

**On *Centrocestus formosanus* (Nishigori, 1924) Price,
1932 and its Experimental Infection in
White Leghorn Chicks**

PREMVATI, G. AND VIBHA PANDE

Department of Zoology, University of Lucknow, Lucknow, India

(Received for publication: Jan. 20, 1974)

The genus *Centrocestus* Looss, 1899 (Trematoda: Heterophyidae: Centrocestinae) occurs as an intestinal parasite of birds and mammals. Experimental and natural infections in man have been reported from Japan and Formosa (Ito, 1964). According to McDonald (1969), *C. formosanus* is widespread in Asia (China, Taiwan, Japan, Philippine Islands) and Oceania (Hawaii). The natural definitive hosts of this species are: *Ardea purpurea*, *Bubulcus ibis*, *Egretta intermedia*, *Nycticorax nycticorax* and *Platalea leucorodia* (Ciconiiformes); *Canis familiaris*, *Felis catus*, *Nyctereutes procyonoides* (Carnivora); and *Rattus norvegicus* (Rodentia). The experimental final hosts include *Anas platynshynchos* (Anseriformes) and *Gallus gallus* (Galliformes); *Cavis porcellus*, *Mus musculus*, *Rattus rattus* (Rodentia); *Oryctolagus cuniculus* (Lagomorpha) and *Homo sapiens* (Primata).

The natural definitive host of *C. formosanus* from India, reported by Nath (1972), is the Pond-heron, *Ardeola grayii* (Sykes). Experimental hosts so far recorded are albino rats, guinea pig, pups, White Leghorn chick and pigeon (Nath and Pande, 1970); pigeons, White Leghorn chick and duck (Nath, 1972); and hamster and rhesus monkey (Pande and Shukla, 1972).

Fresh-water fishes and anurans have so far been recorded as second intermediaries of *C. formosanus* in China, Japan, Taiwan, Philippine Islands and Hawaii. In India, only fresh-water fishes have been reported to be infested with centrocestine cysts (Nath and Pande, 1970; and Pande and Shukla, 1972).

The common occurrence of metacercarial cysts of *C. formosanus*, in some of the local fresh-water fishes, prompted a detailed study on their course of development in hatchery-raised clean chicks, which has not been elucidated so far. This study also includes a comparison with the developmental stages reported by earlier workers from different experimental hosts.

Materials and Methods

The metacercariae of *C. formosanus* were isolated from the infested gills of *Channa* (*Ophicephalus*) *punctatus* (Bl.), *Cirrhinus reba* (Ham.), *Mystus vittatus* (Bl.), *Ompok bimaculatus* (Bl.), *Chela laubuca* (Ham.), *Nandus nandus* (Ham.), *Puntius chola* (Ham.) and *Colisa lalius* (Ham.). Their development was studied in 2-7-day-old hatchery-raised clean chicks. Metacercarial stages (both encysted and excysted) and adult flukes (recovered 3, 3.5, 4, 6, 8, 9.5, 12, 13.5, 14 and 20.5 days post-infection) were studied alive and from stained permanent mounts. Sections of the infested gill-filaments were cut and stained with Hematoxylin and Eosin. Measurements are given in microns unless otherwise stated.

Observations

The intermediate hosts, the infective dose administered, the number of specimens recovered, the age of the flukes and the average number of eggs per worm in each age group are given in Table 1.

Table 1 Number of worms and average number of eggs per worm recovered from White Leghorn chicks infected with different doses of metacercarial cysts of *C. formosanus* infesting fresh-water fishes.

Intermediate hosts	Infective dose	No. of specimens recovered	Age of flukes (in days)	Number of eggs (average in parentheses)	
				In living specimens	In permanent mounts
<i>Channa punctatus</i> .	66	2	3	4, 21 (13)	4
<i>Nandus nandus</i> .	354	1	3.5		16
<i>Nandus nandus</i> .	15	1	4		
<i>Channa punctatus</i> ; <i>Colisa lalius</i> .	330	14	6	20, 50, 22, 25, 20, 26 (27)	34, 53, 13, 26, 59, 53, 16, 42, 23, 7 (33)
<i>Channa punctatus</i> .	178	10	6	107, 65, 46, 58 (69)	49, 56, 55, 51, 111, 47 (62)
<i>Puntius chola</i> ; <i>Cirrhinus reba</i> .	104	1	8	60	61
<i>Nandus nandus</i> .	101	5	9.5		33, 25, 13 (24)
<i>Mystus vittatus</i> .	282	11	12	66, 20, 32, 35, 32, 50, 23, 33, 5, 10, 48 (32)	25, 23, 39, 49, 18, 30, 56, 61, 35 (37)
<i>Nandus nandus</i> .	656	5	13.5		19, 21, 6, 14 (15)
<i>Nandus nandus</i> ; <i>Ompok bimaculatus</i> ; <i>Channa punctatus</i> .	102	8	14	22, 25, 25, 60, 23, 38, 57, 71 (40)	21, 58, 34, 28, 24, 66, 42, 76 (44)
<i>Chela laubuca</i> ; <i>Channa punctatus</i> .	252	31	20.5	40, 30, 58, 55 (46)	46, 43, 44, 60, 25, 16, 31, 21, 54, 24, 50, 58, 58, 55, 44, 7, 33, 82, 27 (41)

Metacercaria :

Metacercarial cysts of *C. formosanus* infested the gill-filaments of eight species of fresh-water fishes (Fig. 1). Structurally, the encysted and excysted metacercaria con-

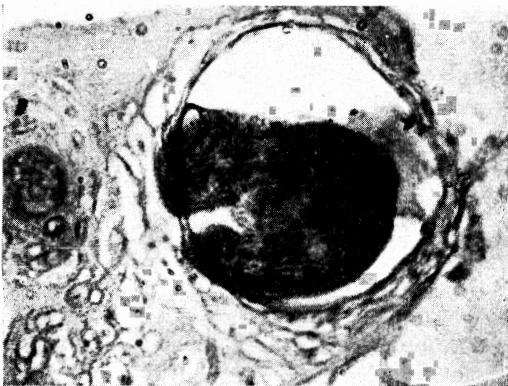


Fig. 1. Photomicrograph of a part of a section of gill-filament of *Nandus nandus* showing a metacercarial cyst of *C. formosanus*.

formed with the accounts given by Chen (1942), Komiya (1965), Nath (1972), and Pande and Shukla (1972). Eight pairs of flame-cells have been observed by Chen. The present authors also counted 8 pairs of flame-cells, arranged as: 2 [(2+2)+(2+2)]. The metacercariae were in advanced state of development and yielded mature flukes 3 days post-infection.

Adult :

(Figs. 2(a), (b) and (c)): Body spiny, flask-shaped, somewhat dorso-ventrally flattened; oral sucker terminal, with two alternating rows of 30-36 circumoral spines, inner spines being larger; prepharynx prominent, pharynx well-developed; esophagus dividing into intestinal ceca extending beyond ventral sucker to ovarian zone; ventral sucker smaller than oral sucker; testes in posterior region of body, post-ovarian, symmetrical, elliptical or ovoidal, with almost smooth



Fig. 2. *Centrocestus formosanus*

- (a) 3.5-day-old;
- (b) 13.5-day-old;
- (c) 20.5-day-old.

margins; seminal vesicle large, constricted near its middle into two parts: a sac-shaped basal portion and a narrower terminal one, the latter being surrounded by a small mass of prostate cells terminally, and opening in front of ventral sucker; ovary smaller than

testes, ovoid or rounded, with almost smooth margins; seminal receptacle well-developed, median and pre-testicular; Mehlis' gland opposite ovary, pre-testicular; uterus ascending, metraterm opening at common genital pore in front of ventral sucker; vitellaria

Table 2 Measurements of *Centrocestus formosanus* recovered at different intervals from White Leghorn chicks.

Age of flukes (in days)	3	6	9.5	12	14	20.5
Body size	609×224	389-615 × 185-273	361-416 × 201-259	505-624 × 238-269	525-690 × 231-292	429-726 × 182-333
Oral sucker	64×69	56-73 × 46-63	33-66 × 53-56	53-76 × 53-76	50-66 × 48-76	59-79 × 53-117
Prepharynx	53	7-69	10-23	21-50	20-53	10-50
Pharynx	43×36	30-46 × 30-43	30-36 × 29-30	31-43 × 31-40	35-53 × 26-43	33-50 × 30-46
Esophagus	53	10-92	13-20	26-63	13-66	13-76
Distance of intestinal bifurcation from anterior end	215	107-261	109-132	158-215	150-201	142-224
Ventral sucker	53×56	36-50 × 46-58	33-40 × 33-51	40-56 × 43-59	46-66 × 56-63	33-63 × 40-66
Distance of ventral sucker from anterior end	358	218-350	160-234	284-350	254-393	221-399
Testes	56 × 76-83	50-83 × 59-116	36-59 × 76-96	59-99 × 86-112	66-99 × 89-135	53-89 × 73-125
Ovary	53×66	40-66 × 46-92	53-59 × 69-79	50-66 × 66-92	56-76 × 63-109	40-66 × 33-106
Eggs (in distal coils)	35×20	30-40 × 13-21	26-36 × 15-20	33-40 × 15-20	30-36 × 15-21	30-40 × 13-22

extracecal, with nearly rounded follicles, extending from pharyngeal level to post-testicular space and tending to meet in the esophageal and post-bifurcal but pre-acetabular regions; transverse vitelline duct from each side passing inwards to form a triangular yolk reservoir; eggs yellowish-brown, operculate, with a pitted or latticed design on the egg-shell. Excretory bladder X-shaped, due to presence of symmetrical testes on either side. Measurements of permanent stained mounts of different age groups are given in Table 2.

Discussion

Among the species of *Centrocestus* described from birds and mammals, Chen (1942) recognised *C. cuspidatus* (Looss, 1896) Looss, 1899; *C. armatus* (Tanabe, 1922) Price, 1932; and *C. formosanus* (Nishigori, 1924) Price, 1932, as valid. Their diagnostic characters

have been based mainly on the number of circumoral spines; margin of the gonads; presence or absence of the latticed design on the egg-shell; presence of unembryonated or embryonated eggs, and their number and size in fully mature specimens.

The number of circumoral spines in the three species has been given as: 36 in *C. cuspidatus*, 44 in *C. armatus*, and 32 in *C. formosanus* (cited by Chen, 1942). Subsequent workers have, however, found variations in the number of these spines: 40-44 in *C. armatus* by Yamaguti (cited by Chen, 1942); 30-36 in *C. formosanus* by Chen (1942), 32-34 by Nath (1972), and 32 by Pande and Shukla (1972). During the present experiments, usually 32-34 spines, and rarely 30 or 36, were observed. Thus, the present authors agree with Chen and Nath who believe that the number of circumoral spines cannot be treated as a reliable character.

Chen (1942) observed that the testicular and ovarian margins revealed indentations when specimens of *C. formosanus* were left in physiological saline, and became smooth when left in tap water, showing thereby that the nature of the margin of the gonads did not have any taxonomic value. Nath (1972) and the present authors also do not attach any importance to this criterion.

The number and size of the eggs are subject to slight variation. Chen (1942) reported the recovery of immature 3-day-old worms of *C. formosanus* from a chick and the presence of eggs in the 8-day-old fluke. During the present experiments, the 3-day-old specimens from a chick had 4-21 eggs while the 8-day-old flukes revealed 60-61 eggs. Evidently, the number of eggs varies even in flukes of the same age group recovered from the same experimental host. Consequently, this character does not have any specific significance.

So far, the latticed design on the egg-shell of *C. formosanus* has been treated as a diagnostic feature by Chen (1942), Nath (1972), Pande and Shukla (1972) and the present authors. As no account on the egg-shell pattern of *C. cuspidatus* and *C. armatus* is available, further studies would alone confirm its importance.

The metacercariae of the three species have been stated to differ in the number of circumoral spines, which, as stated earlier, reveals variations. The number of flame-cells also differs in the metacercarial stages of the three species: at least 6 pairs have been observed in *C. cuspidatus* (Martin, 1959), 7 pairs in *C. armatus* (Komiya, 1965), and 8 pairs in *C. formosanus* (Chen, 1942; and present authors). A detailed study of the life-cycle stages, including the flame-cell patterns of these three species, would help in ascertaining their validity.

The developmental period for metacercariae of *C. formosanus* differs in different experimental hosts. Chen (1942) obtained mature specimens in 2-3 days from white rats, in 3 days from duckling, in 8 days from chicken, in 8 days from white mice,

in 15 days from rabbit, in 11-17 days from kitten, and in 58 days from pup. He further detected variations in the number of eggs in flukes recovered from these hosts; e.g., 20 eggs in 10-day-old fluke, and 21-32 eggs in 12-day-old flukes from white rats. Flukes recovered from other hosts had smaller number of eggs.

Nath (1972) also noticed variations in the number of eggs in flukes recovered from different birds: in the 2, 4, 6, 15 and 40-day-old specimens from pigeon, he observed 1-5, 5, 6-14, 10-26, and 12-17 eggs, respectively; 22 eggs in the 10-day-old fluke from a duck, and 4 eggs in a 15-day-old specimen from a White Leghorn chick. Thus, the number of eggs varies not only with the ages of the parasite but also with the host species. During the present studies also, the number of eggs was found to vary, the maximum number of 111 eggs being observed in a 6-day-old specimen.

The recovery rate depends on the suitability and susceptibility of the host, as evident from the infection experiments conducted with *C. formosanus* in different hosts. The percentage of recovery, calculated with the results of Indian workers, was found to be: 0.4-4 in pigeon, 0.66 in chick, and 0.5 in duck (Nath); 13.3-90 in hamsters, and 40.3 in rhesus monkey (Pande and Shukla), and 0.15-12.3 in chicks (present authors). Evidently, hamsters and rhesus monkey act as more favourable hosts for this species. There is, therefore, every possibility of its incidence in man in India, particularly in those infested areas where infected fishes are eaten raw or semi-cooked.

Summary

Centrocestus formosanus has been recorded in a number of natural and experimental hosts, including man, in China, Formosa, Taiwan, Japan, Philippine Islands, Hawaii and India. Common incidence of its metacercarial cysts in gill-filaments of eight local fresh-water fishes necessitated a study on the course of its development in hatchery-raised clean chicks. A comparison has been made

with the observations of earlier workers from different experimental hosts. Total number of eggs depends on the age of the flukes and on the host species. Recovery rates, from different experimental hosts, show that hamsters and rhesus monkey act as more favourable definitive hosts for this species in India. The validity of the species of *Centrocestus* has been briefly discussed.

Acknowledgement

Thanks are extended to the State Council of Scientific and Industrial Research, Lucknow, India, for a Research Grant for this project.

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