

Table 1. Detection of rabies virus in body secretions of experimentally infected buffalo calves

DPI	Group 1			Group 2			Group 3		
	N	S	R	N	S	R	N	S	R
0	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-
15	-	+	-	-	-	-	-	-	-
20	+	+	-	-	-	-	+	-	-
25	-	-	-	-	+	-	+	-	-
30	-	-	-	+	-	-	-	-	-
35	-	-	+	+	-	+	+	+	-
40	+	+	-	+	-	-	-	+	-
45	-	+	-	-	+	-	-	+	-
50	-	-	-	-	+	+	+	-	-
55	-	-	-	-	+	-	+	+	-
60	+	-	-	-	+	-	-	+	+

DPI: Days post inoculation; N, nasal secretion; S, salivary secretion; R, rectal secretion.

titrated by intra-cerebral inoculation of serial dilutions ranging from 10^{-1} to 10^{-5} into 3 weeks-old albino mice. The calculation was carried out as per Reed and Muench (1938).

Preparation of inoculum for experimental study: After the third passage, all infected mice brains were pooled, weighed and homogenized in a sterile pestle and mortar to make a fine paste. Chilled sterile distilled water containing 2% inactivated normal horse serum was added to make a 10% suspension and was centrifuged at 1000 rpm for 5 min. 100 IU/ml of penicillin and 50 µgm/ml of streptomycin were added to the supernatant and were kept at 4°C for 30 min. This supernatant was inoculated via different routes to experimental buffalo calves divided into 3 groups.

Preparation of normal brain tissue suspension: Brains of normal mice were collected, pooled, weighed and homogenized. Chilled sterile distilled water containing 2% inactivated normal horse serum was added to make 10% normal mice brain (NMB) suspension. This suspension was

Table 2. Detection of rabies virus in impression smears of experimentally infected buffalo calves

DPI	Group 1		Group 2		Group 3	
	C	P	C	P	C	P
0	-	-	-	-	-	-
3	-	-	-	-	-	-
5	-	-	-	-	-	-
10	-	-	-	-	-	-
15	-	-	-	-	-	-
20	-	-	-	-	-	-
25	-	-	+	-	-	-
30	-	-	+	-	-	-
35	-	-	-	+	+	+
40	-	-	-	+	+	-
45	+	-	-	-	-	-
50	+	-	+	-	+	+
55	+	-	-	-	+	+
60	+	-	-	+	+	+

DPI, days post inoculation; C, corneal impression; P, prepucial impression.

centrifuged at 1000 rpm for 5 min. Penicillin @100 IU/ml and streptomycin @50 µgm/ml were added to supernatant and kept at 4°C for 30 min. This supernatant was inoculated to experimental buffalo calves, kept as control.

Experimental study: Male buffalo calves (9) aged 2–5 months were subjected to elementary examination for a week before starting the experimental study. During these days, their general behaviour, hematological parameters, body secretions/excretions and body temperature (°F), were analyzed. Modified counter immuno-electrophoresis was employed to rule out the anti-rabies antibodies in sera samples of these calves before the start of the experiment.

Buffalo calves (9) were divided into 3 groups of 3 animals each, wherein buffalo calves (2) were inoculated with infected mice brain (IMB) suspension and third buffalo calf was inoculated with normal mice brain (NMB) suspension which served as control. In group 1, buffalo calves (2) were inoculated with 5 ml of 656865 MICLD₅₀ of IMB suspension

Table 3. Sensitivity comparison of detection of rabies virus in body secretions and impression smears

		Earliest detection of rabies virus in experimental animals					
		Group 1 DPI		Group 2 DPI		Group 3 DPI	
		Control	Infected	Control	Infected	Control	Infected
1	Salivary	CND*	15 (2)	CND*	25 (1)	CND*	35 (2)
2	Nasal	CND*	20 (1)	CND*	30 (1)	CND*	20 (1)
3	Rectal secretion	CND*	CND*	CND*	35 (1)	CND*	45 (1)
4	Corneal impression smear	CND*	45(2)	CND*	25 (1)	CND*	35 (2)
5	Prepucial impression smear	CND*	CND*	CND*	35 (1)	CND*	35 (1)

*CND: Could not detect, () number of calves showing positive result.

via oral route. 5 ml of NMB suspension was inoculated via ocular route with 131373 MICLD₅₀ of IMB suspension wherein 0.5 ml of SRB was instilled in either eye. 0.5 ml of NMB suspension was similarly instilled in either eye in the control buffalo calf of group 3. Infected buffalo calves from 3 groups were sacrificed at 30 and 60 DPI whereas control buffalo calves of all the groups were sacrificed at 60 DPL.

Body secretions

The samples of salivary, nasal and rectal secretions were collected from zero DPI and subsequently on 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 DPI. The salivary, nasal and rectal secretions were collected as discussed here.

Salivary secretion: Sterile cotton swab was soaked in sterile physiological saline and wet cotton swab was swirled beneath and around the tongue surface to collect saliva. The collected swab was placed in a sterile test tube. Duplicate impression smears were made from cotton swab on the labeled glass slides and were fixed in chilled acetone for direct FAT staining.

Nasal secretion: The sterile cotton swab was inserted into nasal cavities and pressed against walls. Duplicate impression smears were prepared on labelled glass slides and fixed in chilled acetone for direct FAT staining.

Rectal secretion: The sterile wet cotton swab was inserted into the rectum by rotatory method against walls. The swab was collected in sterile test tube. Duplicate impression smears were prepared from the swab on labelled glass slides and were fixed in chilled acetone for direct FAT staining.

Impression smears

The prepuccial and corneal impression smears were prepared at zero DPI and subsequently on 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 DPI as discussed here.

Prepuccial impression smears: The samples were collected by inserting the wet sterile cotton swab into prepuce and rotating it. The swab was collected in sterile test tube. Duplicate impression smears were made from the swab on labelled glass slides and were fixed in chilled acetone for direct FAT staining.

Corneal impression smears: Duplicate corneal impression smears were made on labelled sterile glass slides directly and were fixed in chilled acetone for direct FAT staining.

Fluorescent antibody technique: Duplicate smears were prepared by swirling and circling movement of the swab on the glass slide. The smears were air-dried and fixed in chilled acetone overnight. Similarly, duplicate impression smears were made on either end of the labelled glass slides from preserved tissues kept at -20°C. The smears were air-dried and fixed in chilled acetone overnight.

Anti rabies fluorescence isothiocyanate (FITC) conjugate was mixed with NMB suspension and infected mouse brain (IMB) suspensions to make final conjugate dilution of 1:20 each. The mixtures were kept at 4°C for 30 min. The

conjugate-IMB and conjugate-NMB mixtures were added on the duplicate impression smears on every slide for each smear of secretion as well as that of tissue samples. The smears were covered with cover slips and incubated at 37°C for 30 min in a moist chamber. The slides were washed in 0.01 M phosphate buffered saline (PBS) pH 7.5 and in distilled water for 15 min each thereafter, air-dried and mounted in 90% buffered glycerol (pH 8.5). The slides were examined using an AH₃-RFC reflected light fluorescence microscope.

RESULTS AND DISCUSSION

Body secretions: Detection of rabies virus at different time intervals in different groups of experimental animals indicated the variance in the *in-vivo* progression of rabies virus in the body with variation in the route of inoculation as reported earlier (Singh and Grewal 1999, Singh 2008). Rabies virus in salivary secretion could be detected earliest on 15 DPI in group 1, 25 DPI in group 2 and 35 DPI in group 3 (Table 1). Isolation of rabies virus in saliva for the first time had been reported by Sikes (1962). Later, Baltazar *et al.* (1986) has also demonstrated rabies virus in the salivary secretion of sheep. Further, Fekadu (1988) in another experimental study in dogs has also reported the detection of rabies virus in salivary secretion. While there are reports of successful detection of rabies virus in salivary secretions, there are also reports of the experimental studies wherein rabies virus could not be detected in saliva of the experimentally inoculated animals. One such observation is of Hill and Beran (1992) wherein after inoculation of raccoons, the saliva samples did not reveal rabies virus even up to 92 DPI. While early detection of rabies virus in salivary secretion lends this approach the requisite sensitivity, however, the failure in detection, thereafter, precisely due to the intermittent secretion of the virus in body secretions renders this approach unreliable because of false negative results. Nevertheless, it still is worth an attempt where the clinical investigator needs to be cautioned to ignore the negative results—which could be false negative.

Presence of viral antigen in nasal discharge was first detected on 20 DPI in groups 1 and 3 and 30 DPI in group 2. SRV was earliest detected in rectal secretion of infected buffalo calves on 35 DPI in groups 1 and 2 and later on it was found on 50 DPI in group 2. While some reports are available about the detection of rabies virus in salivary secretion, however, only a few workers have attempted to detect rabies virus from the other body secretions for ante-mortem diagnosis of rabies. Steinhagen (1992) has reported that nasal and pharyngeal swabs examined by immunofluorescence gives promising results, but has recommended further research in this regard. The detection of rabies virus in the nasal discharge has likewise been found to be promising in the present study.

Whereas the approach of detection of rabies virus in the

body secretions might not be quite practical in canines especially those showing furious symptoms, however, this approach can be of practical significance in case of bovines and ovines vis-a-vis ante-mortem diagnosis of rabies.

Impression smears: Corneal impressions revealed the viral antigen on 25 DPI as earliest in group 3, 45 DPI in group 1 and 30 DPI in group 2 (Table 2). The virus in the prepucial smear was detected earliest on 35 DPI in group 1 and 3; however, it could not be detected in group 1 throughout the experimental study.

On comparison of sensitivity of detection of rabies virus in body secretions and impression smear preparations, it was found that detection of rabies virus in secretions is more sensitive approach than detection of rabies virus in impression smears. However, the rectal secretion among all body secretions examined and the prepucial impression smear among all the impression smears tested were least sensitive. Corneal, impression smear though more sensitive than prepucial impression smear was less sensitive than salivary and nasal secretions. Comparison of salivary and nasal secretion for detection of rabies virus revealed that salivary secretion was more sensitive for detection of rabies virus in groups 1 and 2 whereas nasal secretion was more sensitive for detection of rabies virus in group 3 infected buffalo calves (Table 3).

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