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ISOLATION OF *ACINETOBACTER CALCEACETICUS* FROM EXTENDED FROZEN BUFFALO SEMEN USED FOR ARTIFICIAL INSEMINATION

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Knowledge of the genus *Acinetobacter* is relatively a recent one as there was some dispute about its classification and nomenclature (Davis *et al.*[2]). Members of the genus *Acinetobacter* especially *Acinetobacter calceaceticus* have been associated with a number of clinical conditions such as cystitis and chronic haematuria in race horses (Rajashekar *et al.*[3]), metritis and endometritis in mares (Ranganath *et al.*[4]) septicaemia, meningitis, urinary tract infections in children and young adults (Davis *et al.*[2]).

In the present investigation *A. calceaceticus* was isolated from frozen semen samples of a buffalo. The straws containing the extended semen were supplied to the laboratory in liquid nitrogen containers. As per the history the buffaloe bull, maintained in an organised breeding farm, was not performing satisfactorily and majority of the animals inseminated with its semen did not conceive.

The samples were processed for total bacterial load and typing of bacteria involved. From this study it was seen that the viable count was within the standard limits (300 organisms per ml of extended semen) prescribed for frozen samples. The samples when streaked on blood agar plates and incubated at 37° C under aerobic conditions yielded non-haemolytic, grayish white colonies having entire margin. The colonies were visible after 48 hours The organisms were gram negative cocco-bacilli, non-motile, non-sporulating and non-capsulated. This isolate was resistant to 5 IU/ml of penicillin and conformed to the biochemical tests of *A. calceaceticus* (Buchnan and Gibbons[1]).

At this stage it is difficult to comment on the role of this organism in causing genital infections of bovines as controlled studies are necessary to incriminate it as an etiological agent of repeat breeding in buffaloes. But ubiquitous nature of this organism, its association with gastro-intestinal, urino-genital and respiratory tract infections in horses and human beings, survival of this organism in spite of freezing at —196° C in liquid nitrogen warrants a systematic study on its role as a possible potential pathogen of male genital organ in cattle and buffaloes.

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HISTOCHEMICAL LOCALIZATION OF ACID PHOSPHATASE AND ESTERASE IN THE THIRD-STAGE LARVA OF *DIPLOTRIAENA TRICUSPIS*

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The present communication reports the distribution and localization of acid phosphatase and esterase in the third-stage larvae of *Diplotriaena tricuspis*, which is a coelozoic parasite of Indian mynah.

Infective-stage larvae were collected from the experimentally infected grasshoppers and fixed in cold acetone and 5% neutral formaldehyde containing 1% $CaCl_2$ at 4° C for 24 hours for histochemical localization of acid phosphatase and esterase, respectively. Lead nitrate method was used for acid phosphatase and indoxyl acetate method for esterase (Pearse)[1].

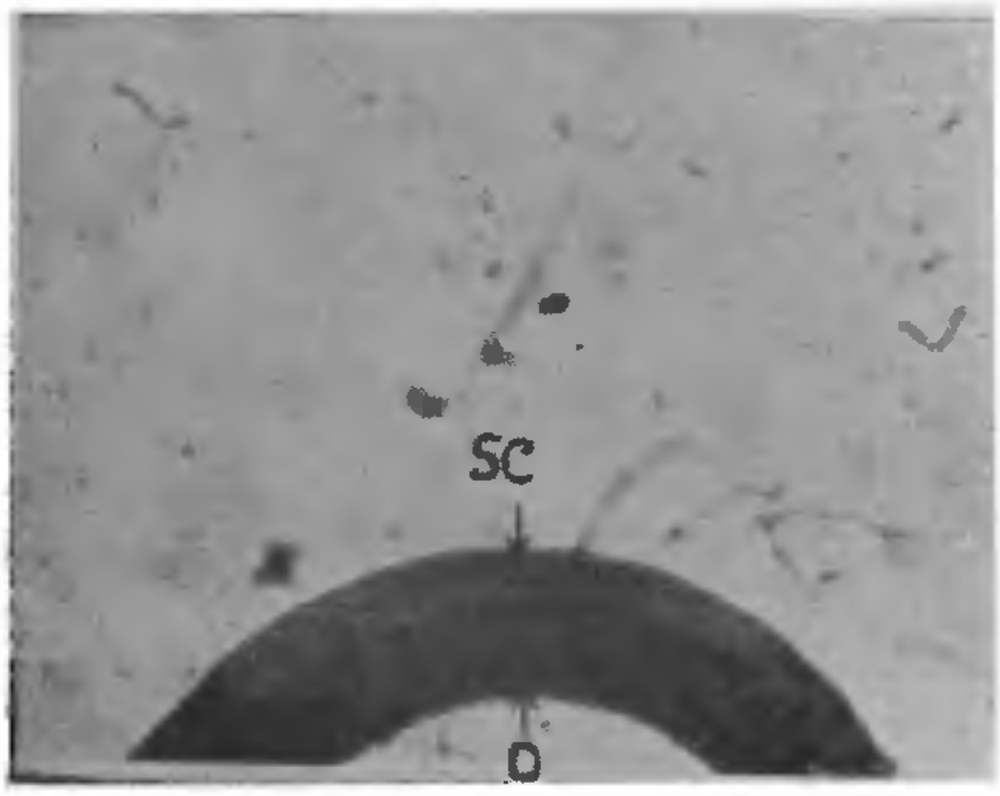

FIG. 1. Portion of third-stage larva showing marked acid phosphatase reaction in subcuticle (SC) and oesophagus (O).

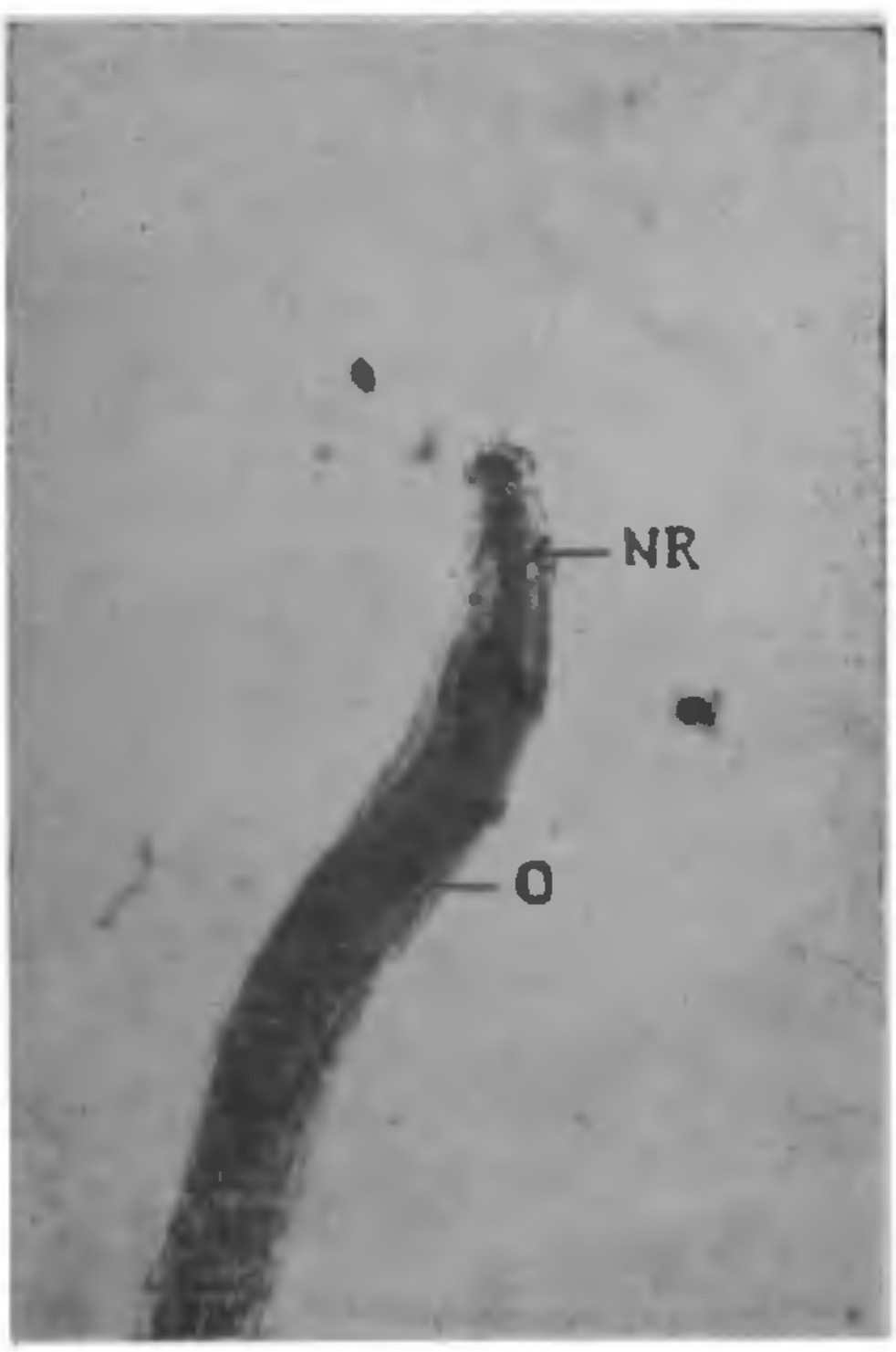

FIG. 2. Anterior end of third-stage larva showing esterase activity in the nerve ring (NR) and oesophagus (O).

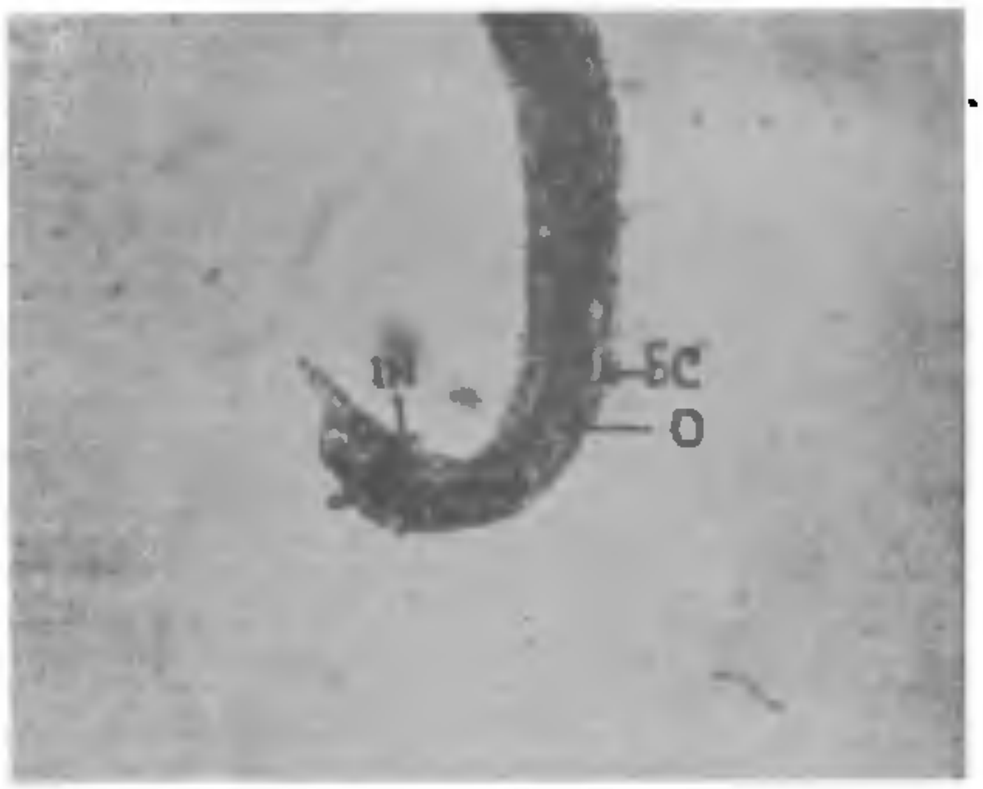

FIG. 3. Posterior end of third-stage larva showing esterase activity in the oesophagus (O), intestine (IN) and subcuticle (SC).

Third-stage larva of *Diplotriaena tricuspis* responded positively to these enzymes. The subcuticle, oesophagus (Fig. 1) and intestine of the larva gave strong reaction for this enzyme, while the nerve ring was found negative. However, esterase activity was detected in higher concentrations in the nerve ring, oesophagus (Fig. 2), intestine and subcuticle (Fig. 3).

Histochemical localization of acid phosphatase in the subcuticle and intestine of adult *Litomosoides carinii* and *Dirofilaria immitis*, and of esterase in the subcuticle, nerve ring, oesophagus and intestine of *Ascaris lumbricoides* has been frequently reported[2,3]. However, Omar and Schulz-Key have reported the distribution of acid phosphatase all over the body of the third-stage larva of *Onchocerca volvulus*[4]. Whereas Ruitenberg[5] detected it only in the intestine of *Anisakis* and *Trichinella* larvae.

Acid phosphatase and esterase activities in the subcuticle of third-stage larva of *D. tricuspis* indicate the presence of a few more enzymes and it is assumed that these enzymes are concerned with the formation of cuticle when the nematode increases in size after the moulting as earlier reported by Lee[6] in adult *Ascaris lumbricoides*. Similarly, intense acid phosphatase activity in the intestine of these larvae indicates that the intestine acts as an absorptive surface. The presence of esterase in the oesophagus and intestine of *D. tricuspis* larva clearly indicates its secretory function as earlier reported by Lee[6].

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NEW RECORD OF A SIMPLE ASCIDIAN, *STYELA BICOLOR* (SLUITER, 1887) FROM THE TUTICORIN COAST OF INDIA

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The present note deals with one simple ascidian, *Styela bicolor*. A perusal of literature[1–4] on Indian ascidians revealed that this form has not been reported so far from India and now it is recorded for the first time at Tuticorin, South East Coast of India.

The important generic characters are, four folds usually on each side of the branchial sac, but sometimes more or less reduced—gonads few, elongate, straight or sinuous but not U-shaped—follicles of testis somewhat separated from the central ovary.

The morphological characters of the species as observed are briefly given below.

The specimens are about 7 mm to 2 cm long—attached to the substratum by one-third of their posterolateral part of the body—siphon ends are brown in colour and are close together—oral tentacles are about 12–32—dorsal tubercle is small—four branchial folds on each side—stomach with numerous folds—a short bulbous pyloric caecum is present—2 gonads of equal length.

This species was seen attached to the glass panels kept for the study of fouling organisms in Tuticorin waters.

Its distribution has been recorded from Gulf of Siam, Java, North of Australia, Banda Sea, Amboina (Moluccas) and Philippines and now it is recorded from India.

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ALL INDIA SYMPOSIUM ON VECTORS AND VECTOR-BORNE DISEASES

An all India Symposium on vectors and vector-borne diseases covering biology and control of vectors of Public Health Veterinary and Agricultural importance will be held at Trivandrum under the joint auspices of the Department of Zoology, University of Kerala and the Association of Microbiologists of India, Trivandrum Unit. The Symposium is scheduled for *February (26th–28th) 1982*. Further details can be had from the Convener, Dr. R. Sripathy Prasad, Department of Zoology, University of Kerala, Kariavattom, Trivandrum 695 581.

NATIONAL CONFERENCE ON CRYSTALLOGRAPHY

The XIII National Conference on Crystallography will be held in Nagpur during March 15–19, 1982.