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The life cycle of *Haplorchis pumilio* (Trematoda: Heterophyidae) from the Indian region

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Abstract

The life cycle of the heterophyid fluke, *Haplorchis pumilio* is elucidated for the first time from the Indian region. Various stages in the life cycle were established based on observations made on natural infections found in snails and fish in a freshwater stream at Visakhapatnam, India and experimental infections carried out in the laboratory. The thiarid snail, *Thiara tuberculata* served as the first intermediate host and a wide range of freshwater fish as second intermediate hosts. Natural infections with adult flukes were found in the piscivorous birds *Ardeola grayii* and *Bubulcus ibis*. Adults were raised experimentally in day-old chicks. Distinguishing features of the cercaria of *H. pumilio* are: a large body size (200–224 × 92–96 μm), body–tail ratio of 1:2.1 and densely distributed pigment granules in the parenchyma imparting a brownish tinge to the body. Natural infections with metacercariae were found in the freshwater fish *Channa punctatus*, *C. orientalis, Puntius sophore, Gambusia affinis* and fingerlings of *Cyprinus carpio* and *Liza macrolepis.* Additionally, experimental infections were established in *Therapon jarbua*, *Esomus danricus* and *Oreochromis mossambica.* Metacercariae were embedded in the caudal muscles of fish and heavy infections induced mortality. Metacercariae were infective at about 15 days of age.

Introduction

The genus *Haplorchis* Looss is a large one comprising species infecting birds and mammals including man. *Haplorchis* spp. have received considerable attention because of their zoonotic potential (Sprent, 1969) and the pathogenicity that metacercarial infections cause in freshwater food fish (Sommerville, 1982b). Pearson (1964) in an extensive review of the genus considered only seven species as valid and later Pearson & Ow-Yang (1982) added one more species. Information on the life cycle is available for *Haplorchis pumilio* (Faust & Nishigori, 1926; Shen, 1959; Khalifa *et al.*, 1977; Sommerville, 1982a; Saad & Abed, 1995), *H. taichui* (Nishigori, 1924; Martin, 1958) and *H. yokogawai* (Martin, 1958).

From the Indian region, several authors reported the occurrence of metacercariae of *Haplorchis* spp. in freshwater fish (Nath & Pande, 1970; Pande & Shukla, 1972; Nath, 1973a,b; Pande & Premvati, 1977) and of adult flukes in birds and mammals (Odening, 1962; Prakash & Pande, 1968). Apparently, cercariae in this group have been recorded from freshwater snails, but to date no detailed life cycle has been elucidated for any species of *Haplorchis* from the Indian region.

During our studies on the helminth parasites of fish in a freshwater stream at Visakhapatnam, India, metacercarial infections of *H. pumilio* were frequently encountered in many species of freshwater fish, with cercariae being found in the snail *Thiara tuberculata*. This has facilitated the present study on the life cycle of *H. pumilio* under both natural and experimental laboratory conditions, together with an identification of specific features which distinguish the cercaria of *H. pumilio* from cercariae of other species of *Haplorchis.*

Materials and methods

Samples of the snail *Thiara tuberculata* and various species of fish likely to serve as second intermediate hosts were collected from the freshwater stream, near the Mehadrigedda reservoir in Visakhaptnam. Snails

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showing infections with *H. pumilio* were separated off from the remainder, whilst fish were examined for the presence of infections with metacercariae of *H. pumilio*.

Experimental infections involving exposure to freshly released cercariae were carried out on the fish species *Channa punctatus, C. orientalis, Puntius sophore, Therapon jarbua, Oryzias melanostigma, Gambusia affinis, Oreochromis mossambica* and *Esomus danricus* and fingerlings of *Cyprinus carpio* and *Liza macrolepis*. Batches of the above fish, free from natural infections with the metacercariae, were exposed to different doses of cercariae (100–1000) for 1 h. Fish were examined 1, 3, 5, 10 and 15 days post-exposure, to follow the susceptibility to infection as well as the growth and development of metacercariae. The latter obtained from experimentally infected fish were fed to day-old chicks to obtain adult flukes. Also birds commonly occurring in the locality were examined for the presence of adult flukes.

The morphology of cercariae and metacercariae was studied using conventional methods. Infected snails were dissected to study the intramolluscan stages whereas adult flukes from avian hosts were fixed in hot AFA solution, stained with alum carmine, cleared in creosote and mounted in Canada balsam. Drawings were made with the help of a camera lucida. Measurements in μm were made from heat-killed cercariae and permanent whole mounts of flukes.

Results

Field and laboratory studies undertaken on the life cycle of *H. pumilio* indicated that the snail *T. tuberculata* acts as the first intermediate host, several species of freshwater fish as second intermediate hosts and the bird species, *Ardeola grayii* and *Bubulcus ibis* as definitive hosts. Chicks served as experimental hosts.

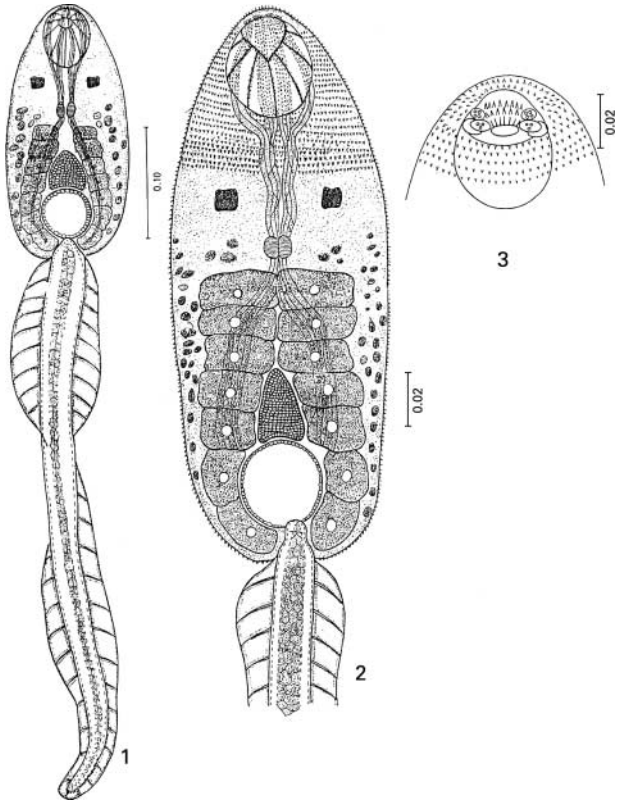
Cercaria

Prevalence of infection

Of 3173 *T. tuberculata* examined during October 1992 to September 1994, one hundred and six snails (3.34%) were infected with cercariae of *H. pumilio*. Monthly prevalence values varied from 0.74% to 6.76%. Although no pronounced seasonal prevalence changes were apparent, there was a tendency for infections to be high during the winter (November, December) and low during the summer (May, June). Double infections involving *H. pumilio* and xiphidocercariae occurred in two snails.

Description (figs 1–3)

Cercaria typical heterophyid, monostomate, biocellate, pleurolophocercous. Body deep brown in colour, opaque, 212 (200–224) long, 94 (92–96) wide. Tail transparent, 448 (420–456) long, 30 (28–32) wide, nearly twice as long as body, broad anteriorly, gradually tapering posteriorly to fine tip, fitting into hollow groove at posterior end of body, provided with two lateral fin folds covering anterior one third, a dorsal fin fold occurring on posterior two thirds of the tail and a ventral fin fold restricted to the posterior one third, both dorsal and ventral fin folds continuous posteriorly. Body–tail ratio 1:2.1. Body spines arranged in regular rows, densely distributed anteriorly becoming sparse posteriorly. Oral sucker subterminal, 46 (40–52) long, 38 (32–44) wide, protrusible through mouth. Postoral ring present. Preoral lobe with three rows of enlarged triangular spines, consisting of 8, 6 and 7 spines, in the first, second and third rows respectively. Eyespots distinct, large, irregular in outline. Parenchyma with densely distributed pigment granules that impart brownish tinge and opacity to cercarial body. Prepharynx long, pharynx globular, post-pharyngeal digestive system not formed. Seven pairs of fairly large, rectangular shaped penetration glands, arranged in two rows, occupy posterior two thirds of body, two pairs of glands paravesicular, remaining five pairs of glands situated in front of the bladder in an arch-like manner. Ducts from penetration glands arise in a single bundle, divide into two bundles near posterior border of oral sucker, each of which subdivides into two bundles, the four bundles traverse dorsal to oral sucker, open out through pores arranged on preoral lobe in groups of 3:4:4:3. Excretory bladder globular, epithelial. Excretory tubules originate from lateral margins. Flame cells arranged in groups of two. Flame cell formula $2\{(2+2+2)+(2+2+2)\}$. Genital rudiment small, triangular mass, $20-24 \times 20$ in size, situated in front of excretory bladder. Cystogenous glands small, numerous confined mostly to lateral regions of the posterior half of body but extending medianly on



Figs 1-3. *Haplorchis pumilio* cercaria. 1, Entire; 2, enlarged view of body; 3, anterior part to show preoral spines and openings of penetration glands.

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dorsal side, overlapping penetration glands, confluent in front of penetration glands.

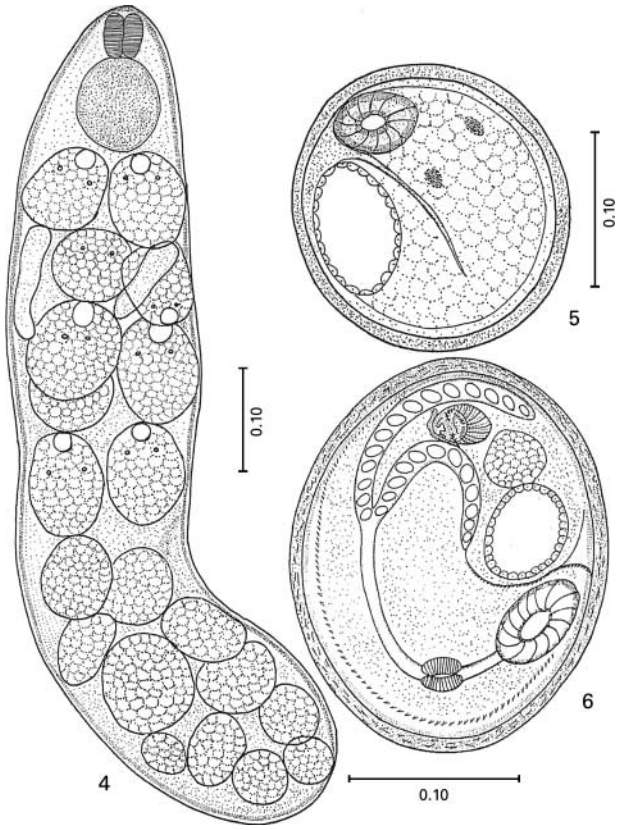
Redia

Spindle-shaped, 992 (880–1040) long, 186 (120–198) wide, light brown in colour, encloses a number of mature and immature cercariae and few germ balls. Pharynx terminal, globular, 55 (48–64) long, 38 (20–48) wide, followed by short gut (fig. 4).

Metacercaria

Natural infections with metacercariae were found frequently in *Channa punctatus*, *C. orientalis* and fingerlings of *Liza macrolepis* and *Cyprinus carpio* and occasionally in *Puntius sophore* and *Gambusia affinis*. Infections could be established experimentally in *Puntius sophore, Oreochromis mosssambica, G. affinis, C. punctatus, C. orientalis, Esomus danricus, Therapon jarbua* and fingerlings of *C. carpio* and *L. macrolepis* while *Oryzias melanostigma* was found to be refractory to the infection.

Cysts were embedded in the caudal muscles of fish, appearing as brown nodules in the caudal region. Cercariae penetrated the caudal muscles within 1 h after exposure and encystment occurred within 24 h. In the newly formed metacercaria, the cyst wall is transparent and the larva lies folded inside the cyst (fig. 5). Genital spines appear in 12-day-old metacercariae. Development of the cercaria to the infective metacercarial stage is completed in 15 days. Fish exposed to heavy doses of 1000 cercariae per fish showed erratic movements and extensive haemorrhaging at the penetration sites of cercariae, resulting in mortalities.



Figs 4-6. *Haplorchis pumilio* redia and metacercaria. 4, Redia from natural infection of *Thiara tuberculata*; 5, one-day-old metacercaria from experimental infection of *Channa punctatus*; 6, fifteen-day-old metacercaria.

Description

Cysts oval, brownish, translucent, 192–198 × 175–178 in size provided with a thick capsule of host origin; larva inside the cyst with fully developed digestive system with caeca filled by disc-like bodies, the genital complex fully formed with typical spine pattern. The single oval testis and globular excretory bladder were clearly seen (fig. 6).

Adult

Natural infections were observed in the piscivorous birds, *Ardeola grayii* and *Bubulcus ibis coromandus*. Infections with adult flukes were established successfully in day-old chicks fed with metacercarial cysts approximately 15 days old. The following description is based on 3-day-old flukes from experimental infections.

Description (figs 7 and 8)

Body pyriform, 359 (320–400) long, 173 (140–200) wide. Forebody flattened, 163 (156–184) long, 17 (112–128) wide, with pigment granules in parenchyma and prominent gland cells on either side of pharynx. Tegument covered with scale-like spines arranged in regular rows, densely distributed in the fore-body becoming sparse posteriorly. Oral sucker subterminal, 40 (32–56) long, 40 (36–40) wide. Ventral sucker small, lying in ventro-genital sac. Prepharynx short, pharynx globular, 31 (24–40) long, 20 (16–20) wide. Oesophagus 83–86 long, bifurcates a little in front of the genital complex, intestinal caeca broad, thick-walled, short, terminating at the level of ovary. Testis single, large, 62 (52–70) long, 55 (45–70) wide, situated at posterior end of body on dorsal side. Cirrus sac absent. Seminal vesicle thin-walled, bipartite, posterior part small, anterior part large, distinct, continues anteriorly as pars prostatica, surrounded by large, conspicuous prostatic cells (fig. 7). Ejaculatory duct and metraterm unite dorsally to the base of the ventral sucker to form a short thick-walled tubular genital sinus. Genital pore median, situated a little posterior to the caecal bifurcation. Ovary sub-spherical, 42 (40–44) long, 40 (36–44) wide, pre-testicular, slightly to right of midline. Mehlis' gland small, compact, situated lateral to ovary. Seminal receptacle large, thick-walled. Uterus provided with three loops, fills entire hind body overlapping testis. Eggs small, 37 (36–40) long, 17 (16–20) wide. Vitelline follicles large, branched, extend from posterior border of ovary to posterior end mostly lying dorsal to testis. Vitelline reservoir prominent situated antero-lateral to ovary. Excretory bladder sac-like with terminal pore. Ventro-gentital sac 46 (36–44) long,

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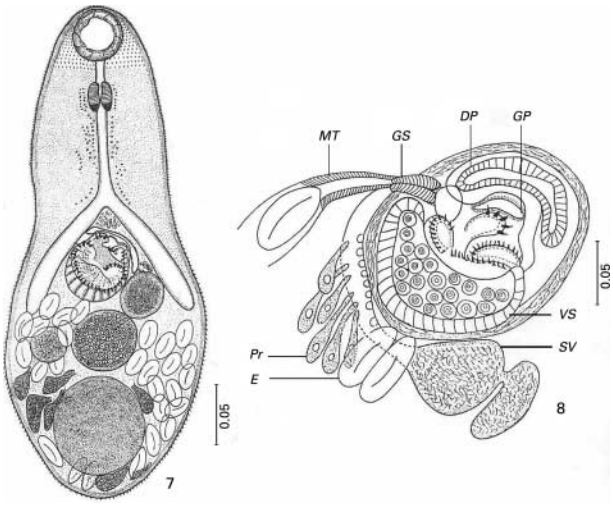

Figs 7 and 8. *Haplorchis pumilio* adult fluke. 7, Ventral view, from experimental infections of chicks; 8, enlarged view of ventro-genital complex (DP, dorsal pocket; E, egg; GP, genital pore; GS, genital sinus; Mt, metraterm; Pr, prostatic cells; SV, seminal vesicle; VS, ventral sucker).

45 (36–60) wide, median, lying just posterior to bifurcation, lined with thick unarmed tegument, consists of ventral chamber containing ventral sucker and transversely elongated dorsal pocket with folded wall (fig. 8). Ventral chamber filled by ventral sucker, apex with crown of 30–34 skeletal bars, interrupted dorsally between latero-dorsal lobes. Mid dorsal lobe tipped with a group of five spines. Ventral sucker divided into two parts, posterior cellular region staining deeply with neutral red, anterior muscular part produced into lobes projecting into the ventro-genital chamber.

Discussion

The pattern of the life cycle of *H. pumilio* is similar to that reported for the species from other geographical regions (Faust & Nishigori, 1926; Shen, 1959; Khalifa *et al.*, 1977; Sommerville, 1982a; Saad & Abed, 1995). It is evident that thiarid snails *Thiara tuberculata* and *Pirenella conica* serve as first intermediate hosts. A wide range of freshwater fish species belonging to the families Cyprinidae, Siluridae, Cottidae, Cichlidae and Mugilidae serve as second intermediate hosts. The final hosts include a variety of piscivorous birds, notably species of *Pelecanus, Nycticorax, Pseudibis, Milvus* and *Ardea.* Natural infections with adult flukes have also been recorded from mammals including man, dog, cat, shrews and foxes and reptilian hosts such as *Varanus* sp. and *Cereberus* sp. (Fischthal & Kuntz, 1965; Sommerville, 1982a). Experimental infections have been established in a number of laboratory hosts including pigeons, rats, mice, guinea pigs, hamsters, cats and monkeys. No specificity is thus exhibited by *H. pumilio* neither in the choice of second intermediate nor definitive hosts, and hence human infections occur due to lack of this host specificity.

The present study is the first report of the cercaria of *H. pumilio* from the Indian region. Morphologically it reflects similarities to cercariae of *H. pumilio* reported from other geographical regions (Faust & Nishigori, 1926; Shen, 1959; Saad & Abed, 1995). Among various heterophyid cercariae reported from the Indian region from *T. tuberculata,* two species *Cercaria pinjorensis* Gupta & Tanega and *C. visakhapatnamensis* 3 Dhanumkumari, Hanumantha Rao & Syamasundari, very closely resemble the present cercaria, particularly in having seven pairs of penetration glands, some of which are paravescicular in their distribution. *Cercaria pinjorensis,* however, differs in the size of body and tail and in the number and arrangement of acicular spines on preoral lobe. *Cercaria visakhapatnamensis* 3 is very different in possessing transparent body, small eye spots and in the contents of the penetration glands.

The cercaria described by Sewell (1922) as Cercariae Indicae VII from the snail *Thiara tuberculata,* also closely resembles the present cercaria, but differs in that it has eight pairs of penetration glands, some of which are lateral to the excretory bladder but do not extend to the posterior end of the body. Sewell (1922) noted its resemblance to *Cercaria pleurolophocerca* Sonsino which Price (1940) considered as belonging to *Haplorchis* sp. and named it as *H. pleurolophocerca.* In the light of that, C. Indicae VII in all probability belongs to *Haplorchis* spp. With the limited information available on the species, it is difficult to comment on its similarity to the cercaria of *H. pumilio.*

A comparison of morphological features of cercariae of *H. pumilio, H. taichui* and *H. yokogawai* (table 1) reveals that the most significant features of the cercaria of *H. pumilio* are the large body size, the body tail ratio of approximately 1:2.1 and the dense distribution of pigment granules in the body parenchyma imparting opacity and a brownish tinge to the body. The cercaria of *H. pumilio* can be separated from that of the other two species by considering the above three characters

Table 1. Comparison of some characteristic features of cercariae of three species of *Haplorchis.*

	H. pumilio	*H. taichui*	*H. yokogawai*
Reference	Present study	Faust & Nishigori, 1926	Martin, 1958
Body size (in μm)	212 (200–224) long 94 (92–96) wide	200 long 50 wide	68–96 long 37–50 wide
Body pigmentation	Deep brown	Yellowish brown	Sparsely pigmented
Number of spines in the first row of preoral lobe.	8	4	6
Body–tail proportion	1:2.1	1:1.8	1: 5.0

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in addition to the differences in the number of spines on the first row of the preoral lobe.

Metacercariae of *H. pumilio* have been recorded from a wide range of freshwater fish. From the Indian region alone, metacercariae have been recorded from freshwater fish belonging to the families Cyprinidae, Siluridae, Cottidae, Cichlidae and Mugilidae. Experiments conducted during the present study showed that *Gambusia affinis, Cyprinus carpio, Channa punctatus,* and *C. orientalis* are highly susceptible to infection with *H. pumilio*. Infections were not established experimentally in *Oryzias melanostigma,* despite the abundance of these fish in the locality from where infected snails were collected. Many other species of freshwater fish also probably serve as potential intermediate hosts.

Metacercarial encystment occurs within 24 h after penetration of the fish *Gambusia,* with metacercarial development being completed within 15 days. Massive invasions by cercariae cause fish mortality, likely to be attributed to extensive haemorrhaging. Mortalities associated with the penetration of the cercariae of *H. pumilio* have earlier been reported by Sommerville (1982b) in the case of *Tilapia zilli* and for other trematode species by Davis (1936), Hoffman (1956), Hoffman & Dunbar (1963), Johnson (1971) and Erasmus (1972). Such a mortality is probably a common feature for fish exposed to massive invasions by cercariae and according to Sommerville, it is possible that many as yet unexplained mortalities of fry in ponds were caused by cercarial penetration. Metacercarial encystment showed a predilection to the caudal muscles of fish, which also occurs in experimental infections in *Tilapia zillii* (Sommerville, 1982a). The formation of nodular tissue at the site of infection and pronounced tissue reactions resulting in the formation of capsules as noted by Sommerville (1982a) have also been observed in the present study. Sommerville (1982b) noted behavioural changes in infected fish, which are likely to enhance the transmission success to the definitive host. In conclusion, it can be said that metacercarial infection of *H. pumilio* constitutes a potential threat to fish fry both in culture ponds and natural habitats.

Metacercarial cysts of *H. pumilio* can be readily identified using the following features: their occurrence in caudal muscles of fish; cysts are light brown in colour, measuring 192–198 × 175–178 in size; the larva has a spined tegument, eye-spot pigment in forebody, caeca filled with disc-like bodies, excretory bladder globular, genital complex with 30–34 lambda-shaped spines arranged in a semicircle.

Metacercarial infections were previously noted in a number of freshwater fish from the Indian region (Pande & Shukla, 1972; Pande & Premvati, 1977). The only report of natural infections with adult flukes in the avian hosts in the Indian region is that of Odening (1962), who recorded their occurrence in the birds *Pelecanus philippinensis* and *Pseudibis papillosus.* In the present study, natural infections were noted in *Ardeola grayii* and *Bubulcus ibis.* The scarcity of reports of adult flukes is not only due to the lack of sustained efforts in obtaining adult flukes from natural infections, but also to the short life span of *H. pumilio*, with adult worms being eliminated from the intestine of the host in a short time. *Haplorchis pumilio* has a wide geographical distribution, having been reported from Egypt, Palestine, Israel, Tunisia, Japan, Taiwan, China, Kenya and India. Human infections with this species have been recorded from Taiwan (Nishigori, 1924) and Thailand (Radomyos *et al*., 1983). Metacercarial cysts survive and retain their infectivity even in fish kept frozen for several days (Sommerville, 1982a) and this feature enhances the chances of human infections.

The stream at Visakhapatnam constitutes a natural focus for infection, with heavy infections of *H. pumilio* prevailing in snails and fish almost throughout the year and birds residing on the trees near the stream playing a major role in transmitting the infections to snails. Such natural foci of infections with *H. pumilio* probably occur in many regions in India and there is thus much potential for humans to become infected with *H. pumilio*. Pande & Shukla (1972) have brought to light the zoonotic significance of heterophyid infections in food fishes from the Indian region and these authors cautioned against consumption of raw and semi-cooked fish. Domestic animals like cats, dogs and foxes serve as reservoirs of infections for *H. pumilio* and are often heavily infected (Africa & Garcia, 1935; Kuntz & Chandler, 1956). Fahmy *et al.* (1976) and Sommerville (1982b) suggested controlling infection by reducing snail populations and the protection of fish stocks from predation. Epidemiological studies, intended to find suitable measures for controlling infections with *H. pumilio* and other heterophyid infections having zoonotic potential, need to be undertaken.

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