them most susceptible to osmotic stress[20]. There is evidence[21] to conclude that trematode cuticle is not only very permeable to ions but also to water in either direction during osmotic exchange. Though the possession of an alimentary canal greatly lessens the necessity for a permeable outer covering, the physiological needs of parasites have forced them to evolve osmotic processes involving cuticular exchange[22], but their tolerance limit to osmotic stress varies. Parasite-induced osmotic stress beyond the tolerance limits of the host and the parasites within host cysts may be postulated to explain the unusual bursting of the oldest cysts in fish skin.

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ISOLATION OF CHLAMYDIA FROM CEREBRAL TISSUE OF BUFFALO CALF

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THE present work on isolation of chlamydia was undertaken as serological studies carried out by various workers[1-3] amply suggested the occurrence of chlamydial infection in domestic ruminants. Since chlamydia were isolated from a variety of tissues and not from nervous tissue, it was considered necessary to attempt isolation of chlamydia from nervous tissue of buffalo calves.

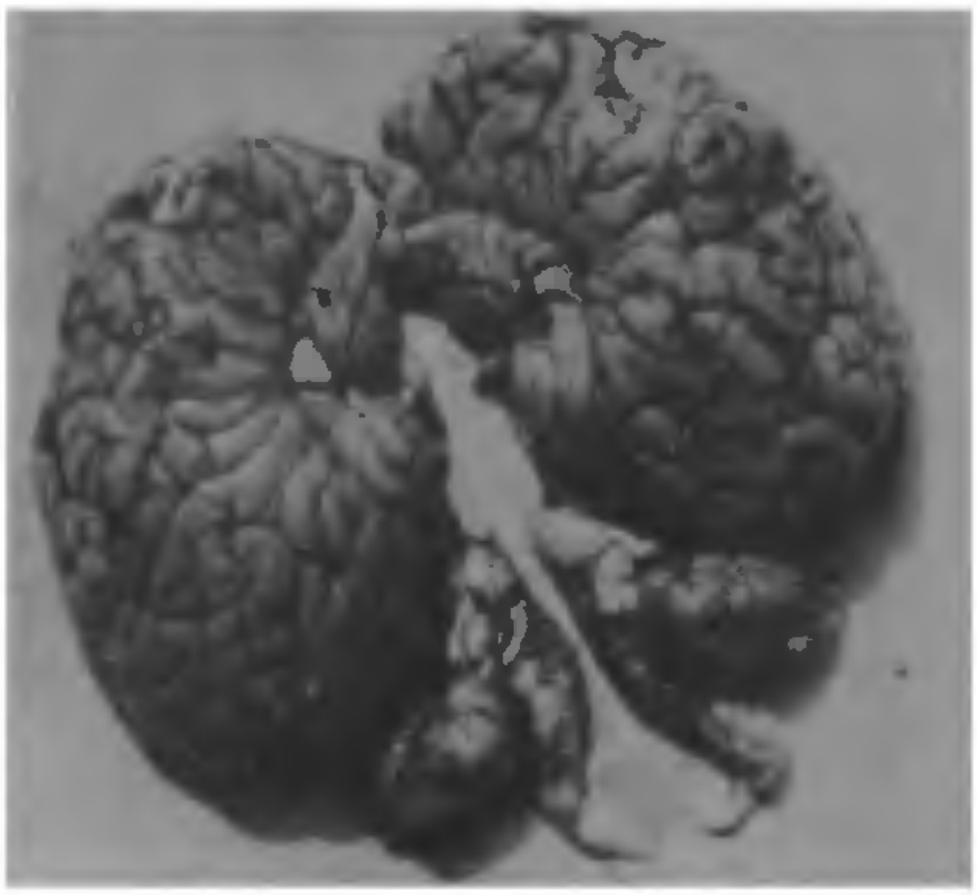
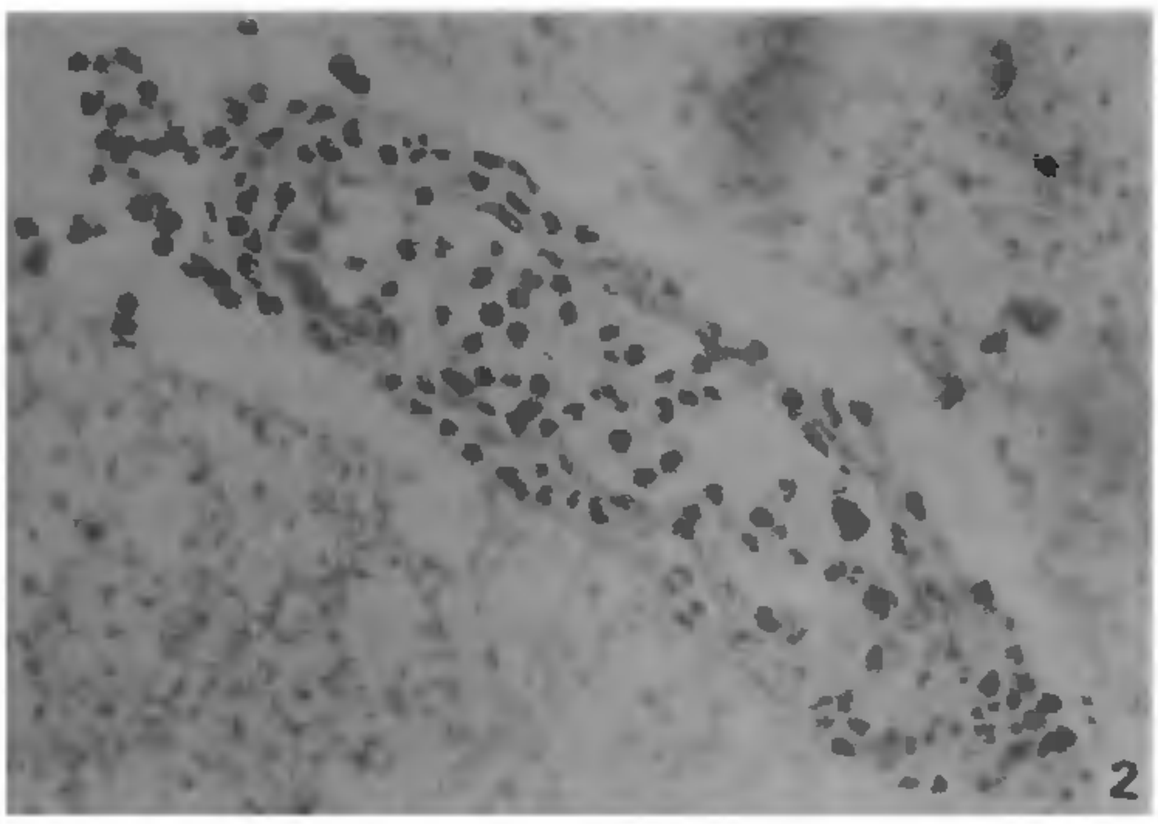
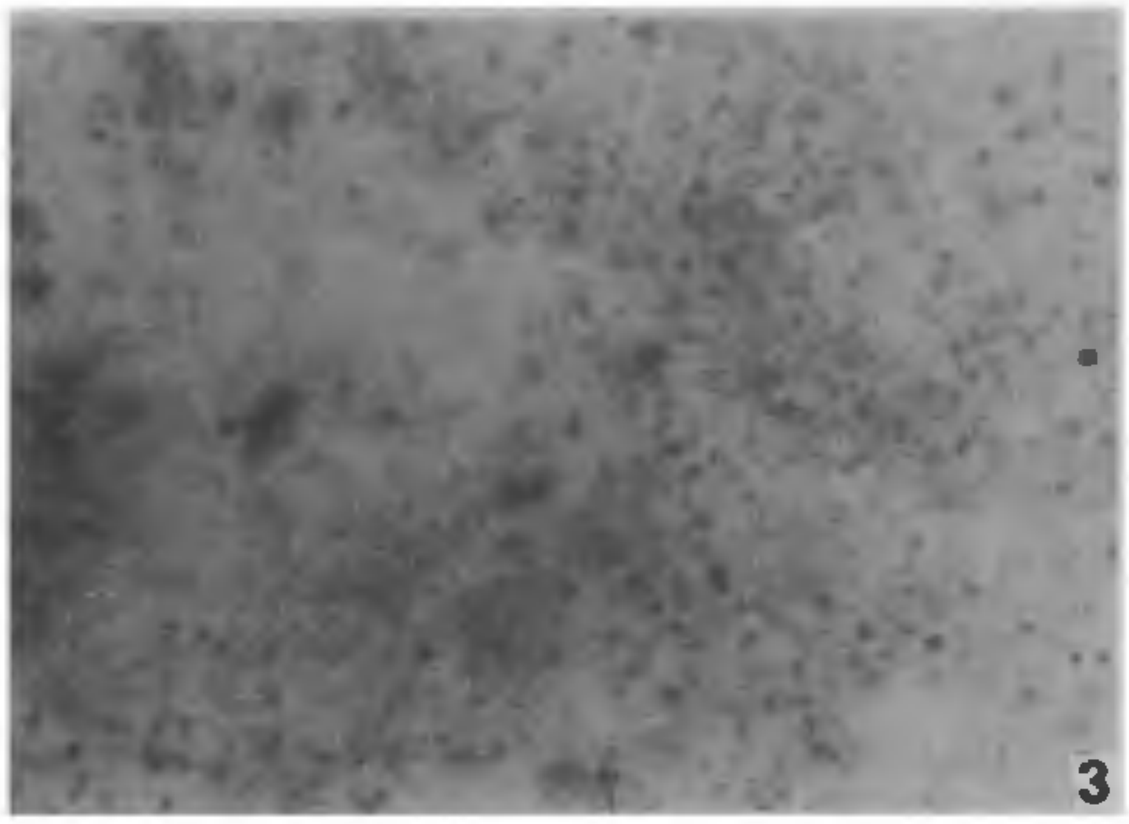


Figure 1. Severe congestion and edema of cerebrum and cerebellum in brain of buffalo calf.





Figures 2 and 3. **2.** Perivascular mononuclear cell cuffing (H & E × 225), and **3.** Infected yolk-sac membrane impression smear showing abundant, small, circular, pinkish chlamydial elementary bodies (Gimenez × 1120).

Isolation of chlamydia was attempted from brain tissue of a buffalo calf which exhibited congestion and edema (figure 1) grossly; revealed chlamydial elementary bodies in the mononuclear cells in Giemsa-stained impression smears[4] drawn from the meninges as well as in Giemsa-stained paraffin sections of cerebrum; exhibited vasculitis, endothelial cell proliferation, perivascular mononuclear cell cuffing (figure 2) in addition to neuronal necrosis, satellitosis and neuronophagia histopathologically; and in which the presence of chlamydial antigen was finally confirmed by fluorescent antibody technique (FAT) carried out in an impression smear of cerebral tissue. After confirmation of chlamydial infection, cerebral tissue collected under sterile conditions and stored at −20°C was used for isolation of chlamydial organisms.

The brain tissue was cut into small pieces of 1–2 cm size and ground in a pestle and mortar with sterile sand. A 20% suspension was made in sucrose–phosphate–glutamate medium to which streptomycin (10 μg/ml) and vancomycin (100 μg/ml) were added. It was centrifuged at 2000 rpm for 15 min. The supernatant was checked for sterility and used as inoculum to inoculate 7–8-day-old embryonating eggs. Inoculum (0.2 ml) was introduced into the embryonating eggs via the yolk sac. The embryos were incubated at 37°C and candled twice daily for 12 days post-inoculation (DPI). Three such passages were given in embryonated eggs. A Giemsa-stained smear[5] from the third passage revealed abundant pinkish, minute, spherical elementary bodies. These bodies were present singly and in clusters both within and outside cells (figure 3). The isolate was identified according to the criteria of Storz[6].

Fifty per cent mortality of chicken embryos occurred between 3 and 7 DPI; the remaining died between 8 and 11 DPI. Congestion of yolk-sac membrane, thinning of yolk-sac contents, and stunted growth were observed in affected embryos.

Giemsa-stained smears revealed scattered typical, minute, spherical, pinkish elementary bodies indistinguishable from the chlamydial elementary bodies.

Yolk-sac impression smears from the third passage were stained with fluorescein–anti-*Chlamydia psittaci* antibody conjugate for direct FAT. Bright greenish-yellow bodies were revealed.

Isolation of chlamydia from a case of meningo-encephalitis in buffalo calf may be considered significant; the chlamydia were able to reproduce meningo-encephalitis in a group of buffalo calves inoculated experimentally (reported elsewhere).

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A SIMPLE, RAPID AND RELIABLE SEROLOGICAL METHOD FOR DIAGNOSIS OF AMOEBIASIS

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COUNTERIMMUNOELECTROPHORESIS (CIEP) is a very simple, rapid, reliable, reproducible and economical method and does not require expensive apparatus or imported materials. A CIEP test has been standardized for quick diagnosis of amoebiasis using axenic *Entamoeba histolytica* antigen and specific hyperimmune serum raised in rabbits. The method detects specific *E. histolytica* antigen in serum samples of patients, and allows timely treatment and cure of the disease. Ganguly *et al*[1] have also found the CIEP test to be suitable for the detection of serum antigen and anti-*E. histolytica* antibodies in patients of amoebic liver abscess.

The antigen was prepared from an axenic culture of *E. histolytica* (strain 200:NIH). *E. histolytica* was mass cultured in modified monophasic Diamond's TPS-1 medium[2–4]. The antigen was prepared from the trophozoites by sonication following the procedure of Das *et al*[5]. Hyperimmune serum was obtained from rabbits immunized with antigen and complete Freund's adjuvant. The antibody was titrated by gel diffusion in 1% agar gel[6]. Blood samples of different categories of clinically diagnosed amoebiasis patients were collected from different local hospitals and King George's Medical College, Lucknow. For separation of serum, the samples were centrifuged at 5000 rpm at 4°C for 15 min. These samples were stored at −20°C until use.

CIEP was performed according to the method of Krupp[7] with slight modifications. The ionic strength of veronal buffer was 0.05 M, instead of 0.1 M. The 1% agarose slides were prepared in veronal buffer of ionic strength 0.01 M. The gels were examined after a run of 60–90 min (current 10 ma/slide) or 180 min (current 3–5 ma/slide) and overnight incubation at 4°C. Positive and negative controls were always run simultaneously.

The results for different categories of amoebiasis patients and normal subjects are shown in table 1. The patterns of bands are shown in figure 1. Serological detection of anti-*E. histolytica* antibodies in serum does not distinguish between presence and previous history of amoebiasis. Detection of specific

Table 1 *Detection of E. histolytica antigen in sera of different amoebiasis patients*

No. of samples	Clinical diagnosis	Serum antigen by CIEP	
		Positive	Negative
33	Amoebic liver abscess	31 (93.9%)	2
8	Amoebic hepatitis	7 (87.5%)	1
3	Amoebic cyst passers	Nil (0%)	3
13	Amoebic colitis	11 (84.6%)	2
41	Normal	6 (14.6%)	35

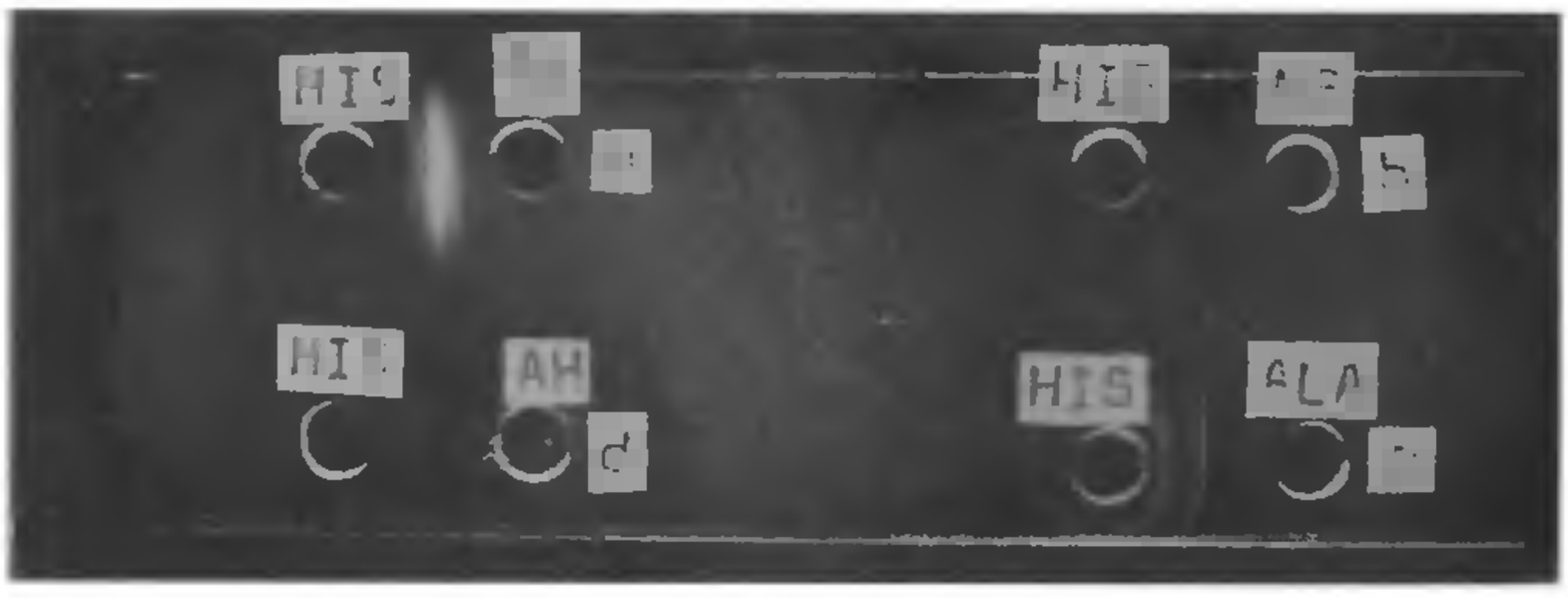

Figure 1. Counterimmunoelectrophoresis patterns of different serum samples. **a.** Positive control, **b.** Negative control, **c.** Amoebic liver abscess (ALA), and **d.** Amoebic hepatitis (AH). [HIS, Hyperimmune serum; Ag, Amoebic antigen; NS, Normal serum.]