Zeitschrift: Acta Tropica
Band: 39 (1982)
Heft: 4

Artikel: Attempts to establish "Onchocerca volvulus" infection in primates and small laboratory animals
Autor: Kozek, W.J. / Figueroa Marroquin, H.
DOI: https://doi.org/10.5169/seals-312992

Acta Tropica *39*, 317–324 (1982)

International Collaboration in Infectious Diseases Research Program, Tulane University – COLCIENCIAS, Apartado Aéreo 5390, Cali, Colombia; Sección de Onchocerciasis, Servicio Nacional para la Erradicación de la Malaria, Guatemala City, Guatemala, and California Primate Research Center, University of California, Davis, California, USA

Attempts to establish *Onchocerca volvulus* infection in primates and small laboratory animals

W. J. Kozek, H. Figueroa Marroquin

Summary

Attempts were made to transmit *O. volvulus* infection to small laboratory animals and serveral species of primates to identify a practical laboratory host for human onchocerciasis. Infective larvae of *O. volvulus* of Guatemalan origin were inoculated into the following animals: rhesus monkeys, bonnet monkeys, golden spider monkeys, black spider monkeys, galagos, opossums, jirds, newborn and adult Swiss mice, kinkajou, cebus monkey, normal and splenectomized multimammate rats, and a calf. The animals were examined for signs of developing infection for up to two years after inoculation. None of the animals tested developed a patent infection, and neither larvae nor *O. volvulus* adults were found during the necropsy of all the animals which died or were killed during or at the end of the examination period. It is concluded that none of the animals tested is susceptible to infection with *O. volvulus*.

Key words: Filaroidea; *Onchocerca volvulus;* animal models; laboratory hosts; rhesus, *Macacca mulatta;* bonnet monkey, *Macacca radiata;* golden spider monkey, *Ateles geoffroyi;* black spider monkey, *Ateles paniscus;* jird, *Meriones unguiculatus;* galagos, *Galago senegalensis;* opossum, *Didelphis marsupialis;* Swiss mice, *Mus musculus;* kinkajou, *Potos flavus;* calf, *Bos bovis;* cebus monkey, *Cebus albifons;* multimammate rats – normal, multimammate rats – splenectomized, *Mastomys natalensis.*

Most of the filariae which infect man have now been successfully transmitted to various primates or rodent laboratory model hosts. Subperiodic *Brugia malayi* has been established in the jird (*Meriones unguiculatus*) by Ash and

Correspondence: Dr. W. J. Kozek, CIDEIM, Apartado Aéreo 5390, Cali, Colombia, S. A.

Riley (1970), in the patas monkey (*Erythrocebus patas*) by Orihel (1971), and in the multimammate rat (*Mastomys natalensis*) by Petranyi et al. (1975). *Loa loa* was transmitted to the drill (*Mandrillus leucophens*) by Duke (1957), and by Orihel and Moore (1975) to the baboon (*Papio onubis*) and to the patas monkey (*E. patas*). Short-term patent infections with *Wuchereria bancrofti* have been obtained in the Taiwan macaques (*Macaca cyclopis*) by Cross et al. (1979). Dissanaike and Mak (1980) obtained adult worms of the rural strain of *W. bancrofti* from immunosuppressed long-tailed macaque *Macaca fascicularis. Mansonella ozzardi* of Caribbean and South America origin has been recently transmitted to the patas monkey (*E. patas*) by Orihel et al. (1981). Most of these successful transmissions made available the parasites and the infections produced by them to numerous investigators for biomedical investigations.

Experimental studies on onchocerciasis have been hindered by the lack of a practical laboratory animal model host. Natural infection with *O. volvulus* have been found in a gorilla by Van den Berghe et al. (1964) in the Congo (presently Zaire). Caballero and Barrera (1958) reported a recovery of a nodule containing fertile *O. volvulus* adults from a golden spider monkey (*Ateles geoffroyi*) captured in the State of Chiapas, Mexico. Duke (1962) was the first investigator to successfully transmit *O. volvulus* to the chimpanzee, but his attempts to transmit this infection to 7 drills, 1 mangaby, 1 Preuss monkey, and 1 goat were unsuccessful. Suswillo et al. (1977) also failed to infect jirds (*Meriones unguiculatus*) and hamsters (*Mesocricetus auratus*) with *O. volvulus.*

There are several inherent disadvantages in the use of a chimpanzee as a laboratory host: it is costly to purchase and maintain, has an unpredictable temperament, and its present classification as an endagered species renders it essentially unavailable for biomedical investigations. In order to identify an alternate and a more practical laboratory host in which *O. volvulus* could be established and maintained, monkeys of several species and other small animals were tested for their susceptibility to *O. volvulus.* The results of these trials are described in this report.

Materials and Methods

Simulium ochraceum adults which fed to repletion on volunteers harboring naturally acquired *O. volvulus* infection were captured in the endemic zones of Guatemala, transported by jeep to Guatemala City, and from there by air to either Davis, California, USA, or Cali, Colombia, S.A. The flies were maintained during the 8 to 12 day period after captured by the method of Figueroa et al. (1977). Beginning at 7 days after capture some of the flies were killed with CO_2 gas or by gently squashing the thorax with an applicator stick inseted into the maintenance tube. The heads of the killed simuliids were cut with #25 gauge hypodermic needles into at least 4 parts, and each part was examined with a dissecting microscope for infective larvae. The thorax was examined in the same manner for developing larvae. When infective larvae were found in the head, all the remaining flies collected on the same day as the flies examined were killed and dissected in small petri dishes which contained either medium 199 or RPMI 1640. Each medium contained penicillin (100 units/ml) and streptomycin (100 μg/ml). The infective larvae maintained good motility in these media for more

than 24 h. The recovered larvae were transferred by means of micropipette into a "holding" petri dish containing the same medium until approximately 20 larvae have been collected. They were then withdrawn into a 3 ml syringe through a gauge #18 hypodermic needle and injected into the recipient animals; newborn mice were inoculated using a gauge #20 needle. The calf and the newborn mice were inoculated without anesthesia, all other animals were anesthetized with either Kataset, Nembutal, or ether, before inoculation. The characteristics of the animals tested, the inoculum, and the routes and sites of inoculation are listed in Table 1.

The laboratory born and reared rhesus and the bonnet monkeys were purchased from the California Primate Research Center, Davis, California, USA. The golden spider monkey (of Nicaraguan origin), the black spider monkey (of Peruvian origin), the galagos (of African origin), and the jirds, were purchased from commercial animal vendors in the USA. Swiss mice were obtained from the colony maintained by the Department of Microbiology, School of Medicine, Universidad del Valle, Cali. The opossums, the kinkajou, and the cebus monkey were trapped near Cali. The multimammate rats were kindly provided by the late Prof. G. Lämmler from his colony at the Justus Liebig University, Giessen, West Germany. Some of these rats were splenectomized two weeks before inoculation. The calf, born and maintained at Finca Terranova, near Guatemala City, Guatemala, was 5 days old when inoculated.

Apart from the calf, which was examined every 3 to 4 months, all other animals were examined monthly for signs of a developing infection. The examination consisted of palpating the animals anesthetized with Ketaset, Nembutal, or ether, to determine wheter subcutaneous nodules were forming. Beginning at 6 months after inoculation skin snips were also taken with a Holth sclerocorneal punch from each inoculated animal during these monthly examinations. Two biopsies, one from the head and one from the lumbar region, were taken from the jirds, mice and rats. Eight biopsies, one from each side of the head and the pelvis, and one from each shoulder and each thigh, were taken from the monkeys, galagos, and the kinkajou. The skin snips were placed on a slide in a drop of cell culture medium 199 or RPMI 1640 and examined for microfilariae with a compound microscope every 20 min for approximately 2 h. Skin biopsies were taken with a razor blade from the umbilical and nuchal region of the calf at 12 and 18 months after inoculation and examined for microfilariae in the same manner as the other biopsies.

One of the mice from each group (adults, newborns) was killed at 12 days, 20 days, and every 2 months after inoculation. One of the jirds was killed every month, and one multimammate rat from each group (normal, splenectomized) was killed every 2 or 3 months after inoculation. The killed animals, and those which died during the course of this study, were autopsied and carefully searched for developing larval stages of *O. volvulus* or any other sign which could have indicated developing infection.

Results

One of the opossums died 3 months after inoculation as a result of intestine perforated by acanthocephalan worms and subsequent peritonitis. Other animals which died from undetermined causes included: the second opossum at 5 months, one galago at 6 months, the kinkajou at 13 months, and one splenectomized multimammate rat at 2 months and another at 4 months after inoculation.

The cebus monkey and the calf were monitored for 18 months after inoculation but were not killed at the end of the screening period. The remaining 3 galagos were killed and autopsied at 8, 10 and 12 months after inoculation. All the rhesus, bonnet and the spider monkey were killed and autopsied between 21 and 24 months after inoculation.

Table 1. Characteristics of the animals into which transmission of Guatemalan *Onchocerca volvulus* was attempted

Animal	Number and sex[1, 3]	Age and status when inoculated[2]	No. L_3 inoculated	Route and site of inoculation[3]
Rhesus	2 M	2 A, LBR	55, 60	SC: shoulders or pelvis
(*Macaca mulatta*)	1 M, 1 F	2 J, LBR	82, 100	SC: shoulders or thighs
Bonnet monkey	1 M, 2 F	3 A, LBR	60–64	SC: shoulders or pelvis
(*Macaca radiata*)	1 M	1 J, LBR	89	SC: lower abdomen
Golden spider monkey	3 M	J, Fe	50–75	SC: shoulders or pelvis
(*Ateles geoffroyi*)	3 F	J, Fe	75 each	SC: ankles, thighs
Black spider monkey	1 M, 1 F	J, Fe	52, 54	SC: shoulders or pelvis
(*Ateles paniscus*)	2 M, 2 F	J, Fe	40–75	SC: thighs, shoulders or abdomen
Jird (*Meriones unguiculatus*)	12 M	A, LBR	9–15	SC: back or abdomen, 5 IP
Galago (*Galago senegalensis*)	2 M, 2 F	A, Fe	100–125	SC: back or abdomen
Opossum (*Didelphis marsupialis*)	1 M, 1 F	A, Fe	80, 110	SC: back
Mice, Swiss, outbred	5 M, 5 F	A, LBR	10–30	SC: back or abdomen, IP
(*Mus musculus*)	5 M, 5 F	5-day-old, LBR	14–24	SC: back
Kinkajou (*Potos flavus*)	1	A, Fe	108	SC: back and abdomen
Multimammate rats	3 M, S, 2 F, S	A, LBR	S: 23–53	SC: back or abdomen, IP
(*Mastomys natalensis*)	2 M, NS, 6 F, NS	A, LBR	NS: 21–73	SC: back or abdomen, IP
Calf (*Bos bovis*)	1 F	J, Fe	124	SC: back
Cebus monkey	1 M	A, Fe	140	SC: shoulders
(*Cebus albifons*)				SC: pelvis, thighs

[1] M = male, F = female
[2] A = adult, J = juvenile, LBR = laboratory-born and reared, Fe = feral animals
[3] SC = subcutaneous, IP = intraperitoneal, S = splenectomized, NS = not splenectomized

Autopsy of the animals which died or were killed during the course or at the end of this study failed to reveal any sign of infection. Microfilariae of *O. volvulus* were never encountered in any of the skin snips examined. Neither larval stages nor adult worms of *O. volvulus* were ever found in any of the inoculated animals. However, three spider monkeys were found to be infected with other filarids. Two harbored *Dipetalonema* spp. in the abdominal cavity, and a *Tetrapetalonema* spp. was found in the fascia of the scapular muscles of another.

Discussion

The animals tested during this study included representatives of the Old World monkeys, New World monkeys, marsupials, prosimians, rodents and a bovid. Since none of these animals was apparently susceptible to *O. volvulus*, the chimpanzee still remains the most practical primate to serve as a laboratory host for both African and American onchocerciasis (Duke, 1962, 1980).

Our inability to transmit *O. volvulus* to jirds confirms the results of Suswillo et al. (1977) who also failed to establish *O. volvulus* in this rodent. The number of the opossums, cebus monkeys, calves, and the kinkajous tested was small, the results obtained with these animals should therefore be interpreted with due caution. Nevertheless, the lack of any indication during this study that *O. volvulus* could develop in any one of them suggests that testing additional members of each of these 4 species may not necessarily produce results better than those obtained to date.

The spider monkeys tested were feral animals. Consequently, the infections with *Dipetalonema* sp. and *Tetrapetalonema* sp. discovered in the 3 spider monkeys during autopsy were probably acquired in the wild, before these animals were captured. The failure to transmit *O. volvulus* to both and the golden and the spider monkeys leaves unconfirmed the implication of Caballero and Barrera's report (1958) that the spider monkey might be susceptible to *O. volvulus*. Conceivably, the Peruvian black spider monkeys used in our study might have been refractory to the Guatemalan strain of *O. volvulus*, but it seems unlikely that the Nicaraguan golden spider monkeys would be markedly different physiologically from those from Mexico. It is also noteworthy that Caballero y Caballero (1962) failed to find any other spider monkey infected with *O. volvulus* in his subsequent surveys of spider monkeys in the endemic zones of Mexico and Guatemala.

The rhesus and bonnet monkeys were tested since they were the primates most closely related to the chimpanzees that were available to us. Furthermore, breeding colonies of these two species have been established in the USA. Thus, had either of these two species been susceptible to *O. volvulus*, a supply of clean, laboratory bred-and-reared animals would have been available for additional inoculations. The jirds, galagos, and the multimammate rats were tried since these animals have been shown to be good experimental hosts for several spe-

cies of filarids (Ash and Riley, 1970; Lämmler et al., 1968; Benjamin and Soulsby, 1976; Petranyi et al., 1975; Wong and Lim, 1975). The cow is a natural host for 3 members of the genus *Onchocerca: O. gutturosa, O. gibsoni* and *O. linealis,* it seemed reasonable, therefore, to test wheter it would also be susceptible to *O. volvulus.* There are no published reports of attempts to transmit *O. volvulus* to mice. The opossums have been used as good models for solid tumors (Jurgielski et al., 1976). Splenectomized multimammate rats and newborn mice were tested to determine whether partially immunodeficient hosts or those with an immature immunological system may be more susceptible to *O. volvulus* than the healthy, normal adults of the same species. The kinkajou and the cebus monkey were tried to determine the susceptibility to *O. volvulus* of some of the small animals commonly found near Cali, Colombia.

When this study was initiated in 1975, the lack of a colonized vector of *O. volvulus* necessitated the collection of infected simuliids in the field and transporting them to the laboratory. The inoculated animals had to be maintained and monitored for at least 15 months, the length of the prepatent period of the Guatemalan strain of *O. volvulus* in the chimpanzee (Duke, 1980). Similar studies may now be greatly facilitated by the recent advances in the laboratory colonization of the simuliid vectors (Cupp et al., 1981), identification of alternate simuliid vectors (Reid, 1979; Lok et al., 1980a) and non-simuliid surrogate intermediate hosts (Zielke, 1977; Zielke et al., 1977; Bianco and El Sinnary, 1980; Lok et al., 1980b) and in the cryopreservation of filarids (Schiller et al., 1979; Ham et al., 1979). However, any potential animal host will still have to be maintained in the laboratory for a long period of time before the infection will become patent. The animals shown to be refractory to *O. volvulus* by this and other studies (Duke, 1962; Suswillo et al., 1977) may assist other investigators to avoid unnecessary duplication of cost and effort during their search for an animal model for human onchocerciasis. Unfortunately, the results of all of these attempts do not provide any clues or direction as to which animals, or groups of animals, might be susceptible to *O. volvulus* and should next be tested. Without such logical indicators and clues, the search for a practical laboratory model host for human onchocerciasis could be long and difficult.

Acknowledgments

This study was supported, in part, by the Rockefeller Foundation grant RF 74085, UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, and grants AI 0050, RR 00169, and a Program Project Grant AI 16315–01 from the National Institutes of Health, Bethesda, Md., USA.

The authors would like to thank Mr. Neri Luttmann and Mr. José Zelada Mota, administrators of Finca Monte de Oro and Finca Montequina, respectively, for their collaboration and permission to work on the premises of their fincas, and to the numerous Guatemalan volunteers whose participation was essential for this study. The calf was kindly provided by Mr. Everico Monzón of Guatemala City, Guatemala, who also allowed it to be kept on the premises of Finca Terranova. Technicians Virgilio López Osorio and Faustino Guate of the Sección de Onchocerco-

sis, Servicio Nacional de la Erradicación de Malaria, Guatemala City, Guatemala, assisted in collection of infected simuliids throughout the duration of this program. Mary Anne Brayton, Paul Lee and John Schmidt, all of Davis, California, and Angela C. de Vargas, Gloria Amparo Chamorro D., Guillermo Arango S., Martha Lucia Arias V., Esmeralda Caicedo R., and Jesus A. Granja, all of Cali, Colombia, ably assisted in various aspects of this study.

Ash L. R., Riley J. M.: Development of subperiodic *Brugia malayi* in the jird, *Meriones unguiculatus*, with notes on infections in other rodents. J. Parasit. *56*, 969–973 (1970).

Benjamin D. B., Soulsby E. J. L.: The homocytotropic and hemagglutinating antibody response to *Brugia pahangi* in the multimammate rat (*Mastomys natalensis*). Amer. J. trop. Med. Hyg. *25*, 266–272 (1976).

Bianco A. E., El Sinnary K.: Infection of *Aedes aegypti* with *Onchocerca gutturosa*. J. Helminth. *54*, 105–107 (1980).

Caballero y Caballero E.:. Estudios helmintológicos de la región onchocercosa de Mexico y de la República de Guatemala. Nematoda. 12a Parte. Filaroidea VI. Investigaciones de reservarios de *Onchocerca volvulus*. Salud públ. Méx. *4*, 978–981 (1962).

Caballero C. E., Barrera A.: Estudios helmintológicos de la región onchocercosa de Mexico y de la República de Guatemala. Nematoda IIa Parte. Filaroidea V. Hallazgo de un nodulo oncocercoso en un mono araña *Ateles geoffroyi vellerosus* Gray, del Estado de Chiapas. Rev. Lat. Amer. Microbiol. *1*, 79–94 (1958).

Cross J. H., Partono F., Hsu M. Y. K., Ash L. R., Oemijati S.: Experimental transmission of *Wuchereria bancrofti* to monkeys. Amer. J. trop. Med. Hyg. *28*, 56–66 (1979).

Cupp E. W., Lok J. B., Bernardo M. J., Brenner R. J., Pollack R. J., Scoles G. A.: Complete generation rearing of *Simulium damnosum* s.l. (Diptera: Simuliidae) in the laboratory. Tropenmed. Parasit. *32*, 119–122 (1981).

Dissanaike A. S., Mak J. W.:. A description of adult *Wuchereria bancrofti* (rural strain) from an experimental infection in the long-tailed macaque, *Macaca fascicularis* (Syn. *M. irus*). J. Helminth. *54*, 117–122 (1980).

Duke B. O. L.: Experimental transmission of *Loa loa* from man to monkey. Nature (Lond.) *179*, 1357–1358 (1957).

Duke B. O. L.: Experimental transmission of *Onchocerca volvulus* to the chimpanzee. Trans. roy. Soc. trop. Med. Hyg. *56*, 271 (1962).

Duke B. O. L.: Observation on *Onchocerca volvulus* in experimentally infected chimpanzees (*Pan troglotydes*). Tropenmed. Parasit. *31*, 41–54 (1980).

Figueroa Marroquín H., Collins R. C., Kozek W. J.: Post-prandial transportation and maintenance of *Simulium ochraceum* infected with *Onchocerca volvulus*. Amer. J. trop. Med. Hyg. *26*, 75–79 (1977).

Ham P. J., James E. R., Bianco A. E.: *Onchocerca* spp.: cryopreservation of microfilariae and subsequent development in the insect host. Exp. Parasit. *47*, 384–391 (1979).

Jurgielski W. jr., Hudson P. M., Falk H. L., Kotin P.: Embryonal neoplasms in the opossums: a new model for solid tumors of infancy and childhood. Science *193*, 328–332 (1976).

Lämmler G., Saupe E., Herzog H.: Infektionsversuche mit der Baumwollenrattenfilariae *Litomosoides carinii* beim *Mastomys natalensis*. Z. Parasitenk. *30*, 281–290 (1968).

Lok J. B., Cupp E. W., Bernardo M. J.: The development of *Onchocerca* spp. in *Simulium decorum* Walker and *Simulium pictipes* Hagen. Tropenmed. Parasit. *31*, 498–506 (1980a).

Lok J. B., Cupp E. W., Braide E. I., Bernardo M. J.: *Aedes aegypti* as a surrogate host for *Onchocerca* spp. Amer. J. trop. Med. Hyg. *29*, 382–388 (1980b).

Orihel T. C.: *Necator americanus* infections in primates. J. Parasit. *57*, 117–121 (1971).

Orihel T. C., Moore P. J.: *Loa loa:* experimental infection in two species of African primates. Amer. J. trop. Med. Hyg. *24*, 606–609 (1975).

Orihel T. C., Lowrie R. C. jr., Eberhard M. L., Raccurt C., Kozek W. J., Tidwell M. A., Tidwell M.: Susceptibility of laboratory primates to infection with *Mansonella ozzardi* from man. Amer. J. trop. Med. Hyg. *30*, 790–794 (1981).

Petranyi G., Mieth H., Leitner I.: *Mastomys natalensis* as an experimental host for *Brugia malayi* subperiodic. S. E. Asian J. trop. Med. publ. Hlth *3*, 328–337 (1975).

Reid G. D. F.: The development of *Onchocerca volvulus* in two temperate blackfly species, *Simulium ornatum* Meigen and *Simulium lineatum* Meigen. Ann. trop. Med. Parasit. *73*, 577–581 (1979).

Schiller E. L., Turner V., Figueroa Marroquín H., D'Antonio R.: The cryopreservation and in vitro cultivation of *Onchocerca volvulus*. Amer. J. trop. Med. Hyg. *28*, 997–1009 (1979).

Suswillo R. R., Nelson G. S., Muller R., McGreevy P. B., Duke B. O. L., Denham D. A.: Attempts to infect jirds (*Meriones unguiculatus*) with *Wuchereria bancrofti*, *Onchocerca volvulus*, *Loa loa*, and *Mansonella ozzardi*. J. Helminth. *46*, 132–133 (1977).

Van Den Berghe L., Chardome M., Peel E.: The filarial parasite of the eastern gorilla in the Congo. J. Helminth. *38*, 349–368 (1964).

Wong M. M., Lim K. C.: Susceptibility of the "bush baby" (*Galago crassicaudatus panganiensis*) to *Brugia malayi* and *B. pahangi*. J. Parasit. *61*, 598 (1975).

Zielke E.: Further studies on the development of *Onchocerca volvulus* in mosquitoes. Trans. roy. Soc. trop. Med. Hyg. *71*, 546–547 (1977).

Zielke E., Schulz-Key H., Albiez E. J.: On the development of *Onchocerca volvulus* in mosquitoes. Tropenmed. Parasit. *28*, 254–257 (1977).