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Full Length Research Paper

Infectiousness of brain and salivary gland suspension from rabies suspected dogs

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Experimental study was conducted on rabies at Ethiopia Health and Nutrition Research Institute to assess the infectiousness of brain and salivary gland tissue suspensions from 13 rabies suspected cases of dog. Fluorescent antibody test (FAT) and mice inoculation test (MIT) were applied. Suspensions from each tissue of individual dog head was prepared and inoculated into intracranial in paired 10 mice pre head of dog. Rabies symptoms from these mice were considered over 30 days of observation and used as evaluation of infectiousness of the suspension. Among both suspensions prepared from 13 rabies suspected cases, 61.5% of each brain and salivary gland tissue were determined rabies positive using FAT. A 53.8% of the brains and 69.2% of salivary glands were found positive by MIT, having a significant difference in sensitivity (P<0.05). Out of the 260 mice inoculated, 55% showed clinical signs of rabies. Of which, 46% (60/130) were those mice inoculated with brain suspension and 63% (83/130) with salivary glands by proportion, showing significant difference (P < 0.05). From 260 inoculated mice, 74% found dead, of which 67% (88/130) and 80% (105/130) was inoculated with brain and salivary gland suspensions, respectively. The salivary glands suspensions were determined to be highly infectiousness than that of the brain (P<0.05). It was concluded that salivary glands suspensions is highly infectiousness than that of brain which could be from the higher virus titer in the salivary gland during symptoms and virus shading by infected cases.

Key words: Rabies virus, brain, salivary gland, infectiousness.

INTRODUCTION

Rabies is a central nervous system viral disease with irreversible fatality, except for few rare reported causes and zoonotic which case encephalitis in many warm-blooded animals and humans (Fauquet et al., 2005; Miah, 2005). Usually transmission occurs by bite with rabid canine and also under unusual circumstances by inhalation of large amounts of aerosolized rabies virus and through organ transplantation from rabies infected patients (Hellenbrand et al., 2005; Smith and McDonald, 2006). Rabies-infected animals have rabies virus in their salivary glands at high titers which can be even greater than in the brain (Charey and McLean, 1983). There is a long and variable rabies virus incubation period in humans and animal which usually last 20 to 90 days but sometimes it takes longer than 1 year (Smith et al., 1991) although there is uncertainty about the precise events during this incubation period. In Africa rabies constitutes a serious public health problem (Bogel, 1984) and also in Ethiopia, it is an important disease that has been recognized for many centuries. WHO (2005) estimated human mortality from endemic canine rabies at about 55000 deaths per year of which 56% in Asia and 44% in Africa.

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The majority (84%) of these occurs in rural areas. The estimated annual cost of rabies in Africa is US$ 20.5 million. These shows public health and economic burden of rabies. Some rare and endangered Ethiopian wolf (*C. simensis*) are further threatened by spillover from rabies in dogs (WHO, 2005). The treatments recommended for individual bitten by rabid animals mainly dogs have been recorded in many Ethiopian medical books since the early 17th century (Fekadu, 1982). The employment of a wide variety of supposed cures by early traditional practitioners illustrated the well-established characteristics of the traditional rabies pharmacopoeia (Pankhurst, 1990).

On the other hand, unique strains of the rabies virus in saliva of infected dogs have remission prosperity with view of great danger which acts as sources of infection for human and animal without noticed (Fekadu, 1972). Additionally, the isolation of two rabies related viruses, Mokola and Lagos bat viruses from domestic animals in Ethiopia by (Matsumoto, 1962) is of public and veterinary concern in view of the difficulty of proper diagnosis and lack of effective vaccines against these agents. FAT was reported to demonstrate rabies virus antigen in brain tissues of experimentally infected mice (Dean and Abelseta, 1973; Goldwasser and Kissling, 1958) and this method was found to be an efficient tool for the diagnosis (Beauregard et al., 1965). More research is needed in good experimental animal models in order for use of better one and to have well understood pathogenesis of the disease (Jackson et al., 2003). This knowledge may be important for the development of good management methods and novel therapies for rabies and other viral disease in the future. Therefore, the objectives of this study were to assess the infectiousness of the salivary gland and brain tissues suspension prepared from rabies suspected dogs.

MATERIALS AND METHODS

Ethical considerations

The research project was approved by the Academic Commission of the College of Veterinary Medicine, Haramaya University and Ethiopia Health and Nutrition Research Institute (EHNRI), Zoonoses research committee, Addis Ababa, Ethiopia.

Sample collection and handling

An experimental study was conducted to compare infectiousness of brain and salivary gland tissues suspension prepared from 13 rabies suspected dogs brought to EHNRI in Zoonoses Research Laboratory Addis Ababa, Ethiopia. These dogs are either clinically ill, whole caracas, sick one at the early or late stage of the rabies disease. All are registered and pairs of tissues were collected from dead one but all clinical subjects were kept under observation for 10 days (Radostits et al., 2007). In the event of death of clinical cases, tissues were also collected in the save for the test.

Laboratory methods

The brain (parts of the hippocampus, cerebellum and medulla oblongata) and salivary gland tissue (rostral part of sublingual glands and paratoid mandibular gland) was dissected to prepare slides for FAT (Dean and Abelseta, 1973), and each tissue suspension were also prepared for MIT using paired 10 each groups of mice for 13 cases of specimens inoculation from each tissue. Hence, a total of 260 mice were used (Delpietro et al., 2001). The specimens were prepared for intra cranial mice inoculation. The different nervous stages of rabies symptoms in inoculated mice were observed and recorded over a period 30 days after inoculation (Koprowski, 1996).

Statistical analysis

All the data collected from FAT and MIT clinical observation were analyzed using SPSS 16.0 and STATA version 7.0 software and student t-test were used. To assess the infectiousness of tissue suspension from suspected case percentage, mean, standard deviation and Chi-square ($\chi 2$) were calculated. P-value less than 0.05 were used to determine the significances.

RESULTS

Out of 13 total rabies suspected cases of dog 8/13 (61.5%) of both brain and salivary gland tissues were positive using FAT. But considering MIT positive when a mice is rabies positive in the group, 7/13 (53.8%) and 9/13 (69.2%) of mice inoculated with suspension prepared from brain and salivary gland tissue were respectively positive having significant difference $\chi 2=6.7$ and $P<0.05$ (Table 1).

As shown in Table 2, 143/260 (55%) of overall mice inoculated with both tissue suspensions showed rabies symptoms. The proportions from brain and salivary gland were 60/130 (46%) and 83/130 (63%), respectively with significant differences ($\chi 2 = 8.22$; $P < 0.05$).

Most of the symptoms manifested in MIT were paralysis and death. Paralytic symptoms of rabies were observed on the mean days of 11th and 12.6th day on mice inoculated with tissue suspension from brain and salivary gland, respectively shows insignificant difference in number of positive ($P > 0.05$) (Table 3).

With regard to the overall mice died from inoculation of

Table 1. The probabilities of diagnosis of rabies using FAT and MIT.

		Salivary gland		Total No. (%)	χ^2	P-value
		No. Positive	No. Negative			
Brain FAT	Positive	7	1	8 (61.5)	5.9	0.015
	Negative	1	4	5 (38.5)		
	Total	8 (61.5%)	5 (38.5%)	13 (100)		
Brain MIT	Positive	7	0	7 (53.8)	6.74	0.009
	Negative	2	4	6 (46.2)		
	Total	9 (69.3%)	4 (30.5%)	13 (100)		

Table 2. Infectiousness of tissue suspension from brain and salivary gland by MIT.

Group inoculation		MIT		Total Count No. (%)	χ^2	P-value
		Negative count	Positive count			
Brain	Count	70	60 (46%)	130 (100)	8.22	0.004
Salivary gland	Count	47	83(63%)	130(100)		
Total	Count	117	143 (55%)	260 (100)		

Table 3. Statistical results between the group and paralysis.

	Group	No. Positive	Mean day	Std. deviation	Standard error mean	F	df	P-value
Paralysis	Brain	34	10.88	2.25	0.39	15.5	75	0.072
	Saliva	43	12.60	5.12	0.79			

Table 4. Associations between group and death.

Group		Died		Total Count (%)	χ^2	P-value
		Negative count	Positive count (%)			
Brain	Count	42	88 (67)	130 (100)	5.8	0.016
Saliva	Count	25	105 (80)	130 (100)		
Total	Count	67	193 (74)	260 (100)		

Table 5. Frequency of rabies clinical sign observed from total of 260 mice.

Ruffled fur No. (%)	Sickness No. (%)	Partial paralysis No. (%)	Paralysis No. (%)	Death with rabies symptom No. (%)	Death without rabies symptom No. (%)	Total death No. (%)
32 (12.3)	26 (10)	64 (24.6)	77 (29.6)	143 (55)	50 (19)	193 (74%)

brain and salivary gland suspension, 193/260 (74%) were found death. From these, suspension from brain accounts 88/130 (67%) but from salivary gland accounts 105/130 (80%) showing significant difference ($\chi 2 = 5.8$; $P < 0.05$) (Table 4).

As shown in Table 5 different stages of rabies were observed in inoculated mice with positive results. Some of them showed one symptom followed by death, some others shows more than one symptom of rabies stages one after another then followed by death, and others died

without showing any rabies symptoms. But others remain alive after the periods of observation.

DISCUSSION

Rabies takes a terminally fatal course following the appearance of symptoms. The fatality could also coupled with the accompanying complications of post exposure treatment which necessitate the use of rapid, sensitive and specific diagnostic methods using appropriate samples for effective medical managements. On the basis of results of (Koprowski, 1996) MIT ensures high accuracy (and preferred as bench marks) to determine the sensitivity and specificity of other diagnostic tests of rabies. But the relative sensitivity and specificity of FAT in diagnosis of rabies was defined on the basis of specimens from rabies suspected cases. Thus, MIT were used as gold standard for rabies diagnosis. The present finding on the experimentally designed tested 13 cases shows 72% sensitivity and 66% specificity of FAT. Each technique was found to be differing significantly with fair agreement in the current study. Likewise the sensitivity and specificity of FAT showing difference from and lower than the report (Koprowski, 1996) which were 98 and 95%, respectively. This difference might be due to difference in laboratory quality. Although there is difference in species, the present overall 55% mice inoculated with suspension as well as the 63% of the groups inoculated with salivary gland suspension by proportion showing symptoms of rabies were similar with the 60 % that of pharyngeal paralysis in cattle reported by Radostits et al. (2007). This might be due to similarity in susceptibility of both species since both of the species are warm blooded with similar susceptibility. Similarly, the present 74% overall mice died from inoculation with both tissue suspensions and 80% died from the groups with salivary gland tissue inoculation were similar with each other and with 40 - 80% mortality occurred in person bitten on the head and face by infected animal (Shah and Haswal, 1976). Moreover the significantly higher proportion (63%) paralysis and (80%) death in MIT by salivary gland tissue suspension than those groups with brain suspension inoculation in the present study clearly shows the higher the infectiousness of suspension prepared from salivary gland than brain. This could be associated to the difference in rabies virus titers in both tissues. We suggested that there is high virus titer in the salivary gland and the saliva of the dogs with the symptoms. Similarly, Charey and McLean (1983) suggested that the rabies infected animals have rabies virus in their salivary glands at high titers which can be greater than in their brain. Radostits et al. (2007) also consider the titer of virus injected into victim is as one of factors responsible for the onset of clinical sign. A symptom of complete paralysis is observed in mice inoculated by suspension from brain and salivary gland on the mean day of 11th and 12.6th day, respectively is similar with each other and just with report of Radostits et al. (2007) in mice. Although it is not in same species, the most of the partial paralysis observed on average of 10th day in the present study is compatible with the 10 day incubation period of rabies in sheep reported by Radostits et al. (2007) in experimental study. This might be due to similar study design and both species are susceptible for the rabies. The ruffled fur, partial paralysis and complete paralysis are most of the symptoms observed in this study. These were agreed with previous work of Bingham and van der Merwe (2002). Hence, regardless of the incubation period, the salivary gland tissue suspensions and the saliva from infected cases have high infectiousness properties. Thus as rabies is economically important; public, livestock and wild-life health significant disease, it requires further epidemiological investigation and application of detailed study on the virus strain diversity in parallel with strategic prevention and control intervention in human, domestic animals and wild-life are recommended. The contribution of the virulence properties of the rabies virus at different tissues of the infected animal as well as strain composition in this study are under question as another gap.

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