a single larva weighing 0·7337 mg has yielded as much as $5·80 \times 10^6$ PIBs[14]. Thus the levels of nitrogen, uric acid and protein show the physiological development of healthy insects and have an important diagnostic value of viral infections.

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CONCOMITANT ISOLATION OF *BRUCELLA ABORTUS* AND *CAMPYLOBACTER FETUS* FROM AN ABORTED BOVINE FETUS

Brucella abortus and *Campylobacter fetus* (*Vibrio fetus*) are recognised pathogens of the genital tract of cattle, the former causing abortions and the latter mostly involved in early embryonic death and repeat breeding. Occasionally, *C. fetus* also causes abortions[4]. The isolation of *B. abortus* and *C. fetus* from aborted fetuses, infected cows and bulls is well documented. However, there is no record of the isolation of these two genital pathogens from a single aborted bovine fetus. This report records the concomitant isolation of *B. abortus* and *C. fetus* from an aborted bovine fetus.

During an investigation of infectious abortions amongst bovines in Karnataka State, an aborted bovine fetus, aborted around six months of gestation, was received in this laboratory from a brucella infected farm. The stomach contents and heart blood of the fetus were aseptically removed and cultured for possible isolation of Brucella, Campylobacter and any other pathogenic bacteria. A few drops of the stomach contents and heart blood were streaked on the following media: Bacto Tryptose (Difco Laboratories, USA) for Brucella, modified Florent medium[2] for Campylobacter and blood agar plates for any other pathogens. The blood agar and tryptose agar plates were incubated in 10% CO_2 tension in an anaerobic jar. Modified Florent medium plates were incubated in an atmosphere of 40% nitrogen, 10% CO_2 and 50% air in a locally manufactured anaerobic incubator. The plates were incubated at 37° C for 4–6 days prior to examination.

B. abortus was isolated in pure culture from the stomach contents in tryptose agar. The heart blood yielded a mixed culture of *B. abortus* and *C. fetus* in this medium. Modified Florent medium showed pure colonies of *C. fetus* only from the heart blood. Stomach contents of the fetus did not yield any growth in this medium. The colonies of *B. abortus* on tryptose agar, when examined on the fourth day, appeared round, with regular margin measuring about 2 mm in diameter. They were transparent and straw yellow coloured. On Grams staining, the organisms appeared as Gram negative coccobacilli arranged singly. When a loopful of the colonies was mixed with a drop of anti *Brucella abortus* serum, visible agglutination was observed in the cavity slide. The isolate conformed to the tests for *B. abortus* as described by Alton and Jones[1]. The isolate was further confirmed as *B. abortus* (biotype 1) by Brinley Morgan of the Central Veterinary Laboratories, Weybridge, U.K.

The colonies of *C. fetus* on the modified Florent Medium were circular about 1 mm in diameter, greyish white, transluscent and glistening in appearance. Under the stereoscopic microscope with a magnification of about 50 diameters, the older colonies had a greyish white opaque central area with a thinning out transparent periphery. Microscopic examination of a Grams stained smear from a young colony revealed Gram variable curved bacilli. Some seagull forms also were seen. Smear from an old culture grown in Bacto-Thiol (Difco Laboratories, USA) showed long spiral forms. Growth in this medium was in the form of a white opalescent ring about 5–6 mm from the surface. The isolate was catalase positive, did

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.

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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