



INCIDENCE OF *ENCEPHALITOZOOM CUNICULI* INFECTION IN DOMESTIC RABBITS IN INDIA

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ABSTRACT

An incidence of *Encephalitozoon cuniculi* infection occurred at the rabbit unit of the Instructional Livestock Farm Complex of Veterinary College and Research Institute, Namakkal, Tamil Nadu, India. The affected animals showed loss of co-ordination in the hind quarters, anorectic and death in 3 to 5 days from the time of first clinical sign. The incidence was first treated nutritionally with vitamins and minerals. When the incidence continued to occur, the suspicion became strong towards infective agents. Based on the clinical signs the suspicion was narrowed down to *E. cuniculi* and accordingly the diagnostic steps were carried out. Nearly 95% of the infected animals died. For diagnostic purpose, 15 affected rabbits were used to collect faecal and urine samples. Faecal sample and urine sediments made into thick smears and were examined by modified trichrome stain. *E. cuniculi* spores could be detected in the faecal and urine samples out of 15 rabbits examined.

KEY WORDS: Rabbit, Hind limb paralysis, Encephalitozoonosis.

INTRODUCTION

Encephalitozoon cuniculi is a eukaryotic organism belonging to the phylum microsporidia, in the kingdom fungi. *E. cuniculi* occurs in laboratory mice and rabbits, monkeys, dogs, rats, birds, guinea pigs, and other mammals including humans. The infective form of *E. cuniculi* is a resistant spore which can survive for a long time in the environment. The majority of rabbits seem to become infected with *E. cuniculi* from their mothers. Infection can also occur when something contaminated by urine containing *E. cuniculi* spores has been ingested. Approximately one month after the rabbit is infected it begins to shed *E. cuniculi* spores via its urine. Spores continue to be shed for approximately 3 months, but can be shed throughout the rabbit's life. The majority of the times, these organisms do not cause any obvious clinical disease. In fact, most often, *E. cuniculi* remains dormant within rabbits, never causing any problems upon initial infection. These parasites rarely migrate to nervous tissue or other organs. On occasions when *E. cuniculi* do reach nervous tissue, rabbits can experience neurological impairment, characterized by partial or complete paralysis, loss of coordination, seizures, and head tilting which is thought to occur when *E. cuniculi* multiply within the rabbit's body inducing the development of granulomas, which are believed to travel through the body via white blood cells.

MATERIALS & METHODS

A commercial cum instructional rabbit unit with 350 meat type rabbits maintained by the Veterinary College and Research Institute, Namakkal, Tamil Nadu State in India was taken for the study. White Giant and Soviet Chinchilla are the breeds maintained following cage system in an elevated platform shed. Bio-security measures namely antiseptic foot bath, rodent proof and bird proof shelter and careful entry of visitors are in practice. In spite of bio security measures, a disease epidemic resulted in high mortality. This episode was studied and discussed. In this outbreak, 95% (33 animals out of 35) of the infected animals died within 5 days of first appearance of clinical signs. The remaining 5% (2 rabbits) of the rabbits were removed from the flock and later disposed for diagnostic purposes. The infection sustained for more than 90 days by the emergence from other healthy animals in the shed. A total of 35 affected rabbits were considered for the study and 15 rabbits were taken for diagnosis. Faecal and urine samples were collected and subjected to microscopic examination. Many samples failed to reveal the any pathogenic organism while some showed the presence organism after continuous examination.

RESULTS & DISCUSSION

The first clinical sign noticed in the present incidence was diminished control of the hindquarters (leg weakness –Fig.1), the assumption was back injury, most likely from improper handling. But this cause was ruled out since the rabbits were not handled for many weeks.



FIGURE 1: Hind limb paralysis

Dullness, listlessness, anorexia and neurological impairment in the form of partial or complete paralysis, loss of coordination, seizures, and head tilt are subsequent signs.

Parasitological examination

From the affected animals, a total of one hundred and five (90 from affected and 15 from severely affected and sacrificed for diagnostic purpose) faecal and urine samples

were collected and examined. Out of which only two faecal and two urine samples revealed *E. cuniculi* spores by modified trichrome stain. This staining procedure revealed the spores (Fig. 2) in isolated fileds. Many samples did not reveal the spores since the organism rarely shed through urine and faeces. As in human cases, *E.cuniculi* could not be isolated both in the urine and faecal samples.

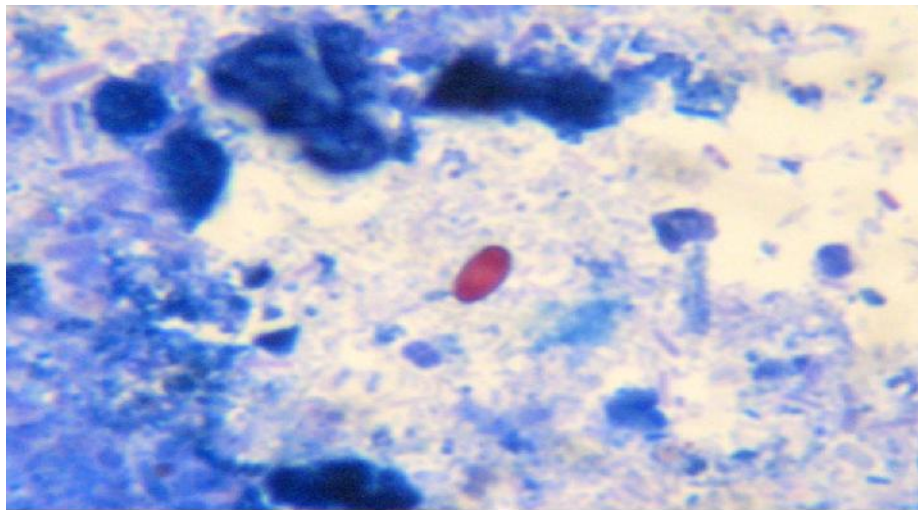


Fig 2. Spore of *E.cuniculi* (1000x) Modified trichrome stain

Many samples collected from affected animals showing typical symptoms failed to reveal the organism or spores while four animals revealed the organism after repeated and continuous examination. There are several staining techniques available for identification of microsporidial spores in faeces, urine and respiratory lavage sediment samples. *E. cuniculi* mainly shed in the urine that too for a very brief period in its incubation period. However, staining methods can, at best, identify microsporidia, but cannot distinguish between species. Current staining methods are also hindered by the fact that *E. cuniculi*

spores are often mistaken for bacteria or yeasts. Calcofluor white M2R is a chemo fluorescent stain that is commonly used for identifying microsporidia in fecal smears, urine sediments and respiratory lavages. Chromotrope-based (Modified Trichrome) staining methods are also used for identifying microsporidia in feces, urine sediment or respiratory secretion samples. Chromotrope stains give spores a pinkish red color, and quick-hot Gram-chromotrope stains color spores a dark violet. Characteristic morphological features of microsporidia that are visible using these stains are a belt-like stripe, a vacuole and Gram-positive granules. Again, spores may be

confused for bacteria or yeasts. Giemsa and Gram stains are not recommended for fecal smears because they do not differentiate between microsporidia and bacteria or yeast that may be present in the sample. However, Giemsa stains may be useful in urine or tissue samples, and spores stain light blue. Transmission electron microscopy is another way to identify and differentiate between species of microsporidia and confirmatory diagnosis is possible.

A major problem with this disease is the difficulty in diagnosing an active infection in live rabbits. It is usually obtained by a combination of examination of faecal and urine samples for parasitological examination by differential staining technique, clinical-neurological/ophthalmological examination, serological diagnosis and by ruling out differential diagnoses by post-mortem examination and performing histopathological and electron microscopic studies. Molecular detection of *E. cuniculi* DNA by polymerase chain reaction (PCR) in secretions or body fluids appears to be a sensitive and specific method to confirm the presence of the pathogen. When the suspicion went toward nutritional causes, vitamin B₁, B₆, B₁₂ and E were supplemented through feed. The drinking water was boiled, cooled and added with an antibiotic for complete sterilization. When the incidence continues to occur, the suspicion of *E. cuniculi* became strong, for which the typical clinical signs were the key factors. Rabbits are infected by ingestion or inhalation of spores passed in the urine or feces, or by transplacental transmission in some animals (Jordan, 2005 and Kathy Smith, 2012). A rabbit may contract it at a young age from an infected mother or from cage mates who are shedding spores, or later in life from an infected companion. The parasite attacks the nervous system and major organs, causing a variety of symptoms including head tilt, liver disease, kidney disease, cataracts, incontinence, loss of function in the legs (back, front, or both), nystagmus (eye twitching), and/or other neurological symptoms. In the present incidence, nystagmus was not noticed. Infection with *E. cuniculi* is usually asymptomatic, except in young or immune compromised hosts. Diarrhoea is the most common symptom of infection in immunocompromised individuals, but disseminated infections can occur, causing conjunctivitis, sinusitis, nephritis and encephalitis (Franzen and Muller, 2001). But, in the present incidence diarrhoea was not noticed. Marinell Harriman *et al.* (1989) handled 25 paraplegic rabbits and when autopsies on several rabbits failed to show identifiable causes they started calling it as "mystery disease" or "old-bunny paralysis." Similarly in the present incident autopsies could not reveal a confirmatory diagnosis.

Post-mortem and Histopathological Examination

Post-mortem is often the only definitive way of testing for the parasite. In rabbits, the central nervous system, kidneys and eyes are predilection sites. Infection of the kidneys leads to polydipsia and polyuria, and gross examination reveals a characteristic pitted appearance of the shrunken fibrotic kidneys. In the present case typical

kidney lesions were noticed in all the 15 sacrificed animals. Although gross lesions are not usually visible, meningoencephalitis can be observed upon histological examination. Histologically, lesions are most frequently recognized in the brain and kidney, consisting of focal non-suppurative granulomatous meningoencephalitis and focal to segmental interstitial nephritis with variable degrees of fibrosis. In the present incidence in addition to above lesions, haemorrhagic lesions were also noticed in the lung, liver and intestines.

Treatment

After the confirmatory diagnosis as Encephalitozoonosis infection, the remaining rabbits showing clinical symptoms were successfully treated with fenbendazole (20mg/Kg body weight) for 7 days as suggested by Suter *et al.*, (2001). The incidence was contained by the administration of fenbendazole daily for seven days.

CONCLUSIONS

Encephalitozoon cuniculi infection is probably first reported in India among meat type rabbits. Hind limb paralysis and death in few days are typical to the infection. Urine samples could be used for parasitological examination using trichrome staining. The infection can be better treated with oral administration of fenbendazole at recommended dose for a period of seven days.

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