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Source: Journal of Wildlife Diseases, 42(2) : 397-401

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-42.2.397>

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***Tetrameres (Tetrameres) grusi* (Shumakovich, 1946) (Nematoda: Tetrameridae) in Eurasian Cranes (*Grus grus*) in Central Iran**

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ABSTRACT: The proventriculi of 11 Eurasian cranes (*Grus grus*) from central Iran were examined for the existence of parasitic helminths. Preliminary reports suggested that the death of these birds was related to untimely cold weather. Nine proventriculi (82%) were heavily infected by the nematode *Tetrameres grusi*. Glandular structure of the infected proventriculi was replaced by epithelial atrophy but significant inflammatory reactions were not observed in any of the infected organs. In serious infections, the nematode produced vast structural and functional changes, causing organ dysfunction and glandular necrosis. The coincidence of heavy helminth infection at times of environmental stress may lead to debilitation, wasting, and perhaps mortality in migratory cranes.

Key words: Eurasian crane, *Grus grus*, helminth infection, Iran, proventricular worm, *Tetrameres grusi*.

Cranes are among the world's most endangered groups of birds, and of the family's 15 species, as many as 11 may be globally threatened (Meine and Archibald, 1996). The Eurasian crane (*Grus grus* L.) is the third most abundant species of crane, second only to sandhill (*Grus canadensis*) and demoiselle cranes (*Anthropoides virgo*). The species' breeding range extends from northern and western Europe across Eurasia to northern Mongolia, northern China, and eastern Siberia, with isolated breeding populations in eastern Turkey and Tibet. The winter range includes portions of France and the Iberian Peninsula, North and East Africa, the Middle East (including Iran), India, and southern and eastern China (Meine and Archibald, 1996). These birds are omnivorous, and tend to make use of a broad range of vegetation and animal foods, perhaps explaining the diverse

parasite species reported from this host (Forrester et al., 1974; Iverson et al., 1983). Although the Eurasian crane is not globally threatened, some populations have been declining in recent years (Markin and Sotnikova, 1995).

Relatively little research has been done on the occurrence of parasitic infections in Eurasian cranes; nearly all of the existing information comes from studies of sandhill crane and whooping crane (*Grus americana*; Spalding et al., 1996). Every year, several thousand birds of the European-Russian population migrate east of the Black Sea to wintering grounds in southwest Iran. Nonetheless, there have been no representative data on the helminth parasites of this host in Iran. The current work, therefore, used an opportunistic sample collection to learn more about the helminth parasites of Eurasian cranes in central Iran.

The study was performed in the Davood-Abad district (49°40'N, 34°09'E) in central Iran (320 km from Tehran, 1,708 m above sea level) in January 2000. According to historical and official documentation, this area has been a permanent sheltering place of cranes over the past two centuries. Temperature ranges between a mean of -5.6 C in January and 32 C in August. Eurasian cranes spend nearly 1 mo in this region prior to migrating to their main wintering sites in Luristan and Khuzistan provinces in western and southwestern Iran, respectively (H. Farhadpour, unpubl. data). The accessibility of diverse plant and animal foods makes this vast and protected area a suitable shelter for cranes during their migration.

In early winter 2000, the Iran Department of Environment reported the unexpected death of more than 500 Eurasian cranes in Davood-Abad district. Unexpected cold weather and premature frost were considered the cause of the mortality. Several dead cranes were scattered over the area in January 2000; however, most were eaten by carnivores. Fifteen partial carcasses were collected, from which 11 birds were appropriate for parasitological investigations.

Each gastrointestinal portion was placed in vials containing 10% buffered formalin, and transferred to the Division of Helminth Taxonomy at Tehran University of Medical Sciences. Each section was opened longitudinally and the contents were washed through a 100- μ m sieve and examined under a binocular microscope. All helminths were removed and placed in vials containing hot 70% alcohol, cleared in lactophenol, and examined at 400 \times magnification. They were identified according to measurement of various structures, including left and right spicules in males, as well as by the arrangement and shape of cuticular spines, buccal capsule, esophagus, anterior and posterior body projections, and by numbers of papillae around the mouth in females (Bush et al., 1973). Representative specimens have been deposited at the Helminthological Museum, School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences (accession no. 6576).

Parts of infected proventriculi were fixed in 10% buffered formalin and prepared for histopathological examination (Luna, 1968). The staining procedure (hematoxylin and eosin) was done by autostainer (Leica Autostainer XL, Leica Instrument, Nussloch, Germany). Stained tissue samples were mounted in Canada balsam and studied under light microscopy (400 \times).

Proventriculi of nine of the 11 birds (82%) were heavily infected by both female and male *Tetrameres grusi* as described by Bush et al. (1973). Intensity

of infection ranged from 79 to 231 worms. Females were blood-red in color, sub-spherical, and without teeth and lips. The mouth in female worms was round with four papillae in an outer circle; the buccal capsule was hexagonal and situated anteriorly. The intestine was saclike. The uterus was coiled, filling most of the body. Eggs were 44–53 μ m long and 26–33 μ m wide, with polar thickening. They were thick shelled and embryonated when laid. Males were white, with an oval, elongate mouth; the buccal capsule had a thick sclerotized lining. The tail was without a terminal papilla or spine, and a gubernaculum was absent. The body had a single row of minute lateral spines 10 to 17 μ m (mean of 13) in length (Table 1).

The glandular structure of the proventriculus was distended and contained larval and adult nematodes. The posterior end of the female worms generally emerged into the lumen of the proventriculus (Fig. 1). Lesions in the submucosa were surrounded by a thin fibrous cyst with or without adjacent inflammatory cells. The glands in close contact with the parasites were attenuated or atrophic and lesions in the submucosa were moderately or completely surrounded by a thin rim of fibrotic cyst with or without adjacent inflammatory cells. Other than partial fibrosis of the infected or uninfected glands, there were no considerable inflammatory reactions or other tissue changes. The copious and fleshy females occupied approximately all proventricular glands and caused severe catarrhal conditions in infected proventriculi. Infected mucosae were compressed as a result of pressure atrophy by the large gravid females. The pressure exerted by the growing nematodes caused atrophy or obliteration of the glandular structures. Grossly, only limited areas were unaffected, suggesting organ dysfunction.

The European-Russian and western Siberian breeding populations of the Eurasian crane migrate to wintering grounds in western and southwestern Iran, and along-

TABLE 1. Comparative measurements of *Tetrameres grisei*.

Measurement ^a	Bush et al. (1973)	Present study	Female	
			Male	Present study
Body length (mm)	3.6-4.6 (4.2) ^b	3.2-4.81 (3.78) ^b	Body length (mm)	3.24-5.50 (4.40) ^b
Body width	135-140 (138)	130-155 (140)	Body width	1.37-2.73 (2.26)
Buccal capsule length	20-23 (22)	19-21 (20)	Anterior body projection	850-1,300 (1,028)
Buccal capsule width	15 (maximum)	18 (maximum)	Posterior body projection	350-790 (577)
Esophagus length (mm)	1.2-1.3 (1.28)	1.0-1.3 (1.14)	Buccal capsule length	14.3-17.5 (15.3)
Esophagus/body length ratio	1:3.1-1:3.5 (1:3.4)	1:2.9-1:3.4 (1:3.0)	Buccal capsule width	8,8
Nerve ring from anterior extremity	240-250 (216)	215-234 (200)	Esophagus length (mm)	1.52-1.91 (1.8)
Excretory pore from anterior extremity	240-260 (251)	231-253 (242)	Nerve ring from anterior extremity	160-210 (201)
Left spicule length	20 (in some worms)	absent	Excretory pore from anterior extremity	210-310 (262)
Right spicule length	625-750 (681)	634-812 (790)	Tail length	130-175 (148)
Left/right spicule ratio	1:4.7-1:6.3 (1:5.2)		Vulva from posterior end	372-478 (442)
Cuticular spines	12	10-17 (13)	Egg length	45-50 (49)
			Egg width	30-34 (31)

^a Measurements are in microns unless otherwise indicated.^b Range (mean).



FIGURE 1. Gross appearance of longitudinally opened infected proventriculus. Each focus represents a female nematode. Some female worms have been detached and their empty places are evident. Bar=1 cm.

side the Iran-Afghanistan border, respectively. In association with anthropogenic changes such as conversion of wetlands and grasslands, deforestation, and environmental contamination, the number of cranes in these two populations is declining (Markin and Sotnikova, 1995). Disease, when added to the stressful conditions associated with long-distance migrations, abrupt temperature fluctuations, and overcrowding during migration, may pose a significant threat to wild crane populations.

Tetrameres grusi was initially described on the basis of five male specimens from the Eurasian crane in western Siberia (Bush et al., 1973). Mature females lie embedded in the crypts of Leiberkuhn and feed on blood, whereas males occur freely in the lumen of proventriculus. In heavily infected birds, the nematodes result in severe proventriculitis and may cause loss of glandular function, leading to a debilitating status characterized by emaciation and anemic conditions (Soulsby, 1982). This condition may be of vital importance in migratory wild birds. *Tetrameres mohtedai*, a species found in fowl, produces severe pathological effects in infected birds, including erosion and desquamation of glandular epithelium. Along with these changes, there is an adenomatous proliferation of the glandu-

lar epithelium together with fibrosis. These tissue changes are responsible for thickening of the walls of the proventriculi in such a way that the lumen is almost blocked (Soulsby, 1982). In heavy infections, worms can be so numerous that only a small portion of the stomach wall remains unaffected. In addition, glandular atrophy and partial fibrosis of the glands may cause malnutrition resulting in emaciation and anemia as seen in heavily infected chickens.

It appears that the *Tetrameres* parasites may be nonimmunogenic or may escape the host immune responses and such a mechanism may be of importance in survival in the bird tissues. The outcome of severe tetrameriasis may be of importance in migratory birds, because these birds have to travel lengthy cross-continental distances. Intense parasitic infections in these migratory birds along with stressful environmental factors may have caused exhaustion in some individuals.

We are grateful to George Archibald, International Crane Foundation at Baraboo, Wisconsin, for comments on the manuscript. We are also indebted to Betsy Didrickson of the same foundation for providing the reprints. Thanks are due to the dean and staff of the Markazi Environmental Protection Organization for their invaluable aids. We thank sincere assistance of H. Farhadpour.

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Received for publication 26 September 2004.