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Transmammary Infection of Newborn by Larval Trematodes

Abstract. *Newborn cats and mice became infected with* Alaria marcianae *if they nursed from females that had been experimentally infected with the parasite. All lactating females showed mesocercarial stages in their mammary glands. This may be the first trematode found to undergo transmission through the mammary glands under experimental conditions. Similarities in the behavior of mesocercariae in humans and in the mouse suggest that an infected human female might infect her infant if she elected to nurse it.*

Infection with the fluke *Alaria marcianae* is recognized as a significant hazard to human health (*1–3*). During our investigations of the larval migration of this parasite in laboratory mice, rats, and cats we noted that the offspring born to infected females also became infected. Although well known in "roundworms" (*4*), maternal transmission is rarely reported in the Trematoda (*5–7*). In this report we outline the route of transmission to the offspring in two mammalian hosts, propose an animal model that would best describe human infection, and discuss the significance of that model in terms of human infection.

Studies of the larval migration of *A. marcianae* in definitive hosts, such as dogs and cats, have demonstrated a complex route in which the passively ingested mesocercarial stage burrows through the stomach wall and diaphragm, enters the lungs, and there develops to the metacercarial stage. After a brief residence in the lungs this stage is coughed up and swallowed; it matures in the small intestine as early as 3 weeks later (*8, 9*). The adult is passed spontaneously from the intestine within 6 months.

Migration of *A. marcianae* in paratenic hosts such as mice and rats does not involve the complex stomach-lung-intestine migration just described for definitive hosts, nor does it result in the development of the mesocercaria to a metacercaria. This lack of further development is a parasitological phenomenon known as paratenicity. In a paratenic host mesocercariae migrate from the stomach to the subcutaneous fat, where they remain as active migratory stages. All reports on human infection have indicated that man is a paratenic host for *A. marcianae* as well. Human infection has resulted from careless handling of contaminated meat during its preparation or through actual consumption of such meat (*10*). In the human the migrating mesocercariae have been found to invade nearly every organ, sometimes with fatal results (*2*).

In our first series of experiments we used laboratory cats as definitive hosts. To determine whether young could be infected prenatally, we orally inoculated two female adults with 200 mesocercariae each and then let them mate. At parturition a total of eight neonates were removed from the two mother cats, killed, and autopsied. None was infected with any stage of *A. marcianae*. Both mother cats were passing eggs in their feces by 3 weeks postpartum.

To determine whether young could be infected postnatally, we allowed four female cats to mate and give birth. Within 24 hours after parturition each mother cat was orally inoculated with 200 mesocercariae, returned to her litter, and allowed to nurse her young. After 21 days of nursing all 14 offspring were passing *A. marcianae* eggs in their feces. Autopsies of ten of these kittens showed metacercariae in the lungs and adults in the intestines of each of them. Apparently the worms were undergoing their typical migration in a definitive host.

Only one mother was allowed to keep her litter. The other three lactating cats were killed and autopsied, and mesocercariae were observed in the mammary glands of all of them.

The surviving mother cat and her infected young were used to determine whether an infected female could transmit *A. marcianae* to a second litter and whether transmammary infection could pass into the third generation. The female was mated again, and she gave birth to a second litter. At 21 days all four offspring had *A. marcianae* eggs in their feces. The first litter was reared to maturity and the females were mated. Only one of the two females gave birth. None of the four kittens in this third-generation litter developed an infection.

In definitive host infection, then, the mesocercariae undergo a stomach-lung-intestine migration; however, if the mammal is lactating the mesocercariae are diverted from their normal migration and toward the mammary glands. The transmammary passage of parasites to the young culminates in a stomach-lung-intestine migration, with eventual elimination of mature worms. Infected females are capable of infecting sequential litters, but passage beyond the second generation does not occur because of the maturation of the worms in the kittens and their subsequent expulsion. The mesocercariae remain in the mammary glands of lactating females, presumably because they no longer receive the cues necessary to lead them to the lung and intestine.

Laboratory mice were used as paratenic hosts in the next series of experiments. To determine whether prenatal infection could occur, we orally inoculated nine female mice with 200 mesocercariae each and let them mate. Their litters were examined immediately before birth (through cesarean section) or

at birth before the neonates had begun to nurse. None of the 81 offspring was infected. The nine dams were killed and autopsied. All had mesocercariae in their mammary glands.

To determine whether postnatal infection could occur, we orally inoculated 11 parturient mice with 200 mesocercariae each, returned them to their litters, and allowed them to nurse the offspring. Before the females were infected, however, three neonates were removed from each litter and examined as controls. None was infected. Three uninfected, age-matched neonates from our rodent vivarium were transferred to each litter to restore its full complement. After 21 days a total of 99 nurslings from ten of the litters were killed and autopsied. All 99 were infected with mesocercariae, including the 30 transfers. Autopsies of ten of the lactating mothers showed mesocercariae in the mammary glands of each.

The eleventh female and her litter were used to determine whether initial infection of a paratenic host could pass to a second litter and then to a third generation. The female was mated again, and she produced a second litter of ten offspring. At 21 days postpartum the offspring were killed and autopsied. All were infected with mesocercariae. Females of the first litter were reared to maturity and mated. Only two produced litters. Autopsies of the 17 third-generation offspring 21 days postpartum showed 13 to be infected with mesocercariae.

The murine paratenic host was similar to the feline definitive host in that transmammary infection occurred in 100 percent of the second generation that nursed from infected mothers. Also, females of both species were able to infect a second litter. The two host species differed significantly in that only paratenic hosts could transmit an initial infection to the third generation. Mesocercariae undergo a complex migration in the definitive host, with diversion to the mammary glands occurring only in lactating females. In a paratenic host mesocercariae behave as though it were perpetually lactating, and in each generation they migrate to the mammary glands, there to remain, without further development, until being passed to the next generation.

Of the two mammalian species we studied, the mouse most closely models the larval dynamics in a human. This is important from an epidemiological point of view because mesocercarial stages are highly pathogenic in humans. If our results in mice are extrapolated to humans, then one may reasonably surmise that a human female, once infected, will transmit mesocercariae to all the children she nurses. Moreover, her female offspring may pass the same worms to their offspring. These migrating larvae might invade vital organs of a newborn, leading to physical or mental impairment, or even death.

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Simian AIDS: Isolation of a Type D Retrovirus and Transmission of the Disease

Abstract. *A type D retrovirus related to but distinct from Mason-Pfizer monkey virus was isolated in vitro from the blood of two rhesus monkeys (*Macaca mulatta*) with simian acquired immunodeficiency syndrome (SAIDS). Three juvenile rhesus monkeys that were injected intravenously with tissue culture fluids containing this virus developed SAIDS after 2 to 4 weeks.*

The simian acquired immunodeficiency syndrome (SAIDS), which occurs endemically in colonies of macaque monkeys in the United States (*1, 2*), resembles the acquired immunodeficiency syndrome (AIDS) in humans in overall clinical manifestations, pathology, and immune deficiency. However, in the simian form of the disease, the ratio of helper to suppressor T cells is not reversed, nor is there a high incidence of *Pneumocystis carinii* pneumonia in affected monkeys. By means of a filterable agent present in tissue extracts and plasma of sick monkeys, SAIDS has been transmitted to healthy monkeys (*2, 3*). Here we report the isolation of a new type D retrovirus resembling Mason-Pfizer monkey virus (MPMV) from the blood of two rhesus monkeys (*Macaca mulatta*) with SAIDS. The virus was grown in tissue culture, and tissue culture fluids were used to transmit SAIDS to juvenile rhesus monkeys.

In a study of some of the biophysical properties of the then unidentified etiologic agent, we obtained plasma from an infected monkey (RM-20265) and mixed it in the cold (for 20 minutes) with an equal volume of ether to destroy the infectivity of ether-sensitive agents. Untreated plasma and ether-treated plasma were each inoculated into juvenile rhesus monkeys. After 6 months, the two animals that received ether-treated plasma remained healthy whereas the four animals that received untreated plasma developed SAIDS as defined previously (*1, 4*). Plasma from a rhesus monkey with SAIDS was subjected to ultracentrifugation, and the pellet, after being suspended in phosphate-buffered saline, was centrifuged to equilibrium in a 20 to 60 percent linear sucrose gradient. The gradient was divided into six fractions, and each fraction was inoculated intravenously into rhesus monkeys. Only the lightest two fractions (1.14 to 1.18 g/ml average density) transmitted SAIDS to recipient monkeys. These data indicated that the SAIDS agent had the physical properties of an enveloped virus.

The studies that led to the isolation of the SAIDS agent are summarized in Fig. 1. RM-18610 was a 3-year-old female with spontaneously occurring SAIDS (*5*). A mixture of tissue homogenates from RM-18610 was inoculated into RM-B883, who developed SAIDS 4 months later. A heparinized blood sample from RM-B883 was inoculated intravenously into two 12-month-old monkeys (RM-20141, RM-20335) and into primary rhesus monkey kidney (Rh-MK) cells (culture C1132). RM-20141 and RM-20335 had an accelerated course of the disease and were moribund from SAIDS by 60 and 65 days after inoculation. Their symptoms were typical, and included generalized lymphadenopathy, splenomegaly, neutropenia, diarrhea, weight loss, and lymphoid depletion (*1–5*).

Cultures C1132 and C1281 (the latter being inoculated with blood from a different SAIDS case, RM-20265) were selected for further study because the blood used to infect these cultures had produced SAIDS in juvenile rhesus monkeys (*3*). Cells from the two cultures were passaged three times and a portion