Alaria alata Infection in European Mink

To the Editor. Alariosis is a re-emerging zoonotic disease caused by infection with larval stages of trematodes of the genus Alaria. The trematodes are found in wildlife that inhabit wetlands, and these animals may serve as possible reservoirs for these organisms that cause human infection (1). The main sources for human infection are suids and frogs (1). In humans, the clinical features of alariosis caused by infections with the North American species of Alaria vary from mild and asymptomatic to moderate with respiratory or cutaneous signs (2) or neuroretinitis (3), to severe-to-lethal anaphylactic shock caused by larva migrans (4,5). The genus Alaria has 7 species; only A. alata is found naturally in Europe (6), a species which has not thus far been shown to be responsible for human infections.

A. alata infection is common in its typical definitive host (red fox, Vulpes vulpes) and in certain paratenic hosts (wild boar, Sus scrofa) (1). However, the role of other paratenic hosts is poorly known. Among these, mustelids are reported to harbor mesocercariae of A. alata trematodes (7). The pathogenic effect of A. alata infection has been poorly studied, because most lesions described were in humans infected with other species of Alaria. Except for 2 experimental studies that described gross lesions produced by A. alata trematodes (6,8), to our knowledge, no data have been published concerning lesions produced by natural infection in nonhuman hosts. Our report provides a detailed description of the lesions, shown by microscopy, which suggests the pathogenic mechanisms.

One adult female European mink (Mustela lutreola) was found dead during standard surveillance operations in which box traps were used; this trapping was part of biodiversity and ecology studies in the central part of the Danube delta in Romania (45°08′ N, 29°19′E) in March 2010. The corpse was deep-frozen and analyzed after 3 months in the laboratory. During necropsy, multiple, well-defined, whitish

Figure. Lesions produced by mesocercariae of Alaria alata in European mink. A) Mesocercariae in the muscle and subcutaneous tissue produce whitish, round or slightly oval, well-defined nodules. B) Free mesocercarium after artificial digestion, showing the characteristics of A. alata mesocercarium: piriform body with anterior oral sucker (OS), acetabulum (AC) positioned in the center of the parasite, 2 pairs of large, finely granulated penetration glands (white stars), limiting the anterior part of the acetabulum, linear ducts of penetration glands (DPG) converging to the oral opening of the oral sucker and large double ceca placed posterior to the acetabulum. C) Histologic section showing an encysted mesocercarium in the muscle (parasite is surrounded by a capsule and pericystic inflammation, which extends to the surrounding muscular tissue). D) Mononuclear leucocytes (arrowheads) scattered between the fibroblastic proliferations (white arrows) and collagen deposits (black stars). Muscle fibers are atrophic due to compression (black arrows). E) Microscopic detail of the inset from panel C. Some mesocercariae (indicated by black arrow) are enclosed in a thin, pale staining capsule (white arrows). Note the lack of leukocyte response. F) Migration route of the parasite (route with center marked by the black star), followed by invasion of nonnecrotic muscle fibers by mononucleate inflammatory cells (white arrow) located mainly in the center of the migration tract and fibrous connective tissue with collagen fibers densely packed at the periphery (black arrows) and more loosely in the center (arrowheads). Hematoxylin–eosin stain (panels C, D, E); Masson trichrome (panel F); original magnifications x40 (panels B and C), x200 (panels D and F), and x400 (panel E). A color version of this figure is available online (wwwnc.cdc.gov/EID/article/19/09/13-0081-F1.htm).
nodules were observed in most muscular and subcutaneous tissues (Figure, panel A), with no evident preferential localization. We collected samples from these tissues for artificial digestion (9,10) and histologic examination, using the routine paraffin-embedding protocol and the following staining methods: hematoxylin-eosin, Masson trichrome, and Gordon and Sweet.

Artificial digestion released parasites (6 larvae/5 gm tissue) with typical larval trematode structures (Figure, panel B). By microscopy, we observed that morphologic features of these larvae were consistent with *A. alata* mesocercariae (6). Histopathologic examination confirmed the presence of parasitic forms in muscle sections (Figure, panel C). The mesocercariae were located in the connective fibrous tissue of the perimysium or between the muscle fibers. The typical structure of muscle fibers was altered around the larvae, with inflammatory cell reactions, represented mainly by lymphocytes, macrophages, and plasma cells (Figure, panel D). In other areas, the inflammatory reaction around the parasite was minimal or absent (Figure, panel E). In certain histologic sections, the damaged muscular tissue was replaced by granulation tissue in various stages of development (Figure, panel F). The maturity of the granulation tissue differed substantially, depending on the muscular areas examined. Some lesions were found in adult connective tissue, formed by mature collagen scar fibers (type I collagen) and few inflammatory cells, whereas other lesions had reticulin fibers (type III collagen) with numerous inflammatory cells. The lesions of the subcutaneous connective tissue consisted of an inflammatory reaction (panniculitis). The inflammation was characterized by a low number of mononuclear leukocytes and fibrinous exudate and fibroplasia.

The polyphasic nature of muscle and subcutaneous lesions produced by *A. alata* infection in its paratenic host appears to be caused by mesocercarial migration. This view is sustained by the presence of mononuclear cells that it infiltrates and by the appearance of the granulomatous tissue in various stages of maturation, which leads to muscle and subcutaneous fibroplasia. The reparatory nature of the lesions suggests that the inflammation is probably the result of direct tissue damage rather than an immune reaction targeted toward the parasitic antigens. This assumption could explain the local absence of inflammatory reaction around the parasites. The lack of inflammation was previously observed also with *A. americana* infection of humans (4). The structure of all mesocercariae observed by microscopy suggested that they were alive and active before the mink carcass was frozen. Because no mesocercariae were surrounded by adult connective tissue or by granulomatous inflammation, together with the multiple presences of migratory routes, the continuous mobility of the parasites through the host’s tissues was strongly suggested.

Although data on the pathologic changes caused by *Alaria* spp. in general, and *A. alata* parasites in particular, are scarce, the migration pattern and the lesions seem to be dependent on the particular parasite and host species. The reparatory nature of the lesions suggests that the inflammation is the result of direct tissue damage rather than an immune reaction targeted toward the parasitic antigens.

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Serologic Survey of Plague in Animals, Western Iran

To the Editor: Plague has been one of the most devastating infectious diseases in human history. The etiologic agent, Yersinia pestis, primarily affects rodents and is usually transmitted to humans through infective flea bites. Endemic plague foci result from circulation of the plague bacillus in its rodent reservoir, the source of human plague cases (1). Carnivores such as dogs and foxes, which prey on rodents and eat their fresh carcasses, are valuable sentinel animals for plague serosurveillance in disease-endemic foci, although their infections are usually asymptomatic (2,3).

Plague epidemics have caused loss of human life in various parts of Iran. During 1947–1966 in western Iran, 9 human epidemics occurred and caused 156 deaths. The last case of human plague was reported in 1966 (4). Field investigations identified 4 Meriones rodent species as Y. pestis reservoirs; 2 were resistant (M. persicus and M. libycus), and the other 2 (M. tristrami and M. vinogradovi) were susceptible to death from infection (4,5). The epidemiologic investigations demonstrated a 3–4 year plague epizootic cycle in Iran (5). The last official report of plague in rodents in Iran dates back to 1978, in Sarab County in the East Azarbaijan Province (6). Plague surveillance was ignored for more than 3 decades and then restarted in 2011 in Iran.

This study was designed to investigate plague among resident animals in western Iran, specifically region localities along the border between the Kurdistan and Hamadan Provinces, where plague in wildlife has been repeatedly reported (enclosed by 47.900° and 48.284° north latitude and 35.4616° and 35.7829° east longitude). The epidemiologic team was based at the Akanlu Research Center of the Pasteur Institute of Iran, in a village ≈100 km from Kabudar Ahang, Hamadan Province, at an altitude of ≈1,600 m. The study was conducted during June–September in 2011 and 2012. In 2011, a large area (2,000 km²) was selected and, because only 1 Y. pestis-positive dog sample was found, in 2012, the study area was reduced to 1,200 km² and confined to localities in which the Y. pestis–positive dog sample was identified the previous year; 3 additional Y. pestis–positive dogs and 1 Y. pestis–positive rodent were found in 2012.

The average number of traps used per night per locality was 13. A total of 46 rodents were entrapped from 26 localities in 998 traps (4.61% success) during the first year, and 52 rodents were captured in 30 localities in 1,164 traps (4.46% success) during the second year. They were mostly members of the Meriones genus, although a few Microtus socialis irani and 1 Ellobius lutescens rodents were also caught (Table). A total of 281 fleas were collected on 70.41% of trapped rodents (Table), corresponding to an average flea index of 4.10 for infested rodents. All fleas were Xenopsylla spp. ELISA was performed as described (7) to detect antibodies against Y. pestis F1 capsular antigen. Samples positive by ELISA were confirmed by using the inhibition ELISA method (8). Of 98 trapped rodents, 1 (1.02%) had IgG against F1 (Table), an M. persicus jird caught in 2012.

Sheepdogs that lived in the study areas were also used as sentinel animals. Blood samples were collected from 58 sheepdogs in 15 villages in 2011 and from 59 sheepdogs in 8 villages in 2012. Of 117 dog serum samples analyzed, 4 (3.42%) had IgG titers against F1, 1 in 2011 and the other 3 in 2012 (Table). Finally, wild animals such as jackals, foxes, rabbits, and hedgehogs were hunted in the study areas, and blood samples were taken immediately. None of the serum samples obtained from 3 foxes, 2 jackals, 8 rabbits, and 1 hedgehog had IgG against F1 (Table).

Because a well-established plague focus existed in Iranian Kurdistan, with animal cases occurring until 1978 (9), complete extinction of this focus is most unlikely. Our study demonstrates that animal reservoirs (Meriones rodents) and flea vectors (Xenopsylla spp.) shown to be central to the plague epizootic cycle in Iran still are found in high numbers in a previously active focus. The fact that 70% of trapped rodents were infested with fleas, with an average Xenopsylla spp. index of 4.10, may be considered as circumstances most favorable for the onset of plague epizootics. Furthermore, the detection of Y. pestis–specific IgG in 1.02% of trapped rodents and 3.42% of sentinel dogs is highly suggestive of active circulation of Y. pestis in its natural animal reservoir. Because Y. pestis antibodies last only for ≈6 months in dogs (2), seropositivity of these dogs indicates newly acquired infections.

This fact that Y. pestis–positive animals were found over the 2-year surveillance period suggests that this area could be an active plague focus. Therefore, although no official reports of human plague in Iran have been made since 1966, this study indicates that the epidemiologic conditions needed to trigger an outbreak have been met. It is thus of utmost importance to maintain and strengthen the health system with plague surveillance in western Iran.