not produce hydrogen sulphide. It did not grow in media containing either 3·5% NaCl or 1% glycine. Deep stab inoculation in Bacto Thiol medium resulted in growth only near the surface. The orgnisma conformed to the tests for *C. fetus* as described by Smibert[5].

As already mentioned, there is no record of the simultaneous isolation of *B. abortus* and *C. fetus* from the same fetus. Therefore, the present observation is interesting. The abortion in this particular animal was probably due to brucella because of the fact that the aborted cow had a titre of 320 I.U. one week after abortion, when its serum was tested by rapid plate test and standard tube agglutination test. Moreover, uterine discharge of the cow showed the presence of brucella organisms both by direct microscopic examination using modified Ziehl-Neelsens technique[6], and also on culture, one week after abortion. It is not possible to indicate the role *C. fetus* played in this particular case of abortion although according to Laing[3] this organism also is occasionally responsible for abortions in bovines during any period of gestation and most commonly between the 5th and 7th months.

The authors acknowledge the financial support of the Indian Council of Agricultural Research for the scheme on Investigations on Infectious abortions amongst bovines in Karnataka State. Thanks are due to Dr. Brinley Morgan of the Central Veterinary Laboratories, Weybridge, U.K., for identification and typing of the brucella isolate.

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*July* 8 1978.

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## ISOLATION OF INFECTIOUS BURSAL AGENT (IBA) FROM DISEASE OUTBREAK IN BROILERS IN MAHARASHTRA STATE

A CONDITION causing considerable mortality in young broiler chickens described by Cosgrove[3] has been designated "Gumboro disease". The same year Winterfield *et al*[12] isolated and identified the causative virus and suggested the name "Infectious bursal agent (IBA)" for it. They have also described the lesions in the bursa of Fabricius.

Infectious bursal disease (IBD) is recognised as an important disease Angstrom[1]. The disease tends to recur in successive flocks of broiler chickens (Cosgrove *loc. cit*) and mortality in each successive outbreak usually decreases on some farms. However severe losses occur in flocks in spite of good sanitary conditions (Snedeker *et al*[11]). Mohanty *et al*[8] described pathoanatomical changes in infectious bursal disease. Jayaramiah and Mallick[7] isolated IBA in Andhra Pradesh. Severe outbreaks of mixed disease, Newcastle virus (NDV) disease and IBD, in young broiler chickens of 3 to 7 weeks occurred in July 1978 causing heavy mortality in spite of vaccination against NDV. This paper deals with the isolation of IBA from mixed infection of NDV and IBA for the first time in Maharashtra State.

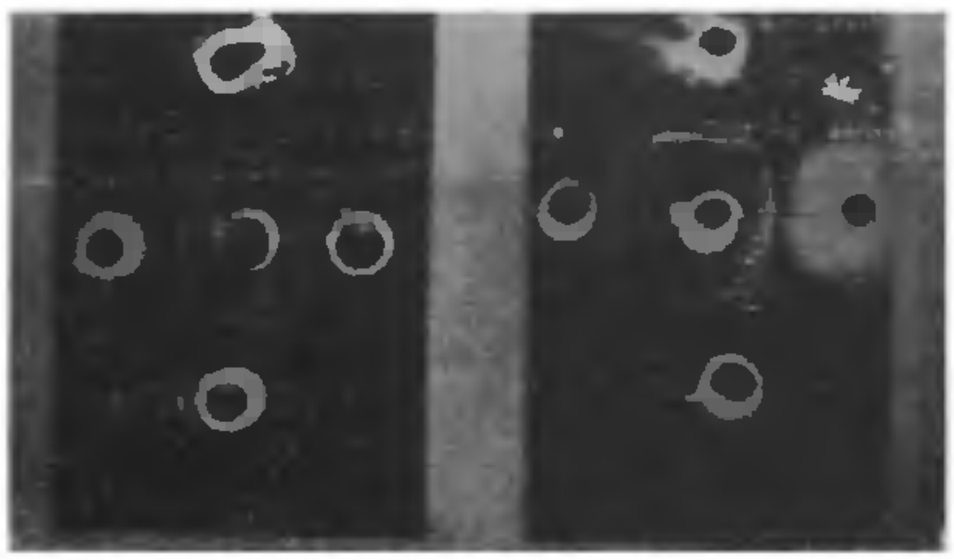
*Materials and Methods*

*Specimen for isolation of* IBA.—Spleen, kidney and bursa of Fabricius from dead and ailing birds, with or without gross lesions of NDV at the time of autopsy were collected in sterile test-tubes. These materials were triturated in morter with the addition of sterile sand and a 20% suspension was prepared in sterile nutrient broth. Penicillin (10,000 units) and Streptomycin sulphate (10 mgs per millilitre) were added. The suspensions were centrifuged at 2,000 rpm for 10 minutes and the supernatants were collected. Their sterility was checked. Kidney supernatant formed the inoculum for isolation of IBA in chicken embryo (Hitchner[5]). Supernatants of kidney, spleen and bursa were used for immunodiffusion test.

*Embryonating Chicken Eggs.*—Fertile eggs were obtained from the disease free flock from the Poultry Farm of Bombay Veterinary College, Bombay. Inoculum of 0·2 ml each was inoculated in 10 days old embryonating eggs by chorioallantoic membrane (CAM) and allantoic routes (Benton *et al*[2]) using three embryos for each route. Eggs were incubated at 37·5° C and candled twice daily. Embryos died between 48 and 72 hours. Dead embryos were chilled and later on harvested. Allantoic fluid (AF) and CAM were collected for further work. CAM was pooled, triturated and a suspension

was prepared with Penicillin 1,000 units and Streptomycin sulphate 1 mg per ml. AF was subjected to haemagglutination (HA) test and haemagglutination inhibition (HI) test to detect and confirm the presence of NDV. Same AF and CAM were subjected to immunodiffusion test to detect the presence of IBA and for elimination of NDV.

*Immunodiffusion tests.*—Immunodiffusion was carried out on glass slide (25 mm by 75 mm) by pouring 2·84 ml of 1·5% Noble agar (Difco) in veronal buffer (0·04 M), pH 7·8 (Rebeyrotte and Labbe[9]). Wells of 2 mm diameter, 12 mm apart from each other were cut out. Hyperimmune serum against IBA was placed in the centre and suspensions made separately of kidney, spleen and bursa were placed in other wells (Plate 1). Similarly, AF and CAM



PLATES 1–2. Plate 1. Centre well—IBA Hyperimmune serum, top well—Kidney suspension, bottom well—Bursa suspension, Right well—Spleen suspension, Left well—Kidney suspension, Plate. 2. Centre well—IBA hyperimmune serum, Right well—CAM suspension, Left well—NDV ($R_2$ B) Vaccine, top well—CAM suspension, bottom well—Allantoic fluid.

harvested after primary inoculation and also CAM harvested from embryos after inoculating them with purified CAM suspension were also subjected to this test in the same manner for the detection of IBA (Plate 2). The slides were observed for precipitation lines everday for 6 days (Jayaramiah and Mallick *loc. cit.*)

*Haemagglutination and Haemagglutination Inhibition tests.*—HA and HI tests of harvested AF were carried out in the perspex plate in two fold dilution. HI test was carried out by Beta procedure. Eight HA units of AF virus were used for HI test. $R_2B$ strain of NDV vaccine and normal saline were kept as positive and negative controls respectively. AF harvested after inoculating purified CAM supernatant in chicken embryos was used for HA test for detection of NDV. One per cent (Three times washed) chicken erythrocytes were used in the tests.

*IBA hyperimmune serum.*—IBA hyperimmune serum prepared in rabbit was supplied by Dr. B. D. Survashe, Department of Pathology, Bombay Veterinary College, Bombay-400 012.

*NDV hyperimmune serum.*—It was prepared by immunising four healthy white leg-horn, four months old birds with NDV vaccine ($R_2$ B). HI titre of the serum was 512.

*Purification of IBA.*—Kidney suspension supernatants, described under specimen for isolation of IBA, were divided into three portions. One portion was used for chicken embryo inoculation and second portion was subjected to heat treatment at 56° C for 90 minutes in water bath (Hanson[4]). Third portion was heated to 56° C for 2 hours and 40 minutes in water bath (Benton *et al*[2]). These were inoculated in chicken embryos after heat treatment. AF, harvested after inoculating kidney suspension and after the death of embryo during 48 to 72 hours which were positive for the presence of NDV by HA test was also subjected to heat treatment at 56° C for 2 hours and 40 minutes.

*Results.*—Kidney suspension inoculated without heating and after heating at 56° C for 90 minutes caused death of embryo within 48 to 72 hours. AF was positive for NDV by HA test (128 HA units). HI test confirmed the presence of NDV. This NDV positive AF and CAM suspension were negative for HA test after heating at 56° C for 2 hours and 40 minutes but IBA was detected in the same by immunodiffusion test indicating that both IBA and NDV replicated in chisken embryos. When the same CAM suspension was inoculated in chicken embryos after heating at 56° C for 2 hours and 40 minutes caused death of embryo between 72 to 120 hours (Hitchner[6]). Harvested AF from these embryos was negative for the presence of NDV by HA test but was positive for the presence of IBA by immunodiffusion test.

Similarly, the third portion which was heated at 56° C for 2 hours and 40 minutes caused death of the embryos between 72 to 120 hours and harvested AF was negative for NDV by HA test but AF and CAM showed precipitating lines by immunodiffusion test. All the embryos died when inoculated with the CAM suspension in the first passage after heating at 56° C for 2 hours and 40 minutes. But when the suspension of CAM harvested from these embryos was passaged for second time in the embryos, one embryo died out of three. The CAM route was used in both passages. The live embryos were sacrificed on the 5th day of incubation (Hitchner *loc. cit.*) and AF and CAM from these embryos gave precipitating lines by immunodiffusion test indicating the presence of IBA. AF was negative for NDV. Thus only

IBA was isolated by purification procedure and designated AMVP 1 isolate.

*Discussion.*—Bursa of Fabricius have been used for the isolation of IBA (Benton *et al*[2]). In the present study kidneys were found to be good specimen for isolation of IBA (Hitchner[6]). When kidney suspensions were heated at 56° C for 90 minutes, infectivity of NDV was not lost. Heating at 56° C for two hours and 40 minutes killed NDV but IBA survived (Hitchner *loc. cit.*). Growth of NDV in chicken embryos did not mask the growth of IBA as this was mixed infection of NDV and IBA in broilers. Mortality in chicken embryos due to IBA was 100% in the first passage and it was 30% in the second passage (Hitchner *loc. cit.*). Mortality in broiler chickens in the present study was not due only to IBA but also due to mixed infection with NDV. IBA was precipitating cause of heavy mortality. This has been explained by Rosenberger and Gelb[10].

*Summary.*—Isolation of IBA has been described from mixed infection of NDV and IBA in broiler chickens. Heating at 56° C for 2 hours and 40 minutes killed NDV but IBA survived which was deteted by immunodiffusion test. Heavy mortality was due to mixed infection of NDV and IBA. The isolate has been designated AMVP 1.

Thanks are due to Dr. Survashe for providing IBA hyperimmune serum and Prof. P. D. Sardeshpande for providing materials for isolation. Thanks are due to Prof. S. M. Ajinkya for his valuable guidance during the work.

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*August* 16, 1978.

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## A REPORT ON THE LARVAL FORMS OF THE NEMATODES BELONGING TO *GNATHOSTOMA* SP.; *CONTRACAECUM* SP.; AND *EUSTRONGYLIDES* SP. FROM NEW PISCIAN HOSTS, FROM VIDARBHA REGION OF MAHARASHTRA STATE, INDIA

*Gnathostoma* sp.

ONLY four larval forms were obtained from the heart of a fresh fish, *Macrones seenghala* (Skyes) collected from a local fish-market, at Gondia, District–Bhandara.

The larval forms ($n = 4$) are 2·77–3·15 mm long and 0·225 mm broad; head bulb 0·075–0·105 × 0·165–0·210 mm; cervical sacs 0·450–0·525 mm long; oesophagus measures 0·930–1·065 mm long; nerve ring at 0·200–0·212 mm from the anterior end; tail 0·052 mm long.

*Contracaecum* sp.

Numerous larvae of *Contracaecum* sp. were found in the outer wall of the stomach withir the cysts (Fig. 1) and mesentery of two fershwater fishes, *Bagarius bagarius* (Ham) and *Rita rita* (Ham.) obtained from river Vainganga, Tumsar; District Bhandara and river Paunar, Wardha; District Wardha

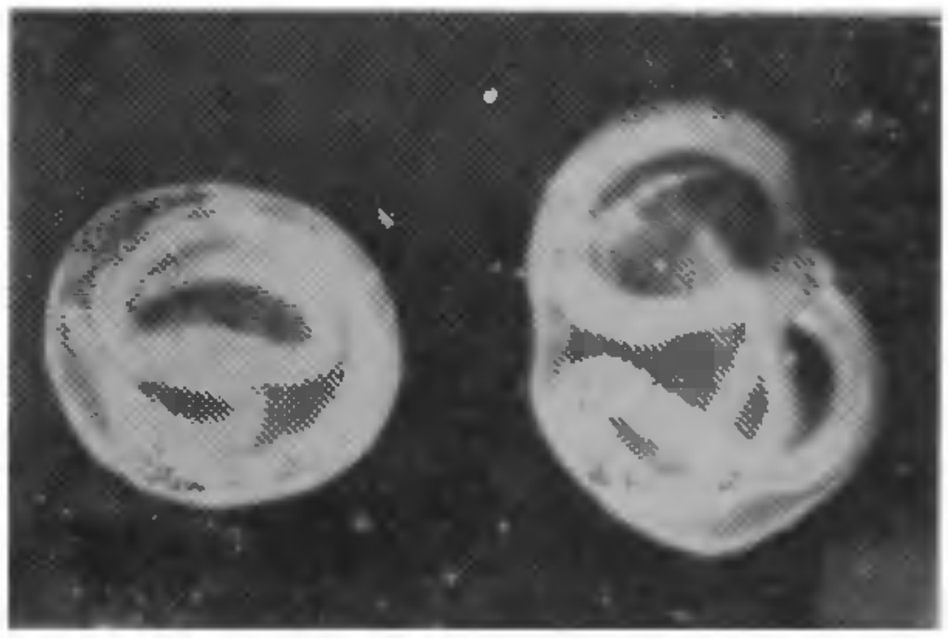

FIG. 1. Microphotograph of *Contracaecum* larva within cyst.

The larvae ($n = 15$) measure 7–20 mm long and 0·405–0·675 mm broad; head diameter 0·075–0·240 mm; oesophagus measures 0·900–1·755 mm long and 0·060–0·195 mm wide; nervering and excretory pore at 0·225–0·375 mm and 0·405–0·435 mm respectively, from head end; ventriculus 0·042–0·120 mm long and ventricular appendix 0·485–6·375 mm long; intestinal caecum 2·475–7·575 mm long; tail 0·225–0·375 mm long.

Karve and Naik[2] have recorded the larvae of *Contracaecum* sp. from *Mystus seenghala* of Poona.