### **ORIGINAL ARTICLE**

# Rabies, an emerging threat in livestock - A field perspective in Shivamogga District, Karnataka

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#### Abstract

Rabies control in livestock is entirely dependent upon either eradication of the disease in vector animals or immunization of livestock liable to be exposed. Considerable number of livestock died of the rabies, the actual number greatly exceeding those included in the annual report of Department of Animal Husbandry, Dairying & Fisheries ministry of Agriculture, government of India.

Totally, 37 samples of brain were collected by employing occipital foramen magnum method from different species of livestock which died due to suspected rabies and had history of being bitten by rabid dogs. These samples were collected from different geographical locations of the Shivamogga district, Karnataka. During Jan 2017 to May 2019. The brain samples were tested by Lateral Flow Assay (LFA) in OIE Twinned Rabies diagnostic laboratory, Dept. of Microbiology, Veterinary College, Hebbal, Bangalore for further confirmation was done by Direct Florescent Antibody test (DFA) The number of brain samples that showed presence of viral Ag were as fallows; Cattle (15/19) Sheep (1/1) Goat (2/2) Buffalo (1/1) Dog (11/14).We attempted one heath approach in control of rabies in Shivamogga District of Karnataka State by organizing "World rabies day" and also created awareness among the public regarding the importance of the disease.

Key words: Rabies, Control of rabies in Livestock, LFA, DFA

#### Introduction

Rabies in livestock is associated with bite of an infected animal of other species, usually of dogs and cats in cities and wild animals in near forest villages. Therefore, control of disease in livestock is entirely dependent upon either eradication of the disease in vector animals or immunization of livestock liable to be exposed. Rabies is prevalent throughout India and South Asia, occurring primarily in dogs, domestic cats. Although rarely in serious proportions, it has been reported in wild foxes, wolf and other wild animals. At present times, rabies in dogs and other livestock is quite prevalent in all the states of India.

In India, states such as Punjab, Haryana, Utter Pradesh, Uttaranchal, Maharashtra, Gujarat, Kerala, Tamil Naidu, Manipur, Meghalaya, Sikkim, Arunachal Pradesh, Mizoram and Nagaland have reported occasional case of rabies while substantial deaths have been reported in all other states of India. Except in Lakshdweep, Andaman and Nicobar, Dadar and Nagar Haveli1. This could be due to low population density of stray dogs and isolated geographical location of these places with rest of the country. The control of disease in endemic region becomes easier if there is an effective vaccine and efficient diagnosis. Karnataka has the greatest incidence of infection, particularly in dogs and livestock's. Rathnaprabha2et al., 2016, observed that 12 out of 27 (44.44 %) livestock brain sample which are tested for rabies antigen found positive during Jan 2011 to march 2016, which are presented to Madras veterinary college, opined that incidence of positive cases were highest in canines which are major carrier host and source of infection for rabies virus. also Prasanna3, et al., 2016, reported prevalence of rabies in livestock and wild animals like mangoose and squirrels from northern Kerala. Manjunath4et al., 2016, reported the rabies antigen in brain of sloth bear from Tumkur, Karnataka. According to Swapna5 2016, drastic increase in incidences in ruminants was recorded. Shift in animal reservoir & cases from unnatural hosts were observed. Considerable number of livestock (cattle, buffalo, sheep and goats) died of the rabies, the actual number greatly exceeding those included in the annual report of Department of Animal Husbandry, Dairying & Fisheries ministry of Agriculture, government of India. Many areas of the country did not report any case during this long period of 7 years despite the endemic status of rabies there. The actual numbers, therefore, may be substantially higher considering the possibility of under reporting due to weak rabies surveillance and inadequate reporting mechanism in the country.

Livestock production notably of cattle, buffalo, sheep and goats constitutes the major agriculture industry in almost all the states of the country in which rabies is known to exist. Livestock are particularly vulnerable to attack by dogs, and livestock loss from rabies corresponds quite closely to the intensity of the infection in these animals. One species of animal vector cannot be incriminated to the exclusion of the others, because a rabid animal will bite any moving object, animate or inanimate, which may come within its vision. If cattle are in the immediate vicinity, 12 to 15may be bitten by one rabid animal, as evidenced by death of one after another in rapid succession. According to WHO 2018 report an estimated 35172 human death (59.6 % of global death) and loss of approximately 2.2 million DALYs (Disability-Adjusted Life Year) occur per year on Asia and due to dog mediated rabies6.India accounts more death in Asia (59.9 % of human rabies death) and globally (35% of human rabies death). The annual economic loss of rabies has been estimated to be US \$ 583.5 million and livestock loss is to the tune of US \$ 12.3 million in Asia and Africa.

#### Methodology

In the present study, totally 37 samples of brain (Table 1; Fig. 1) were collected by employing occipital foramen magnum method (Fig.2,3) from different species of livestock which died due to suspected rabies and had history of being bitten by rabid dogs. These samples were collected from different geographical locations of the Shivamogga district, Karnataka. during Jan 2017 to May 2019.

The brain samples were tested by Lateral Flow Assay (LFA) using the Anigen Rapid Rabies Ag Test Kit of BIONOTE, Korea..In brief, the brain tissue was mixed with equal quantity of the assay diluent in a micro centrifuge tube A negative control, a brain tissue sample confirmed negative by DFA previously) was also simultaneously tested. The test device was placed on a horizontal surface and four drops of the virus diluent mixture was added to the sample well. The results were read within 5-10 min. Presence of two bands in the result window at position "T" (Test sample) and "C" (Control) indicated the presence of virus.

Furthermore, the brain samples were transported to OIE Twinned Rabies diagnostic laboratory, Dept. of Microbiology, Veterinary College, Hebbal, Bangalore for further confirmation by Direct Florescent Antibody test (DFA) as per the standard protocol of CDC, Atlanta, GA, US.. In brief, the DFA was performed essentially following the method as described by the CDC, Atlanta. In brief, touch impressions were made from brain tissue samples on microscope slides. For each of the test samples, three impressions were made one each for the anti rabies nucleoprotein IgG-FITC conjugate (Millipore-Light Diagnostics, Rabies DFA III, Cat # 5600), negative control FITC conjugate (Millipore-Light Diagnostics, Cat # 5102) and normal goat serum FITC conjugate (Millipore-Light Diagnostics, Cat # 5202). A known healthy brain sample (as negative) was also included in the test as an internal control. The impressions were blotted onto paper towels to remove excess of moisture, tissue remains and the blood stains. Then were initially air dried for 5 min. before fixing in high grade chilled acetone (80 % v/v) either for an hr at -20 °C or overnight at 4 °C. The fixed impressions were briefly air dried to ensure that the acetone traces on it evaporated and were stained using 1:100 dilution of the above said FITC conjugates by incubating in a humid chamber at 37 °C for 45 min. Then the impressions were washed with 1x PBS for 5 min. and the wash step was repeated twice to remove excess stain. The stained impressions were observed under a fluorescent microscope (AxioVert A1, M/s Carl Zeiss, Germany). Presence or absence of typical granular intra-cytoplasmic apple green fluorescence of aggregated nucleocapsids was used as a criterion in declaring positive and negative samples respectively.

#### **Results:**

In all, 37 brain samples from animals were tested by both LFA and DFA. The details of testing are provided in Table 1; Fig.1. Further, Fig. 4 shows the rabies viral antigen by LFA and rabies viral inclusion by DFA.

	Cattle	Sheep	Goat	Buffalo	Dog
Positive by LFA and DFA	15	1	2	1	11
Negative	4	0	0	0	3
Total	19	1	2	1	14

Table 1: Outcome of testing brain samples of various species of animals by DFA and LFA



Fig.1: Outcome of testing brain samples of various species of animals by DFA and LFA



Fig. 2: Collection of brain sample from carcass of goat by occipital foramen magnum method



Fig. 3: Collection of brain sample from carcass of cattle by occipital foramen magnum method



Fig. 4: LFA showing the positivity of brain samples of cattle, goat and dog for rabies viral antigen



Fig.4: Direct Florescent Antibody test (DFA) showing rabies viral inclusions.

#### **Discussion**:

The onset of rabies in cattle is marked by prodromal symptoms which last from few hours to one to two days. In this period a clinical diagnosis of rabies is very difficult. There may be temperature rise of 1 to 3 oC, and general picture of malaise, anorexia, and abrupt cessation of lactation. Usually show marked depression which may be readily confused with some intestinal disturbance, such as impaction of rumen or rectum, or early stage of an infectious condition (FMD). Bellowing sound changes. At this stage, a good history and knowledge that rabies is prevalent in that area is invaluable and may save human exposure. The saliva may carry virus four to five days prior to the onset of symptoms. If in doubt, or if there is anything about the patient that arouses suspicion, it is advisable not to handle the mouth. Give only hypodermic medications and observe the patient in isolation. The vast majority of human exposure occurs during this early stage when one is groping for diagnosis. The transition from the prodromal to the furious stage is usually rapid within few hours. There is a pronounced change of behaviour. The patient become nervous, often shows persistent tenesmus, has tendency to attack. It does not eat and although it may attempt to drink water but unable to drink water and dehydrate rapidly. The facial expression changes rapidly and becomes quite diagnostic, have a tense, alert appearance: the head is up, the eyes are wide open and follow any moving object. Bellowing is very common. The head is extended, the back arched, the flanks tucked in a hoarse, deep bellow is emitted. The tone of the bellow is quite characteristic. It is difficult describe but once heard, veterinarians can make tentative diagnosis of rabies. Due to paralysis of the throat and inability to swallow, saliva may drool from the mouth. Occasionally, animal lie down and refuse to get up, however, if a chicken or dog is tossed in front of them, they jump up and chase it. They will attack and butt a barrel, box or other loose object. They are particularly sensitive to movement and sound. Small amount of urine are voided at frequent intervals and many females will accept a male repeatedly. The excitement stage rarely lasts over two days. The animal weakens rapidly, becomes prostrate and dies within a few hours of cardiac or respiratory failure due to extensive brain damage.

Clinical rabies in cattle may be diagnosed in the field without difficulty in many cases. However many rabid cattle show atypical symptoms and diagnosis may be difficult. Therefore, in areas where rabies exist sick animals should be handled as though they have rabies until one is sure they do not have this disease, livestock owner and veterinarians attribute salivation to the possible presence of a foreign body, such as stone or corncob or vegetables lodged between teeth or pharynx, with the bare hands, this may lead to scratches and abrasion on the hands from animal teeth, contamination of these wound with probable virus bearing saliva. Rabies is primarily an encephalomyelitic disease and may be confused with any other encephalitides or with the nervous type of acetonemia. A history of rabies or group of dog/ wild canines/ new dog in the immediate area two to three week before is very useful.

To confirm the disease, better not to destroy the animal but allow it to die a natural death. And collect the brain sample from dead animal through foramen magnum method and submit the same for Laboratory diagnosis.

We undertook Post Exposure Prophylaxis antirabies vaccination in the animals which were bitten from suspected or confirmed cases also. Pre exposure prophylactic vaccination undertaken in the owner dogs in the area of rabies report. There are scanty reports on post exposure prophylactic vaccination trials in livestock including cattle,7.8,9,10.Post exposure treatment in animals is stillcontroversial despite the fact that it has been practiced on large scale in certain situations in developed countries11(Clark and Wilson,2001). The Post exposure prophylaxis treatment has been described for variety of species both experimentally and for field condition11, Furthermore, controlled challenge experiments are necessary to establish definitely the duration of immunity, the minimum age limit and the value of postexposure vaccination. We have no field or experimental data to indicate the efficacy of postexposure administration of rabies vaccine.

Rabies is probably never transmitted from cattle to cattle. Control of the disease in livestock is therefore dependent entirely on control or complete eradication of the disease in the vector animals. The principal vectors are dogs and wild animals. Rabies control is essentially a program of reducing to a practical minimum the number of susceptible hosts within an infected area. The Control of the disease in wildlife is based entirely on maintaining a disease free dog population. We have made very little actual progress to date in eradication of rabies, therefore at present mass immunization of cattle in known infected area appears to be the only available protective measures.

The history of rabies eradication in this country has been and still is a masterpiece of lack of leadership confusion and incoordination. There is no uniform pattern of approach to the problem. The course of action pursued on the local level depends upon political expediency, enforcement officials are close to and responsive to local pressure groups. The result is that instead of being able to act immediately on the occurrence of one proved case of rabies to prevent its spread we must wait for an epidemic in order to have popular support, appropriation of funds or even demand.

Rabies in livestock is almost entirely a problem in animal husbandry economics. Incidence of rabies in livestock is directly related to incidence of disease in dogs. National rabies control programme implemented by Dept. of Health and Family welfare have taken much of rabies control activities. We believe that joint action by the three responsive agencies (Health and Family welfare, Animal Husbandry and Municipal/Local govt) is imperative and that the presence of rabies is disgrace. It could be eradicated without great difficulty but this would involve prompt coordination effort wherever the disease exists. We have more rabies than probably any other country in the world and the time is now past due for National programme of control and eradication. We attempted one heath approach in control of rabies in ShivamoggaDistrict of Karnataka State by organizing "World rabies day" attended by both veterinarians and medical doctors and discussing about the role of each of department and coordination, exchange of information, of disease existence. So able to maintain rabies vaccine in all Primary Health Centres and rabies immunoglobulin in Dist. Level Hospitals. In Karnataka, about 200 field veterinarians have been trained



on collection of brain samples in suspected animal died of rabies. Attempts are being made to create awareness among animal handlers of the Dept. of Animal Husbandry, also they are provided with Pre Exposure prophylaxis rabies vaccine.Furthermore, awareness to animal owners and school children is made about the prevention of dog bite and need of first aid.In addition to all these attempts, it is most important to convince the policy makers to consider that rabies is an economically important disease as more and more livestock rabies cases are being reported.The current study also emphasises the need to undertake PreEP even in livestock with special reference to cattle and buffalo.

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