

Original Article

Esophageal gongylonemosis in ruminants slaughtered in Hamedan and Babol, Iran

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Summary

Members of the genus *Gongylonema* are thread-like nematodes that infect the upper digestive tract of birds, various mammals, and humans. To date, the infection with *Gongylonema* species has been reported in over 60 human cases, including one in Iran. This study was planned to investigate the state of infection in cattle, sheep, and goats slaughtered in Hamedan industrial abattoir, and sheep and goats slaughtered in Babol industrial abattoir. From March 2018 to June 2019, full-length esophagi were collected from 384 cattle (Hamedan) and 584 sheep and goats (384 from Hamedan and 200 from Babol). The esophagi were cut longitudinally and inspected for observation of zigzag burrowed tunnels in the mucosa. The tissue-embedded worms were carefully retrieved, preserved in ethanol/glycerine and examined microscopically. Out of 968 examined animals, three (0.8%) cattle harbored *Gongylonema* spp. nematodes in their esophagus. The relative length of left spicule/body length in the only male nematode was 48.94, suggestive of *G. pulchrum*. This study provides the first information on *Gongylonema* infection of livestock in Hamedan province. The low infection rate of cattle and absence of infection in small ruminants suggests that climate change and/or improvement of rearing conditions and hygiene measures might have led to this. The combination of molecular and morphological methods is crucial in speciation of *Gongylonema* spp. in ruminants, especially *G. pulchrum* and *G. nepalensis*, which are almost identical morphologically wise.

Keywords: Gullet worm, Livestock, Small ruminants, Vector-borne, Zoonoses

Introduction

The genus *Gongylonema*, a member of the superfamily Spiruroidea, includes thread-like species capable of infecting birds, various

mammals, and humans. The life cycle of the parasite is indirect and the mammalian host gets infected through ingestion of the infected arthropod intermediate (beetles and

cockroaches) and paratenic hosts or via drinking contaminated water (Liu et al., 2017). Although *G. pulchrum* is a common parasite of domestic herbivores, the numerous other *Gongylonema* species usually parasitize rodents and birds (Setsuda et al., 2018). So far, over 60 *Gongylonema* infected human cases, including a patient in Iran, have been reported (Liu et al., 2017; Molavi et al., 2006). The typical clinical presentation in the majority of patients is sensation of something migrating within the epithelia of the oral cavity, which could be interpreted as delusion and psychosis (Molavi et al., 2006). There is also one case of *Gongylonema* infection reported in the eyeball anterior chamber of an adult patient (Waisberg et al., 2018). However, species-level identification of the parasite has rarely been performed in human-infecting *Gongylonema*.

In Iran, adult nematodes have been reported from the esophagi of sheep, goats, buffaloes, and cattle (Tavassoli et al., 2007), camels (Mirzayans and Halim, 1980), donkey (Movassaghi and Razmi, 2008), wild boars (Eslami and Farsad-Hamdi, 1992), rats (Homayouni et al., 2007), domestic and wild canids (Heidari et al., 2017), and pigeons (Borji et al., 2013). However, except for one molecular-based study on *Gongylonema* of cattle slaughtered in Mazandaran Province, north of Iran (Halajian et al., 2010), gullet worms of ruminants have been reported

traditionally as *G. pulchrum* based on morphological features of the anterior end and the site of infection. However, recently a novel *Gongylonema* species (*G. nepalensis*) has been isolated from domestic and wild ruminants in Asia and Europe (Setsuda et al., 2016; Varcasia et al., 2017).

This study was performed to investigate the rate of esophageal infection in cattle, sheep and goats slaughtered in the Hamedan industrial abattoir (west of Iran), and sheep and goats slaughtered in Babol industrial abattoir (north of Iran).

Materials and methods

From March 2018 to June 2019, full-length esophagi were collected from 968 ruminant livestock, including 384 cattle (*Bos taurus*) and 584 sheep (*Ovis aries*) and goats (*Capra hircus*). All the cattle and 384 small ruminants were slaughtered in Hamedan, and 200 small ruminants were slaughtered in Babol. Hamedan is located in western Iran with a cold and mountainous climate, and Babol is in northern part of the country with a Mediterranean climate. The esophagi were cut longitudinally and inspected thoroughly for observation of superficial zigzag burrowed tunnels in the mucosa of the organ. The worms embedded in the tissue were carefully retrieved, preserved in ethanol containing glycerine, and studied morphologically.

Results

Out of 384 examined esophagi of cattle, three (0.8%) harbored worms. Parasites were found in the proximal ($n=2$) and middle portions ($n=1$) of the esophagi. Typical cuticular bosses arranged in longitudinal rows on the anterior end of the worms were consistent with *Gongylonema* spp. The only fully isolated male *Gongylonema* was measured 46 mm in length and 264 μm in width with a 22.1 mm long left spicule. The relative length of the left spicule/body length was 48.94, suggestive of *G. pulchrum*. Although special care was paid for isolation of the other two female nematodes, they were not recovered fully to be measured precisely, hence those are reported as *Gongylonema* spp. Infection was not observed in the esophagi of small ruminants. The *Gongylonema* spp. in esophagus shown in Figure 1.



Fig. 1. The *Gongylonema* spp. in esophagus

Discussion

In this study, three nematodes including one male specimen with morphological characteristics of *G. pulchrum* and two female *Gongylonema* spp. specimens were isolated from the esophagi of infected cattle in Hamedan, western Iran.

Due to the paucity of critical morphological data on herbivores-infecting *Gongylonema*, all of the gullet worms have been named *G. pulchrum* by tradition. However, in 2013, a morphologically and genetically distinct *Gongylonema* species was isolated from buffaloes in Nepal (Makouloutou et al., 2013), which was further recognized as a new species, and was named *G. nepalensis* (Setsuda 2016). Subsequently, the newly described species was found to be distributed in Sardinia, Italy, where steer, sheep, goat, and mouflon were reported as natural hosts (Varcasia et al., 2017). Very recently, red fox and wild boar were recognized as definitive hosts for *G. nepalensis* (Setsuda et al., 2020). Two *Gongylonema* species known to infect ruminants are almost identical in morphology, except for distinctly different sizes of left spicule relative to the entire body. Based on the latest morphometric analyses, these ratios range between 24.6–64.1 and 15.5–21.2 for *G. pulchrum* and *G. nepalensis*, respectively (Setsuda et al., 2020). The male nematode

found in the present study had morphological characteristics of *G. pulchrum*, since the relative length of its left spicule/body length was 48.94. Molecular examination of the specimens isolated in this study will be performed upon collection of more specimens from other regions of Iran.

To track the possible presence of *G. nepalensis* in Iran, a thorough search through published articles, postgraduate theses, and proceedings of conferences was performed. Only in a few studies on gongylonemosis of livestock, morphometric analyses have been done. In a study on 1038 cattle, buffaloes, sheep, and goats in Urmia, 225 adult nematodes were isolated. Morphometrics were performed on 50 nematodes. Male nematode lengths were 20–43 mm (average 29 mm), and mean length of the left spicules was 14 mm (Tavassoli et al., 2007), suggestive of *G. pulchrum*. The measurements of single worms are not available for further analyses (personal communication of the first author with M. Tavassoli and M. Nazarimanesh). In another study from Iran, 12 out of 114 recovered *Gongylonema* nematodes (six males and six females) from cattle were measured. Six male nematodes were measured 36.7–48.6 mm (mean = 41.9±4.8 mm) with length of the left spicules ranged from 10.60 to 27.86 mm (mean = 18.87±6.46 mm) (Halajian et al., 2010), suggestive of *G. pulchrum*. The measurements

of single worms are not available for further analyses (personal communication of the first author with A. Halajian). In other reports, gullet worms from livestock have been reported as *G. pulchrum* without providing the measurements. In the sole human gongylonemosis report of Iran, the morphology of both isolated male and female worms is different from *G. pulchrum* and *G. nepalensis* (Molavi et al., 2006). Further morphologic analysis of the specimen is set to be performed for precise speciation (personal communication of the first author with G. Mowlavi). It is strongly suggested that precise measurements be documented by researchers in future studies.

In this study, molecular analysis of the samples was not performed. However, in the only molecular biology-based study from Iran, two out of 114 recovered *Gongylonema* nematodes from cattle were subjected to polymerase chain reaction (PCR) and nucleotide sequencing techniques, by which, the nematodes were identified to be *G. pulchrum* (Halajian et al., 2010). The combination of molecular and morphological methods is crucial in the speciation of *Gongylonema* species, especially *G. pulchrum* and *G. nepalensis*, which are almost identical morphologically wise (Setsuda et al., 2016; Setsuda et al., 2020; Varcasia et al., 2017).

In the present study, nematodes were found in proximal ($n=2$) and middle third ($n=1$) of the esophagi. Due to the low number of worms, distribution of adult nematodes in the esophagus cannot be discussed, however, in a previous research from Iran, 26, 41 and 47 worms were collected from the upper, middle, and lower esophageal parts of native dairy cattle (Halajian et al., 2010). Although esophagus is the most investigated organ for gongylonemosis in animals, the buccal, lingual, pharyngeal, and gastric mucosae are other sites which could be inspected in future studies (Kudo et al., 2003).

We found three infected cattle (0.8%), which is the lowest in comparison with previous reports from Iran, with a range from 11.3% to 49.7% (Anwar et al., 1979; Eslami and Fakhrzadegan, 1972; Halajian et al., 2010; Kheirandish et al., 2013; Tavassoli et al., 2007). In addition, no esophageal infection was found in the examined sheep and goats, while in previous studies from Iran, the infection rates of 4.08–8.5% in small ruminants were reported (Eslami et al., 2010; Tavassoli et al., 2007; Youssefi et al., 2011). The observed decrease in ruminants gongylonemosis in Iran might be resulted from: i) major changes in husbandry system from traditional grazing to industrialized farming and improving the hygiene measures, which may have resulted in subsequent less

exposure of livestock to infected intermediate vectors, ii) regular administration of effective anthelmintics to livestock, and iii) microclimatic changes which may have led to shift in habitats of coprophagous beetles (Menéndez and Gutiérrez, 2004).

This study provides the first information about *Gongylonema* infection of livestock in Hamedan. The low infection rate of cattle and absence of infection in small ruminants of the region shows that infected intermediate hosts are not abundant in the area, therefore, the risk of human infection might be low. The decrease in ruminants gongylonemosis probably suggests that climate change and/or improvement of rearing conditions and livestock management are effective factors. Further epizootological studies with the aid of molecular biology tools are necessary to find out if *G. nepalensis* does occur in Iran.

Ethical approval

Not applicable.

Conflict of Interest Statements

The authors declare that they have no conflict of interests.

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