

not germinated was evident. Hence, while assessing the seed viability through topographical tetrazolium staining, the seed showing unstained portion at the tip of the scutellum can be considered as non-germinable.

19 March 1984

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### ISOLATION OF *CANDIDA* *GUILLIERMONDII* FROM SUSPECTED BOVINE LYMPHANGITIS CASES

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INFECTION due to *Candida* species is usually restricted to the alimentary canal but association of this organism in causing diseases of the respiratory and reproductive systems and as cutaneous/subcutaneous mycoses are also reported. *C. albicans* is by far the commonest species associated with clinical condition but *C. tropicalis*, *C. krusei*, *C. parapsilosis*, *C. pseudotropicalis* may occasionally have causal roles<sup>1</sup>. Mathias<sup>2</sup> observed thrush of the rumen. Systemic bovine candidiasis was first reported in cattle by McCarty<sup>3</sup>. Farley<sup>4</sup> isolated *C. albicans* and other yeasts from cultures of foot and mouth disease virus in strips of bovine epithelium. Stuart<sup>5</sup> isolated *Candida* sp from an outbreak of bovine mastitis and attempted to reproduce the condition experimentally. Prasad and Prasad<sup>6</sup> isolated *C. parapsilosis* in mastitis milk following antibiotic therapy from a pre-existing bacterial infection. Mills and Hirth<sup>7</sup> reported systemic candidiasis in calves as a result of prolonged antibiotic therapy.

A number of cattle suffering from abscesses throughout the body were not responding to antibiotics therapy. The infection spread to healthy and aged bullocks and was clinically suspected for bovine lymphangitis. In the present investigation *C. guilliermondii* was isolated from pus swabs.

The samples were streaked on Sabouraud's agar plate containing chloramphenicol and cycloheximide and were incubated at 37°C under aerobic conditions. Creamy growth was noticed within 48 hr of incu-

bation. The organisms were slightly oval-shaped. On Sabouraud's broth no surface growth was visible but bubbles were noticed. The isolate failed to produce germ tube in fresh rabbit serum. It was negative for urease activity and fermentation of maltose and lactose while it was positive for glucose and sucrose. On both corn meal agar and chlamydo-spore agar, very fine mycelium with small clusters of blastospores at the septa were observed and chlamydo-spores were absent. On the basis of these characters the isolate was identified as *C. guilliermondii*.

It is difficult to comment on the role of the organism in causing bovine lymphangitis as pathogenicity studies with the isolate in a homologous host are necessary to establish it as an etiological agent. The ubiquitous nature of the genus *Candida*, its association with gastro-intestinal, respiratory, reproductive tract infections and its involvement in cutaneous/subcutaneous mycoses in different species of animals, birds and humans warrant a systematic study on its involvement in the etiology of the disease.

The authors thanks the Director, Indian Veterinary Research Institute for facilities.

4 August 1984; Revised 1 October 1984

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### ARNETH COUNTS OF NEUTROPHILS IN THREE HILL STREAM FISHES

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VERY little information is available on the Arneth count of granulocytes of fishes<sup>1-3</sup>. Arneth count is an

Table 1 Arneth count of neutrophils in the three species of fishes.  
All values are mean (%).

Groups:	I	II	III	IV	V	VI
Species and number of observations	'Stab'	Number of nuclear lobes				
		1	2	3	4	5-or more
<i>S. richardsonii</i> (20)	9.74	22.72	34.94	22.82	6.8	3.00
<i>S. plagiostomus</i> (20)	9.10	22.12	39.90	21.60	6.1	3.20
<i>P. sulcatus</i> (20)	3.00	77.00	20.00	—	—	—

enumeration of the number of lobes present in the nuclei in the first 100 granulocytes of all types seen on a single blood slide<sup>3</sup>. The present study aims at describing the Arneth count of neutrophils of *Schizothorax richardsonii*, *Schizothorax plagiostomus* and *Pseudecheneis sulcatus*.

Live specimens of fish were collected from different tributaries of Alakananda of Garhwal Himalayas and acclimatized under laboratory conditions. Blood was drawn by cutting caudal peduncle. The blood without anticoagulant was directly used for smear preparations. Blood smears were air-dried and stained in Leisman's and Wright's stains<sup>6</sup>. A hundred neutrophils were studied in each smear and placed in six groups. The results obtained for the three species are presented in table 1.

**Group I:** This group consists of neutrophilic granulocytes which contain a "stab-shaped nucleus." This type of neutrophils was observed in all the three species of fish studied here, being 9.74% in *S. richardsonii*, 7.1% in *S. plagiostomus* and 3.0% in *P. sulcatus*.

**Group II:** This group consists of neutrophils with a single-lobed nucleus. Such neutrophils was abundant in the three species, but its highest number (77%) was observed in *P. sulcatus*. It was 22.72% in *S. richardsonii* and 22.12% in *S. plagiostomus* (table 1).

**Group III:** Neutrophils with two-lobed nucleus were included in this group. Their maximum number (39.9%) was noted in *S. plagiostomus* and minimum number (20%) was recorded in *P. sulcatus*. They were 34.94% in *S. richardsonii*.

**Group IV:** Neutrophils having three lobes in the nucleus were entirely absent in *P. sulcatus*, while they were 22.82% in *S. richardsonii* and 21.6% in *S. plagiostomus*.

**Group V:** Neutrophils with four-lobed nuclei were present in very low number and in *P. sulcatus* they

were not seen in the blood picture. The percentage of such neutrophils in *S. richardsonii* was 6.8% and in *S. plagiostomus* they were 6.1% only.

**Group VI:** Neutrophils having five or more lobes in the nucleus were very scanty. Such cells could be seen only in *S. richardsonii* (3.0%) and *S. plagiostomus* (3.2%), while they were absent in *P. sulcatus*.

Arneth count of neutrophils showed that one-lobed and two-lobed neutrophils were abundantly present in the species studied. However, *Schizothorax* species had a higher percentage of the three-lobed cells. The percentage of "stab-shaped", four-lobed and five-lobed neutrophils were poor in *Schizothorax* species. On the other hand "stab" neutrophils were very poor in *P. sulcatus* and three, four and five-lobed neutrophils were totally absent in this fish. The single and bi-lobed nuclei predominated among the granulocytes and constituted up to as much as 77% (single-lobed) in *P. sulcatus*. *Schizothorax* species showed a good number of bi-lobed nuclei as compared with *P. sulcatus*. Kawatsu<sup>2</sup> noted hypersegmentation in the granulocytes, which he referred to as supersegmentation in a diseased fish. It is said that higher the segmentation of the nucleus of polymorphs, the older it is<sup>7</sup>. In the present study it was found that *P. sulcatus*, a carnivorous sluggish fish, does not possess three-, four- and five-lobed neutrophils. This suggests that the life span of *P. sulcatus* neutrophils is shorter than that of the neutrophils of *Schizothorax* species, which has five lobes or more than six.

1 September 1984; Revised 22 October 1984

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### INHERITANCE OF GREYISH BROWN (*gb*) AND DARK (*da*) LARVAE, AUTOSOMAL RECESSIVE MUTANTS IN THE FILARIA VECTOR *CULEX QUINQUEFASCIATUS*

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LARVAL colour mutants have been reported in *Culex pipiens* complex<sup>1-7</sup>. However, to-date only a few morphological larval colour mutants are available in *Culex quinquefasciatus*, one of the members of *C. pipiens* complex. The present paper describes the mode of inheritance of two larval colour mutants, dark (*da*) and greyish brown (*gb*) larvae for *C. quinquefasciatus* one of the important vectors of Bancroftian filariasis in South East Asia.

**Greyish brown larvae (*gb*):** The mutant larvae are greyish brown in colour and the colour differences can easily be seen from second instar onwards. The colour, however, darkens in the later instars, particularly in the fourth instar. The colour persisted throughout the pupal stages and in the freshly emerged adults.

The mutant was isolated from Bangalore, Delhi, Poona and Kolar strains and maintained in two large population cages. This mutant has been designated as *gb*.

**Dark larvae (*da*):** The larvae are deep dark in colour, and can easily be distinguished even during the early instars (second instars). However, the colour darkens during the late larval instars and persist throughout the pupal stages and in the freshly emerged adults. This mutant, designated *da* was isolated from the Bangalore strain and maintained two large cages.

Single pair matings in *C. quinquefasciatus* have not occurred sufficiently often for regular use in genetic experiments. Mass matings were, therefore, made in all crosses. Individual egg rafts were, however, collected, reared and scored separately for wild type and mutant forms in each cross. In all crosses 25 males and 25 females were placed in an 8 × 8 × 8 inches cage made of an iron frame covered with nylon mosquito net. The males and females used in the experiments were all originally isolated as single pupae in vials, then sexed

before being introduced into the experimental cage.

The gross colour differences between the mutants and the wild type phenotypes were conspicuous to the naked eye and could readily be detected from some distance. The counting of the larval phenotypes was comparatively easy due to the marked differentiation of the colour, especially during the third and fourth instars. Therefore, counts requiring colour segregation were made even without recourse to any visual aid.

Rearing of all stages and the experiments themselves were done in a controlled environment. The temperature was maintained at 25 ± 1°C and the relative humidity of 80 ± 10%. The rearing procedure was kept constant throughout the course of this investigation.

The mutants greyish brown and dark larvae were crossed with wild type and were scored in the fourth instar and these larvae were kept separately, sexed in pupal stages, and they were again scored as adults for further verification of the results. The results of these crosses are given in the tables 1 and 2.

The appearance of normal larvae in the  $F_1$  crosses (1 and 2 in tables 1 and 2) suggested that the genes *gb* and *da* were recessive to its normal alleles. The  $F_1$  heterozygotes were then back crossed with the presumptive homozygotes of both sexes. The results of these back crosses (crosses 3, 4, 5 & 6 in tables 1 and 2) revealed the expected 1:1 ratio of wild type to mutants.  $F_1$  adults were sib-mated to produce an  $F_2$  generation. The results of the crosses 7 and 8 involving the inbreeding of each mutant of the  $F_1$  fit the expected 3:1 ratio of wild type to mutants (tables 1 and 2). Thus, the above crosses clearly show that the gene *gb* and *da* are recessive and autosomal.

The mutant greyish brown larvae has not been reported so far in *C. quinquefasciatus*. The melanotic larvae reported in *C. pipiens* is lethal in homozygous condition<sup>4</sup>. However, the mutant dark larvae reported here is known for good viability.

Crosses were made between the larval colour mutants of *C. quinquefasciatus*<sup>7</sup>. These mutants include golden yellow (*go*), greyish brown (*gb*), brown (*br*) and green larvae (*g*). The experimental results proved that all the above four mutants belong to an allelic series.

The larval colour mutants greyish brown and dark are excellent markers with full penetrance, uniform expression and high viability in both the sexes.

This work was supported in part, by a grant from the Indian Council of Medical Research, New Delhi.

17 July 1984; Revised 2 November 1984