



Clinical Findings and Diagnosis of Rabies Using Direct Fluorescent Antibodies Test (FAT) in Domestic Animals

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Abstract: Rabies is an important zoonotic disease of all warm-blooded animals. The present study was carried out in Jodhpur city of Rajasthan, India. In cattle and buffaloes, important clinical signs of rabies were bellowing, anorexia, high rise of temperature and hypersalivation whereas, in dogs changes in behaviour, aggressiveness and anorexia were the most common clinical signs. Biting and paralysis were the main signs in camel. In goat, head pressing and continuous bleating were observed the main symptoms. The diagnosis was done through brain sample collected from suspected cases of rabies after death. In present investigation, fluorescent antibody technique (FAT) was used in brain tissues of 82 rabies-suspected animals for diagnosis. Out of 82 brain tissue samples, 52 dogs, 8 buffaloes, 15 cows, 4 goats and 3 camels were examined for FAT test. Out of 82 rabies-suspected cases, 49 (59.75%) cases were found positive by FAT. On the basis of findings of our study it is proposed that FAT can be used as rapid diagnostic test for diagnosis of rabies in animals.

Key words: Rabies, clinical symptoms, FAT, animals.

Rabies is an enzootic posing serious public health concern in India. The disease is caused by highly infectious neurotropic negative-sense, single-stranded RNA (ssRNA) virus results in acute encephalitis in warm-blooded animals (Pringle, 1991). An estimated 55,000 people die of rabies worldwide every year, based on a probability model which takes into account under-reporting of this disease. The majority of human fatalities occur in developing countries (Knobel *et al.*, 2005) with India accounting for 20,000 human casualties annually (Sudarshan *et al.*, 2007). In India, children aged between 5 to 14 are in the highest risk group (Singh *et al.*, 2001). Rabies is mostly associated with stray dogs in India. It is transmitted through biting of dog in 96.2% cases (out of which 62.9% are due to stray dogs and 37.1% are due to other pets). No-one is responsible for veterinary and nutritional care of stray dogs (Butcher, 1999). Despite of 35% of world human death occurring in country though rabies is not notifiable in India, (Sudarshan, 2005 and Knobel *et al.*, 2005). The reporting of rabies cases is not properly due to fear of touching cadavers, not properly transporting of samples and limited number of

laboratories for definitive diagnostic test for rabies in India.

For routine histopathological diagnosis of rabies, the tissue sections of brain are examined for negri bodies. However, these are not present in all cases. Hence, there is a need for a better method of diagnosis of rabies. Present study was conducted in suspected rabies cases in domestic animals after death. Before the death of animal's, clinical finding was observed and confirmation was done through fluorescent antibody test (FAT) in brain tissue samples collected after death of animals.

Materials and Methods

Sample collection

Before collection of brain tissue samples of rabid suspected animals, appropriate biosafety measures were taken such as wearing full hand gloves, full sleeve aprons, masks, goggles, gum boots and face shield to prevent any exposure. The staff involved in collection of brain tissue samples of suspected rabid animals are vaccinated prophylactically.

The brain samples were collected from 82 rabies-suspected animals (dogs-52, buffalo-8, cattle-15, goat-4, camel-3) after death which were referred from the veterinary polyclinics and other veterinary hospitals and dogs brought for the animal birth control programme at the Marwar Animal Protection Trust (MAPT), Jodhpur (Rajasthan). MAPT established the first rabies diagnostic laboratory in the Rajasthan. The brain tissue samples were collected directly through foramen magnum with the help of 5 ml syringe using 16-gauge needle (Kachhawaha *et al.*, 2006). Tissue samples were stored in deep freeze at -20°C in 50% glycerol saline solution.

Fluorescent antibody test (FAT) technique of brain tissue impression smears:

The FAT was employed as diagnostic technique because of its excellent sensitivity, accuracy and speed as recommended by World Health Organization (Meslin *et al.*, 1996). Lyophilized, adsorbed anti-rabies nucleocapsid fluoresces in isothiocyanate (FITC) antibody conjugate was acquired from Canada. Each vial of lyophilized, adsorbed anti-rabies nucleocapsid conjugate was reconstituted with 3 ml of distilled water as recommended by manufacturer and centrifuged at 1500 rpm for 5

minutes for clarification. The clarified conjugate (0.1 ml) was added on the duplicate impression smears on every slide for each tissue samples. Control positive slides from infected animal brain and control negative from respective normal animal brain were prepared along with the test smear and 0.1 ml conjugate was also added on positive and negative control slides. The smears were covered with cover slips and slides were incubated at 37°C for 30 minutes by placing in a dark humidified chamber. The slides were twice washed in 0.01 M phosphate buffered saline (PBS) pH 7.5 for 5 minutes each. Thereafter, air-dried and mounted in 90% buffered glycerol (pH 8.5). The slides were examined using an AHBT3 - RFC reflected light fluorescence attachment.

Results and Discussion

Clinical observations in rabid animal:

In our study 100% (30/30) dogs showed hyper-salivation, 76.66% (23/30) showed biting behaviour/aggressiveness and pica in 40% (12/30) (Table 1). Whereas, anorexia was reported in 100% (30/30) cases, hence these signs should be taken seriously while examining any case of dog for rabies. Silva *et al.* (2004) and Eng and Fishbein (1990) reported aggressiveness in 77% and 31% cases, respectively. Paralysis was also considered as characteristic symptoms of rabid dogs, which were observed in 93.33% (28/30) cases, respectively. Low incidence (29%) of paralysis was recorded in rabid dog by Eng and Fishbein (1990). There is a common belief that rabid animal shows febrile condition but, in this study, we observed fever in 76.66% (23/30). However, 80% (24/30) rabid dogs showed circling movement.

In rabid buffaloes, anorexia was in 100% cases followed by pica, difficult in taking feed and hyper-salivation (Table 1). However, bellowing and congestion of eyes were observed 62.5% (5/8) and 87.5% (7/8), respectively. Frequent micturition, fever and paralysis were observed in 37.5% (3/8) cases. 12.5% (1/8) rabid buffaloes did not recognize the owner. Similar symptoms were also reported by Singh and Grewal (1998) and Rissi *et al.* (2008).

In rabid cattle, anorexia was observed in 100% (7/7) cases followed by hyper salivation in 71.42% (5/7) cases, congestion of eyes and bellowing in 71.42% (5/7) cases (Table 1).

Table 1. Clinical sign in rabid dog, buffalo, cattle, goat and camel

Signs	Dog (30)		Buffalo (8)		Cattle (7)		Goat (2)		Camel (2)	
	No.	%	No.	%	No.	%	No.	%	No.	%
Anorexia	30	100	8	100	7	100	2	100	2	100
salivation	30	100	4	50	5	71.42	1	50	1	50
Fever	21	70.00	3	37.5	4	57.14	1	50	1	50
Biting/aggressiveness	23	76.66	6	75	5	71.42	2	100	2	100
Not recognise the owner	-	-	1	12.5	5	71.42	1	50	1	50
Circling/head pressing/aim less wandering	24	80.00	2	25	3	42.85	1	50	1	50
Difficult in standing/ paralysis	28	93.33	3	37.5	5	71.42	0	0	1	50
Pica	12	40.00	4	50	4	57.14	2	100	2	100
Frequent micturition	Nil	-	3	37.5	4	57.14	0	0	1	50
Bellowing/bleating	Nil	-	5	62.5	5	71.42	2	100	0	0
Exophthalmia and congestion of eyes	Nil	-	7	87.5	1	14.28	1	50	0	0

However, frequent micturition in 57.15% (4/7) and difficulty in standing and not recognise the owner in 71.42% (5/7) and circling in 14.28% (1/7) rabid cattle. Exophthalmia and congestion of eyes were observed in 1 cow i.e. 14.28% (1/7). Similar to the findings of present study several researchers were also reported by Srinunthapanth *et al.* (1985), Tanyi (1988), Aytakin and Mamak (2009) and Pedroso *et al.* (2009).

In rabid goat, anorexia, aggressiveness, pica, bleating and difficult intake feed were observed in 100% (2/2) cases. Salivation, exophthalmia and not recognise to owner were recorded in 50% (1/2) cases. However, fever and head pressing were observed in 50% (1/2) cases.

In rabid camel anorexia, aggressiveness (biting) and difficult intake feed were observed in 100% (2/2) cases. Paralysis and not recognise the owner were observed in 100% (2/2) cases. However, salivation, fever and head pressing were observed in 50% (1/2) cases.

Fluorescent antibody test (FAT):

Out of 82 cases, 49 were diagnosed positive for rabies by FAT of brain samples. The overall incidence of rabies was 59.75% in suspected cases. Characteristic apple green immunofluorescence was observed intracytoplasmic in neurons (Fig.1) as well as in form of diffused fluorescence in the smears of brain tissues. The findings in this study are in accordance with other author findings (Jayakumar and Ramadassa,1991; Dutta *et al.*, 1992; Tepsumethanon *et al.*, 1997). Many

researchers compared the efficacy of FAT with other diagnostic test viz. cytological examination and histopathological examination in diagnosis of rabies and have reported that FAT was extremely sensitive while cytological examination remained the least sensitive followed by the histopathological examination (Dean and Abelseth 1973; Tepsumethanon *et al.* 1997; Praveena *et al.* 2003 and Singh and Singh 2011).

In developing countries where rabies is endemic and every laboratory cannot afford to be equipped with costly sophisticated equipments for diagnosis of rabies. Prompt and accurate diagnosis is of utmost importance for the surveillance and control of rabies in man and animals. The world health organization (2005) and world organization for animal health (2013) recommended golden test for

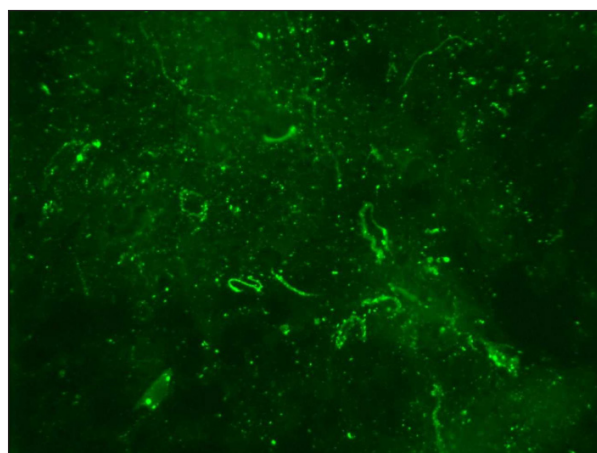


Fig. 1 Fluorescence in the brain tissue smears of rabid animal.

rabies diagnosis is detection of virus antigen in the brain tissue by the direct fluorescence assay (DFA).

Conclusion

It can be concluded that direct FAT test can be an important tool for rabies diagnosis. The technique can be used for rapid and prompt diagnosis for surveillance and control of rabies in developing countries. However, for these countries, widespread adoption of FAT tests may present certain limitations. For example, import of expensive fluorescent microscope along with requirement of well trained personnel, chemicals, personal protective equipments and a laboratory.

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