

## Ultrastructural Diagnosis of Rabies in a Goat

**M. Asokkumar\*, L. Gunaseelan and S. Ramesh\*\***

*\*Department of Veterinary Public Health and Epidemiology  
Madras Veterinary College, Chennai-600007*

*\*\*Central Instrumentation Laboratory  
Madras Veterinary College, Chennai-600007*



### Introduction

Rabies is a deadly disease caused by virus belonging to the family *Rhabdoviridae* and is highly fatal disease of viral etiology affecting the nervous system of all warm-blooded animals on invasion. It is of great importance as a zoonosis and predominantly a disease of carnivores posing serious threat to man and animals. Productive animals get infected mainly through bite of a rabid dog. Rabies virus is transmitted primarily through the saliva of infected animals, most commonly through bite. The ultrastructure of rabies can be viewed through electron microscope as a bullet shaped structure with an average diameter of 75 nm and a length of 100-300 nm. The electron microscopical traits allow the virus particles to be identification up to the family or genus level. The direct fluorescent antibody test (FAT) is one of the most accurate microscopic tests available as gold standard test for diagnosis of rabies (WHO, TRS 931). Rabies is always fatal once clinical symptoms are exhibited with reports of rabies in goats being sporadic; however the furious form of rabies appears to be more common. The present paper reports a case of rabies in goat.

### Materials & Methods

A two year old goat was brought to the private veterinary clinic with a history of inability to take feed, excitement, drooling of saliva and tendency to attack over 3 days. A further detailed anamnesis revealed that the goat had been bitten by a stray dog about 2 months back on its thigh region ( category II bite) and the owner had applied turmeric powder after cleaning the area with warm water. No post exposure prophylaxis was performed. On examination, the animal showed symptoms specific of rabies aggressiveness, biting inanimate objects without provocation. Paralysis of the hindquarters and total recumbence (Fig.1) after examination were observed. The animal tried to bleat but no sound produced. The animal died within few hours of recumbency.



**Fig.1 Rabid Goat**

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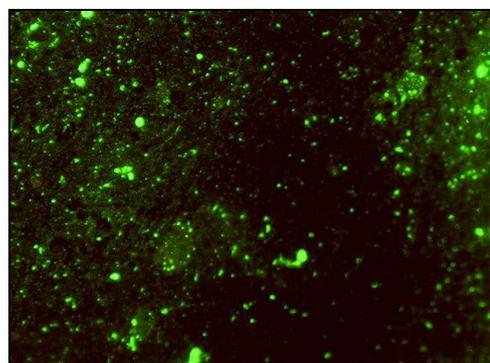
*\* Corresponding Author: Assistant Professor,  
E-mail: drashokpmd@gmail.com*

On post mortem, the brain sample (Hippocampus) was collected from the dead animal through retro-orbital route without opening the skull and brought over ice for laboratory processing. The impression smears were prepared on a clean slide and subjected to direct fluorescent antibody test (FAT) to detect rabies specific fluorescence using rabies anti-nucleocapsid FITC – conjugate (Bio-rad, France). A portion of brain sample was triturated with sterile sand in PBS-A buffer using pestle and mortar, while triturating 2% horse serum was added to the brain suspension in order to stabilize the virions in the sample. The brain suspension was transferred to sterile centrifuge tube and centrifugation was performed at 1200 g for 5 minutes. The supernatant was then subjected to ultracentrifugation at 90000 g for one hour to generate a pellet of concentrated virus particles. The pellet was resuspended in 20µl of sterile water for injection and was used for negative staining with 2% phosphotungstic acid (PTA, pH 7.0) for electron microscopic study (Goldsmith *et. al.*, 2009).

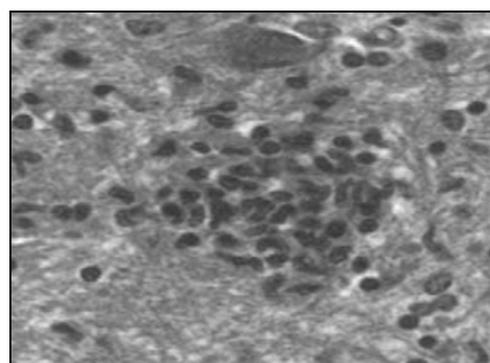
### Results and Discussion

Despite sporadic reports of rabies, the furious form is more common than dumb form in goats. The major clinical signs of rabid goat are aggressive behavior, continuous bleating, excessive salivation and paralysis and the clinical course is usually between one and five days and always results in death (Smith, MC. *et.al.*). Though various methods are employed for the detection of Lyssavirus

of the family *Rhabdoviridae*, the fluorescent antibody test (FAT) as in (Fig 2) remains the “gold standard” and consequently the most commonly used rabies diagnostic technique (Meslin *et al.*, 1996; OIE, 2012). The sensitivity of test may be reduced in autolysed and putrified brain samples. The electron microscopic study of the obtained supernatant showed a typical bullet shaped virus particles (Fig.3), which substantiates earlier studies (Matsumoto, S. 1963).



**Fig. 2 FAT showing rabies specific apple green fluorescence**



**Fig.3 TEM image showing typical bullet shaped rabies virus**

In this case, a tentative diagnosis of rabies was established based on the clinical signs in the animal before death. However, the direct fluorescent antibody test (FAT) and electron

microscopic (TEM) studies aided in a confirmatory diagnosis. Parthasarathy *et.al.*,(1978) reported 19.1 per cent of rabid goats amongst the cases admitted for observation of rabies during the period between 1971-75 at Madras Veterinary College Hospital and a later survey also showed a high proportion of positivity among suspected cases of goats for rabies (Sudarshan, 2005). The aggressiveness in captive animals suggest a greater awareness level among farmers need to be instilled about rabies and its post exposure prophylaxis in farm animals in general and dog bite management in particular.

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