Macroscopic and microscopic examination of pulmonary *Crenosoma striatum* in hedgehog

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Abstract The aim of study was to necropsy and histopathology evaluation of lung *Crenosoma striatum* in hedgehog. In July 2012, 10 porcupines were collected from Urmia city and transferred to parasitology lab of the veterinary faculty where they were euthanized by ketamine (over 40–90 mg/kg) intraperitoneally. In this study the lungs were assessed through naked eyes regarding parasite presence upon washing. The lung tissue was examined under loop microscope in order to finding small worms in lung parenchyma. The worms were removed by Anse forceps and kept in AFA solution, and collected for diagnosis. In order to carrying out pathological tests, some samples prepared and placed in formalin 10 % for fixation. The counted worms frequency in high severe and moderate lungs were as 86 (50 females and 36 males) and 19 (13 females and 16 males) worms respectively. But no worms were observed in healthy lungs. The infestation severity was as; low infestation (1–7

worms), moderate infestation (8–20), severe infestation (21–50) and very severe infestation (more than 50 worms). The lung examinations of non-infested lungs indicated that the lung tissues had no parasite. In addition, no inflammation reactions as inflammatory cells presence were observed, and the air spaces with alveoles' wall in some regions were observable. On histopathological examination, the observed alteration was primarily inflammatory changes, and in some cases the proliferation was also observable. Hyperemia and inflammatory cell infiltration, somehow the alveolar space was filled, representing bronchopneumonia reaction. The bronchioles had various changes as hypertrophy and hyperplastic in different parts of respiratory system. Hyperemia and hemorrhage were very severe in some cases caused hemosiderosis in the lung. In severe inflammations the pneumonia along with increasing of bronchial cells in the lumen rose as well, leading to severe verminous infestation of the lung. In regard to the obtained results, the verminous infestation of the porcupines' lung with *C. striatum* indicated inflammatory and proliferative alteration which was as inflammatory changes in mild cases, and inflammatory and proliferative stances in severe cases.

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Introduction

Hedgehogs (*Erinaceus europaeus*) are common hosts to a diverse range of both endo- and ectoparasites (Sykes and Durrant 2005). Hedgehogs are small, nocturnal, spiny-coated animals that have been growing in popularity as exotic pets. Thus, they are often submitted to veterinary surgeons or animal rescue centers. These animals are host

to a wide variety of viruses, bacteria, fungi and parasites, some of which are of zoonotic character (e.g., *Herpesvirus*, *Salmonella* spp., *Trychophyton mentagrophytes* var. *erinacei*, *Eucoleus aerophilus* syn. *Capillaria aerophila*) (McCarthy and Moore 2000; Riley and Chomel 2005). Hedgehogs play an important ecological role due to their position within the food chain. Natural predators include eagles (Accipitridae), stoats (*Mustela ermine*), polecats (*Mustela putorius*), foxes (*Vulpes vulpes*) and more commonly badgers (*Meles meles*) (The Mammal Society 2012). In turn, hedgehogs predate a large number of invertebrates and small vertebrates, which has been found to significantly influence localised populations. Therefore, the equilibrium between hedgehog predators and their prey could be detrimentally affected by further decline or potential extinction. Furthermore, hedgehogs serve as a reservoir for a variety of pathogens; therefore loss of this species could consequently cause a significant disparity between parasites and wildlife in the UK (Gaglio et al. 2010). Additionally a number of these pathogens are zoonotic and therefore carry the risk of transferring disease to humans (Khaldia et al. 2012). Although there have been several studies of hedgehog parasites in other countries (Laux 1987; Boag and Fowler 1988; Lowenstein et al. 1991; Slapeta 1999; Poglayen et al. 2003), the parasites of hedgehogs in Iran have received little attention, and the studies that have been published are quite limited. The aim of this study was to macroscopic and microscopic examination of lung *Crenosoma striatum* in hedgehog.

Materials and methods

In July 2011, 10 porcupines were collected from Urmia city and transferred to parasitology lab of the veterinary faculty where they were euthanized by ketamine (over 40–90 mg/kg) intraperitoneally. The visceral organs of animals were removed from the necropsied abdomen. The lungs were assessed through naked eyes regarding parasite presence upon washing. The lung tissue was examined under loop microscope in order to finding small worms in lung parenchyma. The worms were removed by Anse forceps and kept in AFA solution, and collected for diagnosis. In order to carrying out pathological tests, some samples prepared and placed in formalin 10 % for fixation. Furthermore, fixed tissues were cut by microtome set through paraffin and stained by H&E and examined for pathological lesions of *C. striatum* nematode by microscope.

Results

In this study, 10 porcupines were examined, and revealed that the counted worms frequency in high severe and

moderate lungs were as 86 (50 females and 36 males) and 19 (13 females, 16 males) worms respectively. But no worms were observed in healthy lungs (Fig. 1). The infestation severity was as; low infestation (1–7 worms), moderate infestation (8–20), severe infestation (21–50) and very severe infestation (more than 50 worms) (Fig. 2). The lung examinations of non-infested lungs indicated that the lung tissues had no parasite. In addition, no inflammation reactions as inflammatory cells presence were observed, and the air spaces with alveoles' wall in some regions were observable (Figs. 3, 4). Concerning infested group, the observed alteration was primarily inflammatory changes, and in some cases the proliferation was also observable. Hyperemia and inflammatory cell infiltration, somehow the alveolar space was filled, representing bronchopneumonia reaction. In bronchioles the parasite particles along with inflammatory cells, primarily eosinophils, were observed. (Fig. 5). The bronchioles had various changes as hypertrophy and hyperplastic in different parts of respiratory system (Figs. 6, 7). Hyperemia and hemorrhage were very severe in some cases caused hemosiderosis in the lung (Fig. 8). In severe inflammations the pneumonia along with increasing of bronchial cells in the lumen rose as well, leading to severe verminous infestation of the lung (Figs. 9, 10). In regard to the obtained results, the verminous infestation of the porcupines' lung with *C. striatum* indicated inflammatory and proliferative alteration which was as inflammatory changes in mild cases, and inflammatory and proliferative stances in severe cases.

Discussion

The porcupines is a sort of small mammalian, coated with spikes, existing in urban, rural and suburb zones and has

Fig. 1 Lungworm infection of hedgehogs was not with nematode *Crenosoma striatum* in macroscopic pulmonary

Fig. 2 Lungworm infection of hedgehogs was with nematode *Crenosoma striatum* in macroscopic pulmonary

Fig. 5 Alveolar space hyperemia and inflammatory cell infiltration into the alveolar spaces are represented Bronchopneumonia ($\times 400$, H&E)

Fig. 3 Photomicrograph of histopathology sections showing lungworm infection of hedgehogs was not with nematode *Crenosoma striatum* ($\times 400$, H&E)

Fig. 6 Hypertrophic and hyperplastic bronchiolar epithelial cells in the respiratory tract ($\times 400$, H&E)

Fig. 4 Photomicrograph of histopathology sections showing lungworm infection of hedgehogs was with nematode *Crenosoma striatum* or cross sections of trematodes within bronchioles and infiltrating of inflammatory cells ($\times 400$, H&E)

Fig. 7 Bronchovascular bundles hyperplasia (muscular bronchiolar) ($\times 400$, H&E)

Fig. 8 Severe hyperemia and hemorrhage is seen that lead to the formation of hemosiderin (hemoglobinogenic pigment) (×400, H&E)

Fig. 9 Pneumonia demonstrated significant increases of inflammatory cells in the respiratory tract (×400, H&E)

Fig. 10 Micrograph displaying the larvae of *Crenosoma striatum*

close relationship with humans environment. Thus, the evaluations prepare new information regarding ecology and behavior of such animals is of high benefit. The disseminated information concerning verminous parasites in porcupines' lung is slightly available (Akil and William

2010). In 1976–1986 in Norway some researches was carried out in order to detecting zoonosis diseases in porcupines, hence the results indicated the *C. striatum* as a zoonotic disease in porcupines (Keymer et al. 1991). Additionally, some researches in Italy were carried out concerning parasitic infestation in porcupine which demonstrated some rates as: *Hymenolepis erinacei*, *Gongylonema neoplasticum*, *Physaloptera clausa*, *C. striatum* and *Capillaria erinacei*. In this study the dominant species was *C. striatum* (Poglayen et al. 2003). In another study, was carried out in Turkey, indicated that there were 6 worms in porcupine's lung including *P. clausa* (55.5 %), *C. striatum* (72.2 %), *H. erinacei* (55.5 %), *Onchota erinacei* (55.5 %), *Nephridiorenicus major* (50 %) and *Uceleus aerophilus* (22.2 %). In present study the most frequency and severity of infestation was associated with *C. striatum* (Ciraka et al. 2010). In an assessment in Turkey on parasitic infestation in porcupine lung revealed that one of the obvious manifestations was eosinophilic bronchitis originates from or parasitic infestation with *C. striatum*, and allergic reactions were triggered by environment antigens (Ciraka et al. 2010). The researches indicated that eosinophilic bronchitis in porcupines primarily originates from *C. striatum* nematode infestation. The results of this study are similar to others' findings. In such assessment, of 10 porcupines, the counted worms in a lung with very severe, moderate-severe infestations were as 86 and 19 respectively, and no worms were found in healthy lung. In present study demonstrated that the infection severity rate is in direct correlation with tissue reactions, somehow in porcupine with 86 nematodes infestation the created inflammatory reactions to a group with high severe infestation, eosinophilic infiltration and other inflammatory cells were remarkably obvious in comparison with healthy sample. Although the common parasitic infestation in porcupines was *C. striatum*, the *C. erinacei* along with former nematode was also visible, inducing respiratory system failure. *Capillaria* is a nematode containing single-host or voluntary intermediate host life cycle, producing pathologic lesions as inflammatory alterations in lung, trachea, bronchopneumonia and presence of the parasite in the lung (Majeed et al. 1989). Another infestation exist in porcupines' lung is *Metastrongylus elongatus* infestation. The pathological consequences of this nematode are mild pneumonia in association with pulmonary emphysema, bronchiolar thickening and extensive hemorrhage. In addition, its egg and larvae induces granuloma in the lung (Polley et al. 2010). In present study, infestation of *C. ernacei* nematode and *M. elongatus* were not recorded. Consequently, pathological alterations stemming from *C. striatum* nematode in the lung was similar to two previous cited nematodes. The *Angiostrongylus* genus is another nematode was found in various species' lungs. The

pathological lesions of such nematode were recorded such as hemorrhage, inflammatory cells infiltration, multifocal granulomatous pneumonia and sometimes foci of chronic thrombi. The infested animals by such nematode contain more severe pulmonary lesions, causing corpulmonale. For this, the severity of lesions is high for this nematode, whereas is not similar to the current results. The inflammatory reaction of this study indicated direct effect of such parasite in respiratory duct, leading to releasing of inflammatory mediators inducing inflammatory reactions (Bunnell et al. 2011). The allergic reaction in similar an assessment by Majeed et al. (1989) demonstrated similar results. In present study hyperplastic changes, indicating long-period stimuli of cells next to the parasite. According to such studies it can be cited that the hyperplastic changes are being induced due to chronic stimuli which absolutely was ascertained in this study. The rate and severity changes of hyperplastic alterations are in direct correlation with infestation severity, and by increasing the infestation rate, the proliferation changes rise as well. The bronchiolar smooth muscle hyperplasia in this study may be due to large pressure on muscular structure, has been created following passages obstruction, by the parasite and exudates. Indeed, it is a secondary pathologic change due to primary obstruction changes. In present study, the granulomatous changes were not observed; however, the detected cellular classes in the inflammatory reactions were including lymphocyte, eosinophil, and macrophages. The common alterations were as bronchopneumonia along with bronchiolar smooth muscle epithelial hyperplasia, were dependent on severity of cases, can be considered as rate and severity of the observed reactions including host immunity reaction rate and recurrent infections. On the other hand, the repeated tissue exposure to parasite following primary exposure can play important role in lesions severity and the produced lesions through hypersensitivity may be emerged as diffuse forms. In present study, the bronchiolar lesions were diffused and extensive. Furthermore, such study indicated the importance role of *C. striatum* worm in inducing bronchiolar and pulmonary inflammation along with hyperplastic alterations.

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