected 25 samples of horses and 14 from donkeys and in Krčedinska ada 8 samples from horses and 7 from donkey.
For faecal examination we use modified Baermann's technique, before being transferred to a glass slide so that their morphological detail can be examined under the high power of a compound microscope [8,9]. This method is most sensitive once live L1 have had time to leave the faecal mass. The best time to read the test is around 8–24 hours after setting it up; hence it is ideally left to stand overnight. Detection of larvae was based on their morphological characteristics [8,10].

3. Results and discussion

During examinations of the domestic mountain horse which are autochthonous breed, kept in a semi-free system together with donkey at Star Planina Mountain infection with *Dictyocaulus arnfieldi* we established at 83, 33% horses and 59% donkeys. Despite the high degree of infection, the intensity of the infections was low, so that milder clinical symptoms were present in only a few cases.
In Zasavica dictyocaulosis was established at 97% of horses and 100% at donkeys.
At population breed in Krčedinska ada dictyocaulosis was established in all examined animals from both population (100%).
D.arnfieldi is the true lungworm affecting donkeys, horses, mules and zebras and is found throughout the world [11,12]. It is a relatively well adopted parasite of donkeys but tend to be quite pathogenic in horses, where this parasite is endemic. Adult Dictyocaulus worms are slender, medium sized roundworms, up to 8 centimetre long. Females are about one third longer than males. They have a whitish to greyish colour. As in other roundworms, the body of these worms is covered with a cuticle, which is flexible but rather tough. Disease is spread worldwide [13,14,15,16].
The natural host and reservoir of *D. arnfieldi* are donkeys [17]. Donkeys, which usually show few signs of the infection, are the prime source of pasture contamination for horses [13,18,19]. Horses are not the favourite host of this parasite and do not usually transmit the disease to other horses. Horses that share pasture with donkeys or follow them into grazing used by donkeys within a few months are most likely to become infected [20,21].
The epidemiology of lungworm disease is largely concerned with factors determining the number of intensive larvae on the pasture and the rate at which they accumulate. The third stage larvae are long living in damp and cool surroundings. Warm and wet summers give rise to heavier burdens in the follow autumn and spring. Under optimal condition the larvae may survive in the pasture for a year. They are quite resistant to cold although it generally delays their maturations. They can withstand temperature of 4-5 degree Celsius; Larvae can over winter in cold climates [22,23]. Most outbreak of verminous pneumonia occurs during cool season especially autumn and early winter because the larvae stages of the causative worms tolerate and prefer low temperatures [9].
D. arnfieldi has a direct life cycle. Adult females lay eggs in the airways of infected hosts. The endpoint of the parasite cycle emerges when then infective larvae arrive into the lungs from the intestine via the lymphatic system [24]. Final

maturation occurs in the bronchi [10,13]. These eggs are transported to the pharynx within respiratory secretions. Mature worms are present in the smaller airways, and they lay eggs which, being already embryonated, can swiftly hatch into first-stage larvae [9,25]. From the pharynx these eggs are coughed out, into the mouth to be swallowed or directly to the outside. Larvated eggs, and very rarely first-stage larvae, pass in the faeces. L1 hatches almost immediately after eggs are passed in the faeces and spread on pastures [13]. In the environment, the eggs hatch and the larvae released undergo two moults to the infective third stage [5,10]. In faeces on pasture; they may remain infective unless killed by drought or very cold conditions. Infection of the donkey is by ingestion of the infective larvae [8,9,10].

Horses become infected by ingesting second-stage larvae (L2), Once ingested and in the host's gut infective larvae penetrate into the gut's wall and reach the lymphatic nodules where the moult to L4 larvae [24]. Through the thoracic duct and the jugular vein, they reach the heart and are pumped to the lungs. Once in the lungs they are stopped in the lung capillaries, cross their wall and reach the bronchioles, bronchi or the trachea where they complete development to adult worms. The prepatent period (time between infection and first larvae shed with the faeces) lasts about 4 weeks [17]. However, larvae in the lungs may become arrested (dormant, hypobiotic, inhibited) for up to 5 months. These larvae resume development at early spring and re-infect the pastures during the next season [26].

The severity of disease is related to the number of larvae ingested. Such animals can act as a source of further larval contamination, although infected horses do not produce many infective larvae [27]. Previously infected adults can become reinfected if they have not been exposed to the lungworm larvae for over a year (and therefore have lost some of their natural resistance) [12,21].

Although parasites are more often found in older horses, the clinical significance of dictyocaulosis is primarily in young ones [28]. The pathogenic effects of lungworm depend on their location within the respiratory tract, the number of infective larvae ingested, the animal immune status, on the nutritional status and age of the host [14,15]. Larvae migrating through the alveoli and bronchioles produce an inflammatory response, which may block small bronchi and bronchioles with inflammatory exudates. The bronchi contain fluid and immature, latter adult worms and the exudates they produce also block the bronchi. Secondary bacterial pneumonia and concurrent viral infections are of the complication of Dictyocaulosis [25,27,29]. Signs of lungworm infection range from moderate coughing with slightly increased respiratory rates to unthriftiness in older horses [23,28]. Infections with few or no visible signs can occur in foals and donkeys.

4. Conclusions

During our research on the prevalence of pulmonary strongyloidiasis in domestic mountain horse and Balkan donkey, we found an infection with *Dictyocaulus arnfieldi.* Such a high prevalence is a consequence of donkeys and horses living together on the same pasture. It is known that donkeys are the most common carriers of these parasites, which transmit it to horses through contamination of common pastures. Routine deworming of horses and donkeys may help prevent cross infection when kept together. Reducing pasture contamination with infective larvae is a key preventative measure that can be achieved to a large extent with adequate management measures.

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