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DETECTION OF THE ANTIBODY TITER OF GOATPOX VACCINE DEVELOPED BY BLRI IN THE KHULNA REGION, BANGLADESH

M.M.I. CHOWDHURY[1*], N. ARA [2], N. SULTANA[3], M.N.K. AZAM[4] AND K.M. HOSSAIN[5]

[1]Assistant Director, Prime Minister office, The people Republic of Bangladesh; [2]Assistant Professor, Department of Pharmacy, University of Development Alternative, Dhaka, Bangladesh, [3]Lecturer, Department of Microbiology, Faculty of Biological Science and Technology, Jessore science and Technology University, Jessore - 7408, Bangladesh; [4]Biotechnology and Genetic Engineering Department, University of Development Alternative, Dhanmondi, Dhaka, Bangladesh; [5]Professor, Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna – 9208, Bangladesh.

[*]Corresponding author & address: Md. Motiul Islam Chowdhury, E-mail: motiul330@yahoo.com
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ABSTRACT

Chowdhury MMI, ARA N, Sultana N, Azam MNK, Hossain KM (2012) Detection of the antibody titer of goatpox vaccine developed by BLRI in the Khulna region, Bangladesh. *J. Innov. Dev. Strategy.* 6(1), 73-80.

Goatpox is a highly contagious viral disease of goats. The disease is caused mainly by Goatpox virus (GPV) and occasionally by Sheep pox virus (SPV) which is enveloped by double stranded DNA viruses, classified in the genus Capripoxvirus of the family Poxvirade. The objective of the research work was to conduct an efficacy trail of Goatpox vaccine BLRI-4505 on selected goats of Khulna region developed by Bangladesh Livestock Research Institute (BLRI). BLRI developed GPV vaccine was injected into 30 goats of selected farmers in a village of Khulna, to observe the physiological condition and the effects of the vaccine. Rectal temperatures of the experimental animals were recorded for before vaccination and seven day after vaccination. Blood was collected on before vaccination (0-Day), 15th and 30th day of post-vaccination period. Separated serum was used for C-ELISA (Enzyme Immune Slide Assay) to determine the antibody titre of vaccinated goats. Hyper-immune serum (several time vaccinated goats) was collected from BLRI experimental goats. Hyper-immune serum was used as a standard. Temperature fluctuations of the vaccinated goats were normal and no uneven effects were found. OD value of antibody titre was satisfactory level and increased in day 30 than day- 0 and day- 15. This experiment reveals that newly developed GPV vaccine is effective against Goatpox virus in Bangladesh and few more trials in the intensive and semi-intensive goat populated areas would be better about the efficacy result of the vaccine.

Key words: *BLRI, capripoxvirus, goat, goat pox, immunogenicity*

INTRODUCTION

The goat (*Capra hircus*) is one of the domesticated small ruminants, recognized as poor men's friend in Bangladesh, because 64% of the 15 million rural household rear goats and 70% of the goats are in the hands of small holders mostly poor and destitute women who rear goats for milk and hard cash and even the children take care of goats as a docile animal. It plays a vital role in promoting both the health and wealth of the nation. It provides the principal dietary animal protein in the form of meat and milk to improve the national health. On the other hand, substantial amount of foreign exchange is earned from exported skin and by-products. Goat is the second most important livestock of farmers in Bangladesh (Islam 2002). In Bangladesh, goats are valued on account of their short generation intervals, higher rate of prolificacy, and being a useful component of poverty elevation for the rural people. Goat provides income-generating activities for around 9.6 million rural households. Annual income generated per family from goat is estimated at taka 1200. There are 195.28 million of livestock in Bangladesh of which small ruminants estimated 33.27 million of which 32.18 million goats and 1.09 million sheep in the year 1994-95, in case of goat population which was 27.49 million in the year 1992-93, and the annual growth rate is 8.2%, against the value 0.80% in cattle (Amin 1994).

The mortality of goats is one of the major problems confronting goat rearing in Bangladesh. Like other livestock, goat is also susceptible to various diseases or disease condition causing debility and mortality, which lead to huge economic loss. The major problem of goat rearing is low kid survivality (70%) due to malnutrition and diseases. In Bangladesh, the common disease which affect the young or adult goats causing death are parasitism, infectious diseases like PPR, Goatpox, Enterotoxaemia, Pneumonia etc. Diseases are the major constraints of goat production in Bangladesh. Death in all groups is 20% and loss is estimated at Tk.560 crores. This high rate of mortality happened due to non- availability of appropriate disease controlling system in Bangladesh. Preventive measures against fatal disorders in animals are of prime importance (Islam 2002). Goat pox is highly infectious viral disease of goats caused by the Goatpox virus of the genus Capripox virus under family Poxviridae. This disease can cause extensive epidemics in which a large number of goats may die. Goatpox has been described in detail in ancient Veterinary Medicine and has been widely spread since early times. The disease was prevalent throughout the Near and Middle east, India, Pakistan, Bangladesh and North Central Africa. Goatpox was first reported in this Subcontinent in 1936 from the Southern part of India (Kuppuswamy 1936) which gradually spread over the other parts of this continent. Sporadic outbreaks still being reported from European countries (Carn 1993). In Bangladesh, first outbreak was reported in 1984 from the Western districts (Jessore, Magura, Cuadanga, Meherpur and Kustia) that cause severe economic losses in the goat farming in those areas (Kitching *et al.* 1985). Since then, the disease remained endemic in Bangladesh and most recent outbreaks were recorded in the Bangladesh Mission Goat Farm and its surrounding villages (Sil *et al.* 1995).

The disease is acutely progressing febrile infection characterized by vesciculo-papular exanthemas of the skin and mucous membranes. Goatpox viruses spread mainly through aerosol, but direct contact and insects also play a minor role in spreading this disease. The morbidity and mortality rate are 60-100% respectively, in which kid mortality is higher (100%) than adults (50%) (OIE Manual 2000).Besides mortality, the disease causes considerable economic losses, reduced growth rate of recovered animals, deterioration in the quality of the hide due to skin damage and finally trades restriction from the endemic areas. The incubation period of Goatpox in goat is 2-14 days. Two forms of Goatpox disease found in goat. Mild form commonly occurs in adults and malignant form occurs in kids (Bhowmik *et al.* 1986). The mortality rate in this form may reach 50%. In the benign form, the common one in adults, only skin lesions occur, particularly under the tail, and there is no systemic reaction.

In enzootic areas, both live attenuated and inactivated vaccines are useful in the prevention and control of goat pox, but inactivated vaccines give only short-term immunity (Prasad and Datt, 1973; Yadav *et al.* 1986; Pal and Soman, 1992). Live attenuated vaccines are highly immunogenic but have a limitation of setting up a 'pock' reaction and / or mortality in some of the vaccinated animals due to proliferation of disease. Usually, homologous vaccinations incorporating locally prevalent strains of GPV are quite successful in protecting goats against goat pox. Therefore, in different countries and sometimes within a country, various live attenuated vaccines have existed from time to time for goat pox with varying degrees of protective efficacy (Ramyar *et al.* 1974; Dubey and Sawhney, 1978; El-Zein *et al.* 1983; Davies and Mbugwa, 1985; Guo *et al.* 1986; Wang and Jiang, 1988a; Mahmood *et al.* 1989). Goat pox is one of the major goat health hazards in all major goat rearing areas in Bangladesh. The socio-economic condition permits only preventive inoculation to control this malady and this makes it imperative to have a good immunizing agent and could as well provide some parameter for monitoring the vaccination programmes. A member of vaccine candidates has been developed and their efficacy and duration of immunity is not defined. Since the first out break of goat pox disease in Bangladesh imported vaccines were used, the more recent imported vaccine candidate (Pendik strain of SPV used as a vaccine in Turkey) was found not effective against our native virulent strains of goat pox virus, which has prompted the new vaccine to develop an attenuated vaccine using our local goat pox virus (GPV) as a parent stock (Sil *et al.* 1995).

This study has described the efficacy trail of goat pox vaccine BLRI-4505 that has developed by an attenuated virus strains from local isolates and investigated their potency and immunogenecity as a potential vaccine candidate against goat pox disease in our country. The objectives of the study were:

1. To detect the antibody titer of Goatpox vaccine developed by BLRI in the Khulna region.
2. To study the physiological changes of goats after vaccination of newly developed vaccine.
3. Compare antibody titer of post vaccination among the experimental animals.

MATERIALS AND METHODS

The study was conducted in Virology Laboratory, Bangladesh livestock Research institute (BLRI), Savar, Dhaka, during the period of April to June 2006. The detailed outline of materials and methods are given below.

Design of the Experiment

The whole experiment consists of two parts; the first one is vaccination of the goats and observes their physiological condition to determine the side effect of the vaccine, second part was assessment of GPV antibody in goats. The GPV vaccine BLRI-4505 was supplied from Livestock Research Institute (BLRI) Savar, Dhaka. The vaccine was given through subcutaneous route. In the first experiment, trial is given in 30 goats. The serum samples were collected 15^{th} and 30^{th} day of post vaccination. The degree of antibody level of hyperimmune serum and post-vaccination sera were determined by Competitive-Enzyme Linked Immunosorbant Assay (C-ELISA). For determining side effect of the vaccine, the experiments were conducted in farmer's level at Austtagram, Kapalipara, and Daulatpur in Khulna.

Materials for Efficacy Trial of Goat Pox Vaccine

Experimental animals:

For the determination of the antibody of GPV vaccine, the experiment was conducted on goats at farmers' level at Austtagram, Kapalipara, Daulatpur in Khulna and the total no. of goat for trial was 30.

Vaccination: The vaccine was given sub-cutaneously in the cervical lymph node region. Each of the experimental animals was vaccinated at same day.

Blood collection

In this experiment, the sera were collected at 15th and 30th day of post-vaccination. The sera of post-vaccination were tested by C-ELISA for the confirmation whether the animal was vaccinated or not. Blood was collected from Jugular vein puncture using syringe. The blood was left to clot overnight in clot boxes and serum was decanted into sterile tubes and kept in icebox for transportation to the

laboratory. In the laboratory, the serum was centrifuged (at 2000 rpm for 10 minutes) to remove the remaining cells before being transferred to 2-ml eppendrop tube and stored at -20° C until use.

C-ELISA (Competitive-Enzyme linked immunosorbant Assay)

For the detection of GPV antibody all materials, chemicals, etc. were supplied by BLRI, Savar, Dhaka which are described below.

- ELISA Reader: Computerized immuno-scan microplate reader with an interference filter of 450 nm was used for the reading of test samples.
- Pipettes: Multi Channel pipette variable Range from 5-50µl and 50-250µl quality tips, reagent troughs and single channel pipette, variable range from 5-50 µl and 200-1000 µl quality tips were used.
- Glassware, plastic ware: Beaker (20-4000m1), flasks (50-1000ml), graduated cylinders (10-2000m1), graduated pipettes (1-20m1.) with suitable bulbs, storage bottles with closures (1-100m1.), dilution tubes (2-4ml.), racks were used.
- Timer: Timer was used for preferably count down type with an audible alarm.
- Marker pens (water-proof) and adhesive labels: Marker pens and adhesive were used for labeling the samples.

Reagent and Sample Preparation

For C-ELISA following reagent and sample were used:

Goat pox virus antigen stock: One ml Goat pox antigen was supplied with the kit in glass vial.

Anti-species conjugate stock: One ml freeze dried horse radish peroxides (HRPO) conjugate, rabbit anti-mouse immuno-globulin were used. HRPO conjugate stock was further sub-divided into 500µl aliquots in 1 ml cryopreservation vials and used at l: 1000 dilution for the test.

Control serum stocks: Freeze dried strong positive hyper immunized serums were supplied in glass vials at 1 ml volumes. The antibody stock was stored -20°C for further use in cryo-vials. At the time of the test cryo- vial containing antibody thawed at 37°C in water bath and used at 1:1000 for the test. At the time of the test cryo-vial containing antibody was thawed at 37 °C in water bath and used at 1:1000 dilutions for the test.

Chromogen stock: Constituted 3.7 mg ortho-phenyldiamine (OPD) in tablet form and was used as chromogen. One OPD tablets was dissolved in 75ml of locally produced deionized water just before substrate or chromogen incubation step and aliquots in 6 ml prepared and stored at -20°C for further use.

Substrate stock: We prepared 3% (w/v) / 882mM hydrogen peroxide for the test and hydrogen peroxide was supplied in tablet form and then one hydrogen peroxide tablet was placed in the empty container supplied and dissolved with 10 ml locally produced distilled water which constituted at 3% solution. The prepared substrate was stored at 40°C for further use.

Coating buffer: We prepared 0.01M phosphate buffered saline (PBS), pH 7.4 ±0.20 and then one tablet was dissolved in one liter of locally produced distilled water and pH was checked. Then prepared coating buffer was labeled and stored at 4°C for the period of two weeks as stock solution.

Blocking buffer: We prepared 0.01M phosphate buffer saline, pH 7.4±0.20 plus 0.1% (V/V) Tween 20 plus 0.3%(v/v) normal bovine serum (C). Blocking buffer was prepared in 100ml volume of PBS containing 100µl, 200 µl and 300µl normal bovine serum (C). Prepared blocking buffer was labeled and stored at 4°C for two weeks.

Washing buffer: We prepared 0.002 M phosphate buffered saline (pH 7.4±0.20) and then 1 liter of locally produced deionized water and used as 1:4 dilution in distilled water.

Stopping solution: 1 M sulfuric acid was prepared in 1 liter volume containing 55ml of concentrated sulfuric acid in 945ml of locally produced distilled water.

Procedure of C-ELISA Test:

The test procedures consisting of several steps of these procedures are described below.

Coating of GPV antigen: Standard GPV antigen (5µl) was taken on a glass plate with 12 holes, air dried and fixed with acetone for 30 minutes.

Addition of blocking buffer: By fixing the glass plate, 20µl of blocking buffer was added in each hole.

Addition of conjugate: A working dilution of the conjugate prepared immediately before the end of' the test serum incubation in a volume sufficient for all the holes. Both the conjugate stock and its working dilution were handled with care. After one hour of serum incubation, glass plates were removed from the incubator and were washed 3 times by washing buffer and air dried. Immediately after washing 5µl of the working solution of conjugate was added to all holes of the glass plates, then glass plate was covered with aluminum foil and incubated for 1 hour at 37^{o}C. After incubation, all holes were washed 3 times with washing buffer and air dried.

Addition of substrate /Chromogen and stopping solution: Immediately before the end of the incubation a working dilution of the substrate/ Chromogen solution was prepared in a volume sufficient

for all holes of glass plates. 50µl of the substrate solution was added in all holes of the plate and incubated at 37°c for one hour. Then 50µl stopping solution was added in each hole.

Measurement of substrate development: The microplate reader should be turned on and allowed to warm up for at least 15 minutes before reading the first microplate. This warm up period is necessary to ensure uniformity of reading for all microplates. Optical density (OD) was read at 450nm. The plate was placed in the carriage of blanked reader and initiated reader sequences. It was repeated for each microplate.

Result: The result was taken from microplate reader which was OD (Optical density) value. This OD was come out as a printed sheet by the ELISA reader.

RESULTS AND DISCUSSION

The experiment comprises two parts; the first part is to observe the temperature fluctuation of vaccinated goats. The second one is the comparison of optical density (OD) of antibody titre among hyperimmune serum and 15th and 30th days collected serum.

To observe temperature fluctuation of vaccinated goats, the trail was conducted at Austtagram Kapalipara, Daulotpur in Khulna. BLRI developed goat pox vaccine BLRI-4505 was injected 30 goats in farmer levels of the above village. The rectal temperature of selected goats recorded before vaccination and after vaccination of first seven days. The recorded temperature is shown in table -1. For assessment of antibody titre of vaccinated animals, the experiments were conducted at BLRI, Savar, Dhaka. Sample collected from Khulna carefully brought into BLRI and preserve in -20°c freezer. Hyperimmunized serum collected from several time vaccinated goats, this also preserve in -20°c freezer. Antibody titre of sample is deteced by C-ELISA. ELISA reader presents this antibody titre as a OD value. The OD value of hyperimmunized serum and post vaccinated (15 and 30 days) serum is shown in table -2.

Table 1. Comparative study temperature fluctuation of post vaccinated goats

Serial No.	Tag No.	Temperatures ^{0}F								Other side effects
		Before vaccin-ation	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6	Day-7	
1	1	99.5	102.6	102.8	102.3	102.5	102.7	102.5	102.0	No
2	2	99.2	101.7	101.8	101.5	101.7	101.7	101.7	101.5	No
3	3	99.0	101.5	101.6	101.0	101.4	101.5	101.5	101.5	No
4	3b	99.8	103.6	103.6	102.0	102.4	102.4	102.0	101.9	No
5	4	99.6	103.0	103.0	102.8	102.9	102.9	102.8	102.0	No
6	5	99.3	101.0	101.2	101.0	101.2	101.3	101.3	101.3	No
7	5a	100.0	102.0	102.0	101.5	101.3	101.5	101.4	101.3	No
8	6	99.6	102.4	102.3	102.5	102.3	102.3	102.0	101.8	No
9	7	99.5	102.3	102.4	102.0	102.2	102.0	102.2	101.9	No
10	8b	99.8	101.8	101.9	101.8	101.8	101.8	101.7	101.6	No
11	9a	99.3	101.2	101.4	101.3	101.4	101.6	101.6	101.5	No
12	9b	99.7	102.0	102.0	101.8	102.0	102.0	101.9	101.8	No
13	10	99.6	102.4	102.5	102.1	102.2	102.3	102.2	102.0	No
14	11	99.4	102.6	102.6	102.3	102.3	102.3	102.0	101.9	No
15	12	99.3	102.0	102.2	102.0	102.2	102.4	102.4	102.2	No
16	12a	99.7	102.0	102.1	101.7	101.9	102.0	101.9	101.7	No
17	13	99.9	102.3	102.4	102.0	102.2	102.2	102.0	101.9	No
18	14	100.0	102.5	102.3	102.3	102.3	102.2	102.2	102.0	No
19	15a	99.8	102.6	102.5	102.5	102.4	102.5	102.3	102.1	No
20	15b	102.6	104.7	102.6	106.0	106.5	107.0	105.6	106	No
21	16	102.8	103.8	105.7	105.0	106.0	106.0	106.4	105.5	No
22	17	99.8	102.7	104.7	102.5	102.5	102.5	102.2	102	No
23	18	99.7	101.3	101.4	101.3	101.4	101.5	101.5	101.3	No
24	25a	99.9	101.3	101.4	101.2	101.4	101.4	101.4	101.3	No
25	25b	100.0	102.6	102.6	101.5	101.6	101.6	101.5	101.2	No
26	25c	99.9	102.5	102.6	101.8	101.8	101.8	101.8	101.5	No
27	26a	99.8	102.7	102.7	102.3	102.5	102.4	102.0	102.0	No
28	26b	99.8	102.8	102.7	102.3	102.5	102.5	102.2	101.9	No
29	27a	100.0	102.9	102.8	102.5	102.8	102.8	102.5	102.0	No
30	27b	100.0	103.0	102.9	102.8	102.8	102.7	102.5	102.5	No

Table 2. Comparative study of OD value of antibody titre against GPV virus in post vaccinated goats

Serial No.	Sample No.	HS	15th day Serum	30th day Serum
1.	1	0.450	0.190	0.250
2.	2	0.468	0.264	0.400
3.	3	0.455	0.222	0.354
4.	3b	0.479	0.213	0.323
5.	4	0.472	0.250	0.370
6.	5	0.468	0.205	0.312
7.	5a	0.448	0.208	0.309
8.	6	0.462	0.242	0.359
9.	7	0.470	0.252	0.382
10.	8b	0.459	0.267	0.398
11.	9a	0.462	0.279	0.402
12.	9b	0.470	0.282	0.432
13.	10	0.459	0.275	0.413
14.	11	0.462	0.265	0.398
15.	12	0.469	0.292	0.419
16.	12a	0.444	0.270	0.398
17.	13	0.473	0.263	0.405
18.	14	0.455	0.251	0.373
19.	15a	0.449	0.266	0.392
20.	15b	0.466	0.276	0.420
21.	16	0.478	0.259	0.385
22.	17	0.472	*	*
23.	18	0.470	**	**
24.	25a	0.473	0.225	0.344
25.	25b	0.469	0.257	0.387
26.	25c	0.477	0.246	0.355
27.	26a	0.467	0.279	0.412
28.	26b	0.482	0.267	0.390
29.	27a	0.453	0.239	0.357
30.	27b	0.482	0.253	0.389

- Died due to pox ** Infected due to pox

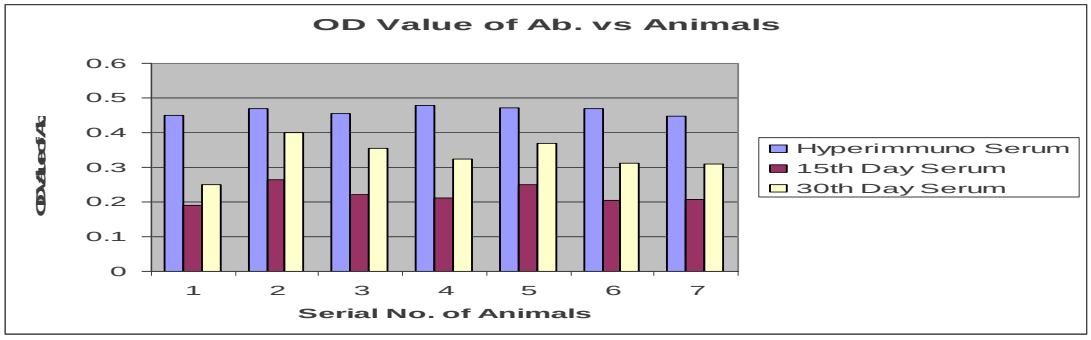

Fig 1. Showing OD value of antibody titre of hyperimmune serum, day-15 and day-30 serum (1-7 goat)

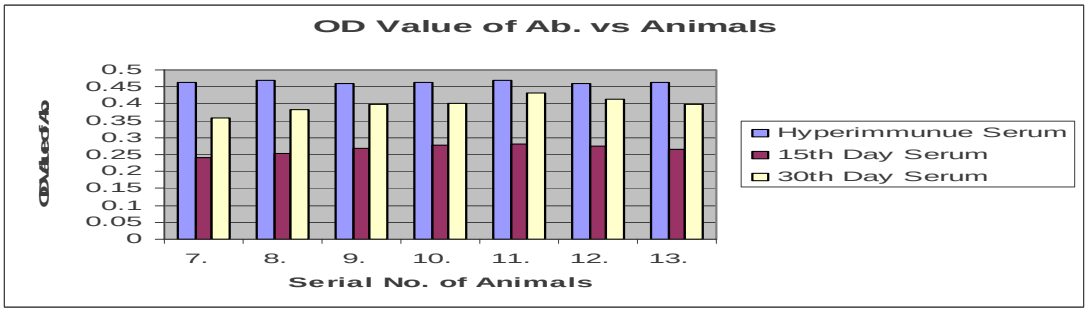

Fig 2. Showing OD value of antibody titre of hyperimmune serum, day-15 and day-30 serum (8-14 goat)

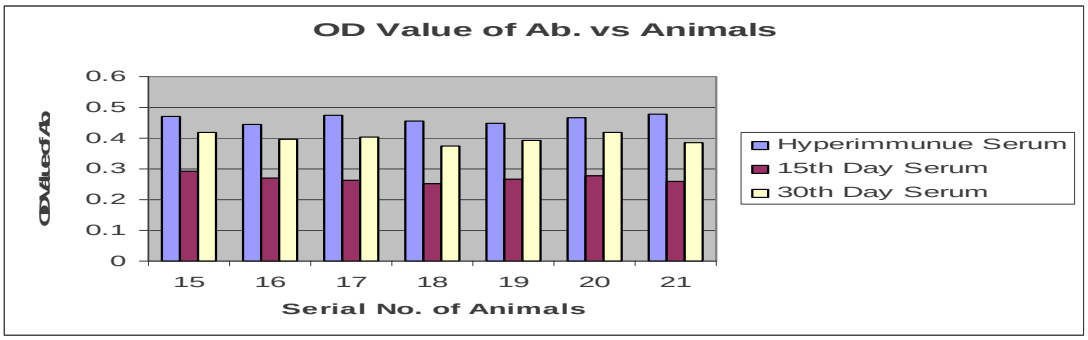

Fig. 3. Showing OD value of antibody titre of hyperimmune serum, day-15 and day-30 serum (15-21 goat)

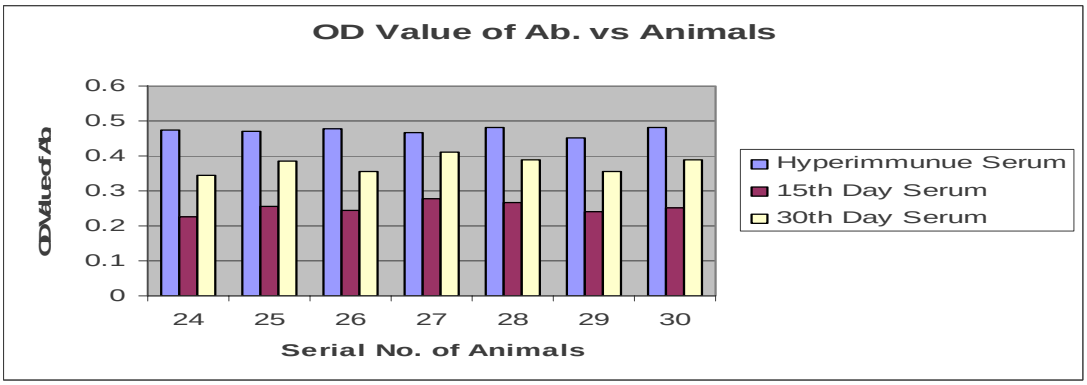

Fig. 4. Showing OD value of antibody titre of hyperimmune serum, day-15 and day-30 serum (24-30 goat)

GPV is a devastating and killer disease of domesticated small ruminants particularly in goats. It causes severe economic loss and hampers the development of goat farm in the developing countries like Bangladesh. The morbidity rates due to goat pox in kids were 81.8% and 80.0% respectively while in adults the corresponding rates were 70.5% and 51.3% mortality rates was 100% in the age group of up to 3 months followed by 95.1% in the age group of 4-6 months (Saha *et al*. 1985).

Field trails of vaccine strains developed satisfactory immunity and this vaccine exhibit fluctuation of body temperature range is moderate, the OD value of antibody titer of day fifteen and day thirteen is increasing satisfactory level. OD value of vaccinated goats is better relative to hyperimmune serum. One vaccinated goat infected and one kid died, the cause of it is unknown. When vaccine was injected the infected goats may remain in latent state of goat pox viruses, for this reason the vaccine may not effective. The mismanagement of the farmer was also responsible for the death of the kids due to suckling its infected mother milk as a result it became infected. Mother was recovered due to appropriate treatment.

Although high level of serum neutralizing antibodies was detected from animals which showed skin reactions following vaccination. This may be due to the attraction of more immunogenic cells during febrile phase. This finding is very similar to that of findings reported by Jadhav *et al.* 1989 and Sing *et al.* 1990 against attenuated sheep pox vaccine.

All vaccinated sheep developed local reaction 96 hours post-challenge with rise of temperature (39.4^0c-40.7^0c) when challenge with SPV (Agarwal and Soman, 1997). It is again suggestive of the satisfactory quality of the vaccine under field conditions. Attenuated GP vaccines can be release in Bangladesh to serve as vaccine candidate against goat pox.

Fig. 5. Showing of vaccination in goat

Fig. 6. Showing of blood collection from goat

CONCLUSION

This study is undertaken with a view to efficacy trail of BLRI-4505 goat pox vaccine in Khulna region. For this reason, 30 goats were selected in farmers level in Austtagram Kapalipara, Daulotpur; Khulna. Animals are vaccinated and blood collected on 15 and 30 days of post vaccination. Temperature of animals was recorded. Serum sample of goats are examined in BLRI, Savar virology laboratory. Antibody titre was detected by C-ELISA. Antibody titre presents as an OD value.

The temperature rise after vaccination is in minimal range. The antibody produced in response to vaccine in 15 day later is quite satisfactory in compare to hyperimmune serum. The antibody titre of day 30 is rising than days 15. The death of one vaccinated animals is unknown, the animals may be affected before vaccination. The post vaccinated animals did not found any exceptionable side effects to be released as a field level. The antibody titre of the vaccine is high as compare to hyper - immune serum.

To control the Goatpox and save the valuable goat and sheep wealth of the country, proper vaccination in the endemic zone is dire necessary. BLRI-4505, Goatpox vaccine will be effective to solve the problem because this vaccine is developed from our local isolates of Goatpox virus. The side effect of the vaccine was not found as well as antibody response was also good. Therefore, this vaccine can be released after conducting few more trials in the intensive and semi-intensive goat populated areas of the country.

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