

Exploring the Feasibility of a New Low Cost Intra-Dermal Pre & Post Exposure Rabies Prophylaxis Protocol in Domestic Bovine in Jawali Veterinary Hospital, District Kangra, Himachal Pradesh, India

—Intra-Dermal Rabies Vaccination with Rabies Immunoglobulin (RIG) Infiltration into Bite Wounds: A Life Saving Protocol in Domestic Bovine

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Abstract

Cattle are the backbone of household economy in rural areas of India and many of them die after bites by potentially rabid dogs, despite being given currently recommended five shots of intramuscular (IM) rabies vaccination as Post Exposure Prophylaxis (PEP). In 2016, seven of 21 bovine bitten by rabid dogs given IM rabies vaccination died due to rabies in Shimla Municipality. This scenario prompted the authors to look for a suitable protocol, based on human studies, to save animals. We tested various schedules of IDRV in bovine and found that a schedule of 0.2 ml given in middle 1/3rd of neck on day 0, 3, 7, 14 and 28 along with local wound infiltration of eRIG is sufficiently immunogenic and life saving in all of them, even if bitten by lab confirmed rabid dogs/mongoose as tested by CRI. Rabivac Vet, a Cell Culture Rabies Vaccine, available as 1 ml per vial was used off level for IDRV. While injecting the vaccine, a raised papule of ≥ 1 cm will appear slowly causing a peau d'orange appearance. All 60 bovine serum samples tested by RFFIT after IDRV, had titers more than 0.5 IU/ml on day 14. Thereafter, a total of 150 animals were given five doses of IDRV as PEP, with or without RIG, after their exposure to clinically or lab confirmed rabid dogs/mongoose and all sur-

vived for more than a year. Serum samples from 15 animals bitten by lab confirmed rabid dogs/mongoose were collected on day 14 and tested for RVNA by RFFIT from NIMHANS Bangalore and all had desired antibody titers above 0.5 IU/ml, without any immunosuppression. The RFFIT titers in 55% bovine in all groups were more than adequate after one year and 100% of them had anamnestic response to a single 0.2 ml booster given at one year. Few of the bovine and even one equine (Horse. **Figure 4**) brought for PEP at some of nearby vet hospitals were given IM rabies vaccine with local eRIG infiltration also survived. Local eRIG infiltration appeared to have covered the lacuna of longer window period required for indigenous antibodies production through IM route in bovine that are not sufficiently produced by day 14. While five times less vaccine was used in this low cost protocol and the survival was 100% compared to traditional IM protocol where survival was 66%. Pre-exposure prophylaxis was found to be effective as 0.2 ml dose of IDRV on day 0, 3, 7 and all bovine had titers higher than the desired by day seven after single 0.2 ml vaccine booster at one year. Our study points towards a possibility of having short schedules of three shots IDRV vaccination in bovine with or without local RIG (depending on presence or absence of wound/s) as PEP and single shot IDRV as PrEP, but further studies are required on a large number of animals. Our study also points out for allowing intra-dermal use in animals as well and labeling vaccines for the same as this is low cost more immunogenic and less painful compare to IM administration.

Keywords

Rabies, Local Wound Infiltration of Rabies Immunoglobulin, Intra-Dermal Rabies Vaccination

1. Introduction

The exposure of domestic farm animals like cattle, buffaloes, horses, goats and sheep to bites of rabid dogs is of great socio-economic concern especially in rural areas where the cattle are the backbone of family economy and a dependable livelihood. In Shimla Municipality in 2016, seven of 21 jersey cows died due to clinical rabies despite having been given post-exposure prophylaxis (PEP) with recommended IM injections of rabies vaccine on day 0, 3, 7, 14 and 28. One of the adult cows died after 20th day of exposure and another after 35th day of exposure. This may have occurred due to a short incubation period consequent to bite wounds mostly near brain (on head and neck region) or non-administration of rabies immunoglobulin (RIG) into wounds or failure of IM vaccination to induce early protective response. IM rabies vaccine in animals has shown protective effect [1] but still PEP failures in cattle have been reported from all parts of the Globe [2]. Even vaccine antigen titers when raised to 4.0 IU or even higher resulted in failure [3] underlining the inappropriateness of rabies vaccine alone in animal post exposure prophylaxis.

Intra-dermal rabies vaccination (IDRV) trials have shown their efficacy in limited studies in cattle [4] [5]. IDRV rabies vaccination has proved to be more immunogenic than IM administration but IDRV protocols have not been formulated till date and no studies are there with IDRV Protocols tested on lab confirmed rabid dog bitten cattle; hence this study was undertaken. The efficacy of IDRV is well documented and practiced in human PEP in many countries including India and in our state of Himachal Pradesh [6]. Since the rabies immunoglobulins given based on body weight in animals have proven their efficacy in saving the life of animals like Sheep [7] (26 IU per Kg on the day of infection), they cannot be practiced in larger animals as the costs are overwhelming. Human rabies immunoglobulins (HRIG), as tried in sheep are @ \$100 per 2 ml vial. A recent study by Bharti and Madhusudana *et al.* has shown that local infiltration of rabies immunoglobulins (RIGs) in and around wounds [8] is effective in preventing rabies even in lab confirmed rabid dog bite human patients and prompted us to do this study in animals to save their lives.

2. Methods

24 of the 60 healthy bovine without any known exposure to rabid animals, were randomly allocated to various groups (**Table 1**) by draw of lots and were given

Table 1. Phase I: exploring the feasibility of a new low cost intra-dermal pre & post exposure rabies prophylaxis protocol, the results are expressed as RVNA titers done through RFFIT in IU/ml of serum on different days (JC MEANS COWS & JB MEANS BUFFALOES).

0.2 ml ID on 0 DAY GROUP (IDRV)-GROUP NO. 1

ANIMAL NO.	0 DAY	14 DAY	35 DAY	90 day	365 day	wt.	age	breed
JC 8	3.7	7.5	1.87	0.93	0.46	150 kg	2 year	jersey
JC 9	3.7	15	1.87	1.87	ANA	300 kg	8 year	jersey
JC 10	0.4	3.7	3.75	3.75	ANA	50 kg	1 year	jersey
JB 1	0.2	7.5	1.87	3.75	0.93	400 kg	5 year	murrah

GMT at Day 14 is 7.47 IU/ml; ANA means Animal not available.

0.2 ml ID on Day 0, 3 DAY GROUP-GROUP NO. 2

ANIMAL NO.	0 DAY	14 DAY	35 DAY	90 day	365 day	wt.	age	breed
JC 13	1.8	7.5	3.75	1.87	ANA	150 kg	3 year	Red sindhi
JC 14	0.4	7.5	7.5	3.75	ANA	200 kg	5 year	Red sindhi
JC 15	0.4	7.5	7.5	3.75	0.46	250 kg	8 year	Red sindhi
JB 2	0.4	7.5	0.4	1.87	0.93	100 kg	2 year	Non descript

GMT at Day 14 is 7.5 IU/ml.

0.2 ml ID on Day 0, 3, 7 DAY GROUP-GROUP NO. 3

ANIMAL NO.	0 DAY	14 DAY	35 DAY	90 day	365 day	wt.	age	breed
JC 1	0.2	7.5	1.87	1.87	0.93	250 kg	2 year	Holstein friesen
JC 2	0.2	7.5	3.75	1.87	0.93	300 kg	4 year	jersey
JC 3	0.2	0.9	3.75	3.75	0.93	400 kg	7 year	jersey
JB 3	0.2	15	7.5	1.87	ANA	500 kg	8 year	Non descript

GMT at Day 14 is 5.2 IU/ml.

0.2 ml ID on Day 0, 3, 7, 14 DAY GROUP-GROUP NO. 4

ANIMAL NO.	0 DAY	14 DAY	35 DAY	90 day	365 day	wt.	age	breed
JC 5	0.4	0.4	15	3.75	0.46	200 kg	2 y	Holstein friesland
JC 6	0.4	7.5	3.75	3.75	ANA	250 kg	7 y	Non descript
JC 7	3.7	3.7	3.75	3.75	0.46	200 kg	3 y	Non descript
JB 4	0.2	1.8	15	1.87	ANA	150 kg	2 y	murrah

GMT at Day 14 is 2.11 IU/ml.

0.2 ml ID on Day 0, 3, 7, 14, 28 DAY GROUP-GROUP NO. 5

ANIMAL NO.	0 DAY	14 DAY	35 DAY	90 day	365 day	wt.	age	breed
JC 11	3.7	3.7	30	3.75	0.93	300 kg	8 y	Red sindhi
JC 12	0.2	7.5	30	3.75	ANA	400 kg	15 y	Holstein friesland
JC 4	0.4	3.7	0.93	0.46	0.46	500 kg	8 y	Holstein friesland
JB 5	0.2	7.5	0.2	15	0.93	100 kg	2 y	Murrah cross

GMT at Day 14 is 5.26 IU/ml.

INTRA MUSCULAR GROUP GROUP NO. 6

ANIMAL NO.	0 DAY	14 DAY	35 TH DAY	90 day	Wt.	age	breed
BUFF A	0.11	0.46	7.5	-	500 kg	8 y	murrah
BUFF H	0.11	0.11	3.7	-	300 kg	3 y	murrah
BUFF M	<0.11	0.9375	7.5	-	100 kg	2 y	murrah
COW HF	<0.11	0.46	3.7	1.87	400 Kg	9 y	Holstein friesland

GMT at Day 14 is 0.38 IU/ml. N.B.: 5 doses of 1 ml rabies vaccine given IM is significantly less immunogenic than required on day 14 compare to 5 doses of IDRv given 0.2 ml. Test of Significance: GMTs ID Vs IM is 5.26 vs 0.38 ($p = 0.0136$).

Group 7: RFFIT RESULTS OF COWS in university Farm not given any rabies vaccination-SERUM SAMPLES

S.NO.	SAMPLE ID	RFFIT TITRE (IU/ML)
1	JC 01 (FARM)	0.23
2	JC 02 (FARM)	0.23
3	JC 03 (FARM)	0.11
4	JC 04 (FARM)	0.23
5	JC 05 (FARM)	0.46
6	JC 06 (FARM)	0.46
7	JC 07 (FARM)	0.11
8	JC 08 (FARM)	0.11
9	JC 09 (FARM)	0.46
10	JC 10 (FARM)	0.23
11	JC 11 (FARM)	0.23
12	JC 12 (FARM)	0.11

GMT: At day 0 are 0.22 IU/ml. NOTE: None of the farm kept cows had antibodies more than 0.5 IU/ml serum.

antirabies ID vaccination in varied schedules on day 0, 3, 7, 14 and 28 as 0.2 ml on Rt side of middle 1/3rd of neck. The volume and site were selected based on the study by Zdenek Beníšek [4]. To develop appropriate PEP protocol, serum samples were collected from all five groups on day 0, 14, 35, 90 and 365 and were tested for RVNA by RFFIT at National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore. The experiment was approved by the ethical committee and animal owner were asked to give written informed consent for injection and blood testing. We did not draw blood from pregnant bovine. Data on animal variables was also collected. Rabivac vet, a Cell Culture Vaccine available in 1 ml per vial in government hospitals and manufactured indigenously by Brilliant Bio Pharma private limited, IDA Pashamytaram, Medak-502 307, T.S., India having potency of 2.5 IU/ml Batch no 147/2015 Mfg Jan 2015 and Exp Dec 2017, was used. Equine rabies immunoglobulin (eRIG) procured from CRI Kasauli HP, India, having potency of 550 IU/ml was used.

2.1. Procedure/Technique of Intra-Dermal Vaccination

This involves injection of 0.2 ml of rabies vaccine ID on middle 1/3rd of neck of the bovine. Using aseptic technique, 1 ml insulin syringe 0.2 ml (up to 8 units in a 40 unit insulin syringe; *i.e.* 0.2 ml per ID site) of vaccine was injected into the dermal layer of middle 1/3rd of neck. A raised papule about 1 - 1.5 cm will appear immediately (**Figure 1**, **Figure 2**) causing a peau d'orange (orange peel) appearance.

2.2. Collection of Samples Post-Vaccination

Blood (Serum) samples for estimation of rabies Virus neutralizing antibodies (RVNA) by rapid fluorescent focus inhibition test (RFFIT) were collected on days 0, 14, 35 and 90 in vaccinated animals. 5 ml of venous blood was drawn with aseptic technique from the jugular vein of the animals. The blood samples were allowed to stand for about 30 minutes, centrifuged at 3000 rpm and the separated sera were collected and transported in cold chain to the Department of Neurovirology, NIMHANS, Bangalore.



Figure 1. Intra-dermal anti rabies vaccination done in a healthy cow in Jawali Clinic, HP, India.



Figure 2. Intradermal anti rabies vaccination done in healthy Buffalo in Jawali Clinic, HP, India.

2.3. Rapid Fluorescent Focus Inhibition Test (RFFIT)

RFFIT for estimation of rabies virus neutralizing antibodies (RVNA) was performed at the department of Neurovirology, NIMHANS using a WHO recommended procedure with minor modifications (Smith *et al.*, 1996 [9]). BHK 21 cell line (ATCC CCL 10) and BHK 21 adapted CVS 11 strain of virus was used and the tests were performed in 96 well tissue culture plates. The antibody titers were expressed in International units (IU/ml) in comparison to an in house reference serum calibrated against 2nd International reference serum obtained from National Institute of Biological Standards, UK.

2.4. Safety Assessment

The adverse events (both local & systemic) were recorded by observing the subjects for about 60 minutes after administration of vaccine and eRIG. Adverse events were evaluated using 4-point scale. 0—None; 1—Mild (an adverse event which has no effect on normal daily activities); 2—Moderate (an adverse event which interfere with normal daily activities); 3—Severe (an adverse event which prevent daily activities).

2.5. Inclusion and Exclusion Criteria

1) Inclusion criteria: Animals reporting within 72 hours of bite, aged 4 months to 15 years, owner willing to consent and animal available for one year to follow.

2) Exclusion criteria: Reluctant owner, animal has received rabies vaccine or RIG in past, Pregnancy or animal having helminth infection or malnutrition.

3. Animal Subject Protection

Ethical approval was obtained from the Institutional Ethical Committee for

CPCSEA of Dr. G.C.Negi College of Veterinary and Animal Sciences, CSK, HPKVV, university Palampur, HP in its meeting held on dated Feb 5, 2016 and subsequent meeting held on dated 9-11-2016 project Sr.no.5/2016 wide letter no-781/2016.

4. Three Phases of the Study Protocol

Phase I: We took 24 domestic bovine out of 60 recruited, in first phase for varied schedule of vaccination to arrive at a suitable pre exposure and post exposure protocols through intradermal route. The animals were randomized into five groups, as follow:

- Group I: Rabivac vaccine ID was given intradermal 0.2 ml in middle 1/3rd of neck on day 0. Group II was given Rabivac on day 0, 3, Group III on day 0, 3, 7; Group IV: on day 0, 3, 7, 14 and Group V on day 0, 3, 7, 14, 28. Details are at **Table 1**.
- RFFIT titers were measured at day 0, 14, 35, 90 and 365 to develop appropriate pre and post exposure prophylaxis (PEP) protocol.

Phase II: 150 bovine, bitten by lab confirmed rabid dogs or mongoose or clinically conformed rabid dogs, were given PEP based on PEP protocol developed in Phase I after RFFIT evaluation and animals followed for more than one year to observe the survival rate. Details are at **Table 2**.

Phase III: To develop a **pre exposure protocol (PrEP) in Bovines** a booster of single 0.2 ml ID to a sub-group of available bovine was given on days 365 and RFFIT titers tested seen again tested on day 7. Details are at **Table 3**.

5. Results and Discussion

Phase I: Out of 60 animals recruited for study protocol, 25 animals, 41.7% (17 cows and 8 buffalos), were having natural antibody titers without vaccination above 0.5 IU/ml in the range of 0.9 IU/ml - 15 IU/ml. Some of these animals were subsequently observed as to how they respond to regular course of antirabies vaccination compare to those having titres below 0.5 IU/ml on day zero.

While we observed that the response in all 5 groups to ID vaccine was robust on day 14 (Group 1 - 5) given 0.2 ml vaccine ID on days 0, 3, 7, 14 and 28. Only one animal could reach the desired titers (>0.5 IU/ml) on day 14 in IM group which is considered adequate. As per WHO guidelines, a minimum level of 0.5 IU/mL is used as a correlate of protection. In healthy vaccinees, this level should be achieved by day 14 of a post-exposure immunization regimen, with or without simultaneous administration of RIG and irrespective of age [10]. Test of Significance: GMTs ID Vs IM is: 5.26 vs 0.38 ($p = 0.0136$), IM vaccine had significantly low titers than IDRIV on day 14 which can be life threatening in cases of wounds on head and neck and may have contributed to observed vaccine failures IM.

To understand this strange phenomenon of existence of natural antibodies without vaccination mentioned above, we took serum samples from 12 cows

Table 2. Phsse II: results of implementation of low cost five dose Intradermal vaccination with local RIG infiltration of wounds in Bovine in real field conditions in villages of Jawali, Himachal Pradesh, India.

S.no.	Age in yrs	Sex	Spp.	Biting animal	Site of bite	Date of bite & date of post bite vaccination injection	Route of vaccine doses 5 on days 0, 3, 7, 14 & 28	Wt. In kg	Volume of eRIG infiltrated in & around the wound	Time interval b/w bite & eRIG (in days)	Status of the bitten animal till Feb 2017	RFFIT at D 14	Status of biting dog
1.	4 years	F	Cattle	Brown dog	Head & muzzle, about 1 inch in length	17-5-16	ID	300 kg	4 ml	Within 24 hours	Healthy	3.75	Lab confirmed rabid dog A
2.	8 months	F	Cattle	Brown dog	Lower mandible, three marks of biting teeth	17-5-16	ID	50 kg	2 ml	Within 24 hours	Healthy	15	Lab confirmed rabid dog A
3.	1 year	F	Cattle	Brown dog	Ear, lower right eye, about 2 inch wound	17-5-16	ID	50 kg	2 ml	Within 24 hours	Healthy	15	Lab confirmed rabid dog A
4.	2 years	F	Cattle	Brown dog	Neck, half inch wound	17-5-16	ID	250 kg	1 ml	Within 24 hours	Healthy	30	Lab confirmed rabid dog A
5.	9 months	M	Buffalo	Brown dog	Head, near eye, each wound half inch	17-5-16	ID	50 kg	1 ml	Within 24 hours	Healthy	7.5	Lab confirmed rabid dog A
6.	1 year	F	Buffalo	Brown dog	Head, wound one inch in length	17-5-16	ID	50 kg	1 ml	Within 24 hours	Healthy	7.5	Lab confirmed rabid dog A
7.	5 years	F	Cattle	Brown dog	Left upper eye lid, about 1 inch	17-5-16	ID	300 kg	2 ml	Within 24 hours	Healthy	----	Lab confirmed rabid dog A
8.	6 years	F	Cattle	Brown dog	Lateral upper muzzle	17-5-16	ID	400 kg	2 ml	Within 24 hours	Healthy	30	Lab confirmed rabid dog
9.	8 years	F	Cattle	Brown dog	Left lateral lip, about 1 inch wound	17-5-16	ID	400 kg	1 ml	Within 24 hours	Healthy	15	Lab confirmed rabid dog A
10.	6 years	F	Buffalo	Confirmed dog bite	Muzzle and neck	14-6-16	ID	600 kg	3 ml	Within 24 hrs	Healthy	3.75	Lab confirmed rabid dog B
11.	7 months	M	male Buffalo calf	Rabid dog	Muzzle	14-6-16	ID	100 kg	Not given as re exposed after ID PEP finished 7 days ago	This calf was given single dose of 0.2 ml booster Within 24 hours	Healthy	30	Lab confirmed rabid dog B
12.	6 years	F	Jersey cow	Rabid dog	Head & ear	14-6-16	ID	400 kg	1 ml	Within 24 hours	Healthy	15	Lab confirmed rabid dog B
13.	5 years	F	Jersey	Rabid dog	Both sides of jaws	14-6-16	ID	300 kg	1 ml	Within 24 hrs	Healthy	30	Lab confirmed rabid dog B
14.	6 years	F	Jersey	Rabid dog	Muzzle	14-6-16	ID	400 kg	1 ml	Within 24 hrs	Healthy	30	Lab confirmed rabid dog B
15.	7 years	F	Buffalo	Rabid dog	Neck & ear	14-6-16	ID	700 kg	2 ml	Within 24 hrs.	Healthy	15	Lab confirmed rabid dog B
16.	2 years	M	Buffalo	Rabid dog	Both ears	14-6-16	ID	200 kg	3 ml	Within 24 hrs	Healthy	15	Lab confirmed rabid dog B
17.	3 year	F	Non descript buffalo	Rabid mongoose	Muzzle wound	6-12-2016	ID	400 kg	1 ml	Within 48 hours	Healthy	-	Lab confirmed rabid mongoose A
18.	7 day old	F	Jersey calf	Rabid mongoose	Muzzle wound	02-01-2017	ID	25 kg	0.5 ml	Within 12 hours	Healthy	-	Lab confirmed rabid mongoose B

Table 3. Exploring the feasibility of low cost Intradermal PrEP in bovine.

0 Day Group I					
Animal Number	0 Day	14 Day	35 Day	365 day	7 Days after single booster dose on 365 Day
JC8	3.7	7.5	1.87	0.46	0.93
JC9	3.7	15	3.75	ANA	Animal not available
JC10	0.4	3.7	3.7	ANA	Animal not available
JB1	0.2	7.5	1.87	0.93	1.87
JB7	<0.1			0.93	1.87

GMT at Day 365 is 0.74 IU/ml and GMT at Day 372 is 1.48 IU/ml.

0, 3 DAY GROUP II					
Animal Number	0 Day	14 Day	35 Day	365 day	7 Days after single booster dose on 365 Day
JC13	1.8	7.5	3.25	ANA	Animal not available
JC14	0.4	7.5	7.5	ANA	Animal not available
JC15	0.4	7.5	7.5	0.46	1.87
JB2	0.4	7.5	0.4	0.93	1.87

GMT at Day 365 is 0.65 IU/ml and GMT at Day 372 is 1.87 IU/ml.

0, 3 & 7 DAY GROUP III					
Animal Number	0 Day	14 Day	35 Day	365 day	7 Days after single booster dose on 365 Day
JC1	0.5	7.5	1.87	0.93	0.93
JC2	0.5	7.5	3.75	0.93	3.75
JC3	0.5	0.9	3.75	0.93	1.87
JB3	0.2	15	7.5	1.87	ANA

GMT at Day 365 is 0.93 IU/ml and GMT at Day 372 is 1.87 IU/ml.

kept in the isolation farm since birth at Agriculture University, Palampur, and all these cows (**Table 1** Group 7) were having RFFIT titers <0.5 IU/ml. This clearly shows that cows and buffalos when left stray for grazing in the open pastures interact with the rabies virus carrier stray dogs [11] [12] and get infected and have rabies antibodies in their blood. Such high antibody titers without any history of exposure were also observed in unvaccinated rag pickers engaged to lift dead bodies of dogs and monkeys in Shimla Municipality [13].

Therefore we are of the view that validation of preventive vaccination schedule in field animals that are basically domesticated but left to graze as stray in rabies endemic countries like India, compare to captive farm animals in Europe/India is entirely different issue due to presence of natural rabies antibodies in domestic animals without any vaccination. These antibodies may interfere with the killed/attenuated antigens used in rabies vaccination and give variable results on RFFIT as is seen in **Table 1**.

We decided to adopt group V protocol for ID rabies vaccination in bovine for

PEP (Phase II) with local infiltration of eRIG to cover the non response of one animal in Group IV as also Group IV had lower GMTs on day 14 than Group V.

Phase II: After assured protection correlates with five dose IDRV protocol in Phase I trials, we decided to test the protocol in real field conditions. In the second phase we started giving PEP with 5 day 0.2 ml IDRV and local wound infiltration with eRIG in animals bitten by suspected or lab confirmed rabid dogs. 150 bovine bitten by suspected or laboratory confirmed rabid dogs were included in Phase-2 of the study. Out of 150 bovine 18 were bitten by lab confirmed rabid dogs/ mongooses and followed for one year. Out of 18, serum samples were taken from 15 bovine on day 14 and tested for RVNA by RFFIT and all had desired titers above 0.5 IU/ml. (**Table 2**). After appropriate wound wash and application of local antiseptics, eRIG was infiltrated locally (**Figure 3**) into all category-III wounds inflicted by suspected or lab confirmed rabid dogs/mongoose. Those animals that were not having any visible wound were not given any eRIG but only vaccine as IDRV and all survived. Brain samples of two rabid dogs and two mongoose were sent for lab confirmation by Fluorescent Antibody Test (FAT) to Central Research Institute (CRI), Kasauli. None of the animal had severe drug reaction and all had only mild redness and some of them had itching sensation that faded away by next day.

Few of the bovine and even one equine (Horse. **Figure 4**) brought for PEP at some of nearby vet hospitals, who were given five shots rabies vaccine IM with local eRIG infiltration also survived. Local eRIG infiltration appeared to have covered the lacuna of longer window period required for sufficient indigenous antibodies production through IM route that are not sufficiently produced by day 14 as demonstrated by our study.

Pre-vaccination of bovine, Phase-III: Pre-exposure prophylaxis was found to be effective as 0.2 ml dose of vaccine on day 0, 3, 7 and all bovine had titers higher than desired by day seven after single 0.2 ml vaccine booster at one year.



Figure 3. Local eRIG infiltration in a Buffalo bitten by rabid dog on nose with insulin syringe.



Figure 4. Local eRIG infiltration in a horse bitten by dog having paralytic rabies (**Figure 5**) on lower lip with insulin syringe.



Figure 5. Dog clinically diagnosed as having paralytic rabies after the attack on the horse lip (observe the jaw drop).

We had serum samples tested for seven calves born to cows during the study period while cows were vaccinated before pregnancy and three of them had more than desired antibody levels at 3 - 4 months of age. It is recommended that we vaccinate calves when they are older than 4 months and then yearly [14] [15].

6. Conclusion

Considering the enormous burden of rabies, we were able to demonstrate clinical effectiveness of a novel low cost protocol based on our previous experience with human rabies vaccination and local infiltration of eRIG and we called this protocol to memorise our guru as “Madhusudana-Bharti-Uppinder” protocol. Failure to achieve desired titers above 0.5 IU/ml at day 14 with IM schedule of rabies vaccination in bovine was identified as one of the reasons of observed PEP failure in bovine vaccinated by IM route in our area. Our study points towards a possibility of having short schedules of three shots ID vaccination with or without local RIG infiltration of bite wound/s as PEP and single shot ID as PrEP but that would require further studies on a large number of animals.

7. Recommendations

We recommend vets to follow this low cost protocol of IDRV with local eRIG

wound infiltration where required for post exposure prophylaxis of bovine or may be in equine bitten by rabid animals to save their lives. We also recommend vet vaccine companies to label rabies vaccines as for “ID/IM” use like human vaccines to facilitate this process for wider pre exposure use that would make them cost effective for poor farmers in rabies endemic countries of Asia and Africa. Another factor that goes in favour of IDRV is less or almost no pain to fine needle insulin syringe compare to stressful response to IM vaccination in cattle as demonstrated by Luis S *et al.* [15].

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Abbreviations

RVNA: Rabies Virus Neutralizing Antibody, **RFFIT**: Rabies Fluorescent Focus Inhibition Test, **eRIG**: Equine Rabies immunoglobulin, **FAT**: Fluorescent Antibody Test, **CRI**: Central Research Institute, Kasauli (HP).