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## KARYOLOGICAL STUDY IN THE DIGENETIC TREMATODE NOTOCOTYLUS ATTENUATUS

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## ISOLATION OF *BORDETELLA BRONCHISEPTICA* FROM A CASE OF BOVINE MASTITIS

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MILK from a cow suffering from clinical mastitis was collected aseptically for bacteriological examination. The sample milk which was yellowish, watery and containing clots was inoculated in serum broth and incubated for 18 hr at 37°C. Streaking of the growth on blood agar plates, resulted in the appearance of tiny, dew drop-like colonies after 48 hr. The organisms were gram-negative, short slender rods, which were motile, non-sporing and non-capsulating. The pure culture was oxidase-positive. Based on other specific biochemical and sugar fermentation tests, the organism was typed out as *Bordetella bronchiseptica*<sup>1</sup>. The organism was sensitive to neomycin, streptomycin, polymixin-B and was resistant to ampicillin, kanamycin, furadantin, erythromycin, penicillin, oxytetracyclin, and chloramphenicol.

*B. bronchiseptica* is known to be widely distributed in nature and is usually transmitted by contact. The association of this organism with bronchopneumonia in rodants, dogs, swine and man has been established<sup>2,3</sup>. But the role of this organism in inducing bovine mastitis is not clearly understood.

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KARYOLOGICAL investigations of digenetic trematodes, although meagre and sporadic, have been the subject of speculation and divergent views. Some of the earlier reports on chromosome number and morphology are based on paraffin sections. Much reliance cannot be placed on these sections for an accurate number and position of centromere. The genus *Notocotylus* has more than 29 species<sup>1</sup>. The diploid chromosome number has been described as 14 for *Notocotylus filamentis* from sectioned material<sup>2</sup>. The chromosome studies investigated in *N. attenuatus* are described here for the first time.

The mature parasites of *N. attenuatus* collected from the intestine of wild duck *Anas creaca*<sup>3</sup>, were processed by the technique described earlier<sup>4</sup>. All the divisional stages were observed from testes squashes. Karyotype analysis was made from six spermatogonial metaphases. Chromosome measurements were made for computing the relative length, arm ratio, centromeric index and construction of karyotype<sup>5-7</sup>.

The diploid chromosome number of the parasite *N. attenuatus* consists of twenty chromosomes studied from gonial metaphases. They fall into three groups. One pair of large chromosomes with total length of 2.57  $\mu$  is found in this group and designated as submetacentric. Median chromosome pairs two to four with total length ranging from 1.77  $\mu$  to 1.48  $\mu$ . Third pair is submetacentric and the rest are telocentric in nature. The rest of the six pairs of small chromosomes are telocentric and ranging from 1.12  $\mu$  to 0.96  $\mu$ . The ratio between the shortest and the largest chromosome is 1:2.6.

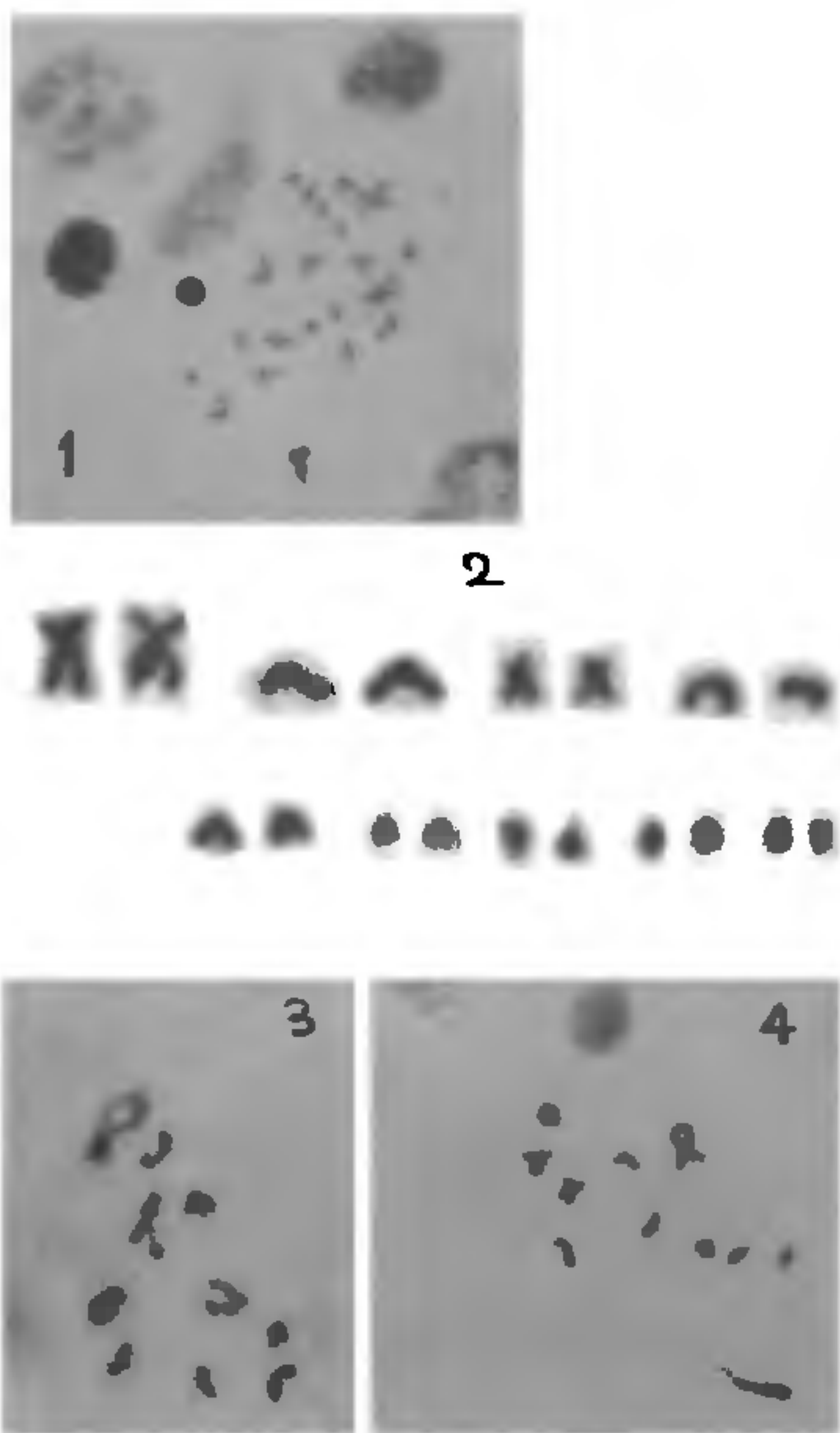
The karyotype constructed from the data (table 1) clearly shows that the first and the third pairs are submetacentric and the others are telocentric. A careful perusal of diplotene and diakinesis indicates one large, three median and six small bivalents which confirm the gonial chromosome gradations.

The chromosome number of *N. filamentis*<sup>2</sup> was found to be 2n-14. These chromosomes can be classified into 2 short pairs (1  $\mu$ ), 2 median pairs, and 3 long pairs (2.4  $\mu$ ) with ambiguous position of

TABLE I

*Quantitative characteristics of metaphase chromosomes of N. attenuatus*

Chromosome number	1	2	3	4	5	6	7	8	9	10
Relative length	18.9	13.0	11.3	10.9	8.2	8.2	7.8	7.0	7.0	7.0
Centromeric index	25.29	0.0	35.94	-----	00	-----	-----	-----	-----	-----
Arm ratio	2.95	$\infty$	1.78	-----	-----	-----	$\infty$	-----	-----	-----

**Figure 1.** Spermatogonial Metaphase X Ca 1300**Figure 2.** Karyotype X Ca 3100**Figures 3 and 4.** Diplotene and Diakinesis X Ca 1300

centromere. Further, the outline drawings of the author are supposed to represent the karyotype (figure 4 of Ciordia<sup>2</sup>) which, however cannot be taken as the accurate classification. In view of the incomplete data, it has become difficult to show any relationship between *N. filamentis* and *N. attenuatus*.

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