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Experimental production of goat russell's viper antivenom

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Snakebite is an occupational hazard of farmers. Specific antidote for management of snakebite is the timely administration of an adequate dose of potent specific antivenom. Because of increased demand of antivenom requirement, ways and means to increase production of antivenom in an alternative host is sought for. Feasibility of raising Russell's viper antivenom in goats was attempted by giving a monthly intradermal injection with a total dose of 2 mg/ml of venom adjuvant mixture at six sites per goat for two years. Antivenom level was monitored on samples collected at 8 days following each boosting by indirect enzyme immunoassay method. Results indicated that antibody reached its peak six months after immunization and sustained at its peak throughout the study. Efficacy of the salt precipitated antivenom was assessed by performing mouse protection test. $ED_{50(s)}$ of $5LD_{50}$ of the antivenom (6, 12 and 18 months after immunization) were 15, 15.8 and 16.2 µl respectively. This study highlights that commercial antivenom could be raised in goat, an alternative to horse and it will help in fulfilling antivenom requirement.

INTRODUCTION

Russell's viper bite is an occupational hazard of farmers. Yearly incidence of Russell's viper bite in Myanmar is 7710 with a case fatality rate of 7.2% [1]. Russell's viper antivenom is used throughout the country for treating Russell's viper bite cases. Myanmar Pharmaceutical Factory is the sole manufacturer of the antivenom in Myanmar. Horses are used for raising antivenom. Since Russell's viper antivenom is used for treating suspected and specific bites in township hospitals throughout the country [2], production of antivenom could not meet the demand. In order to fulfill it, ways and means of production of antivenom other than from horses has become an urgent necessity. Feasibility of raising Russell's viper antivenom in goats was attempted and its neutralizing potency assessed for possible commercial use.

MATERIALS AND METHODS

Immunisation method

Two male goats each weighing 20kg were immunized intradermally at six sites on the dorsum of the goats with a total dose of 2 mg/ml Russell's viper venom mixed with an equal volume of Complete Freund's adjuvant at monthly intervals. Incomplete Freund's adjuvant was used in immunogen mixture in subsequent immunizations. The goats were bled 8 days after each boosting. Serum obtained following centrifugation at 1500 rpm for 10 min at 4°C was stored at -80°C until use. Immunisation of the goats lasted for 2 years.

Monitoring of reactions

Local reactions such as swelling, ulceration, abscess formation and general constitutional symptoms such as alertness, eating habit,

general well- being were recorded daily for a week after each boosting in goats.

Characterisation of immunogen (venom)
Determination of Median Lethal Dose

The lethal toxicity of the venom was assessed by intravenous injection of 0.2 ml of venom in physiological saline into the tail vein of 18-20 gm male Institute of Cancer Research (ICR) strain mice. Six mice were used for each venom dose. For control, six mice were injected with normal saline. A wide range of venom (3µg-9.15µg) was selected. Death following 24 hours after injection was noted. The LD_{50} (intravenous) of the venom was calculated by probit analysis [3].

Determination of neutralising efficacy of goat antivenom (ED_{50})

Neutralisation of lethal activity of the venom by the antivenom (ED_{50}) was performed according to WHO recommended standard test of neutralizing activity. Pooled sulphate precipitated goat antivenom was used for the assay. Peak sera collected from 6-12 month, 13-18 month and 19-24 month durations were pooled into 3 groups and precipitated with ammonium sulphate. Briefly, 100µl of a fixed amount ($5LD_{50}$=24 µg) of Russell's viper venom was incubated with an equal volume of varying amount of sulphate precipitated goat antivenom for 30 minutes at 37°C. Each mixture (0.2ml) was injected intravenously into groups of five ICR mice (18~20gm) and deaths were recorded within 24 hours. Controls received $5LD_{50}$ of Russell's viper venom in PBS. Results were analyzed by probit test and neutralization is expressed as effective dose 50% (ED_{50}), the minimum amount of antivenom that will save 50% of the test animals in 24 hours after injection [4].

Ammonium sulphate precipitation

Ammonium sulphate precipitation was carried out according to Wiyada (1995) method [5]. Briefly, 30 gm of ammonium sulphate was gradually added to 100 ml of goat serum. The mixture was stirred vigorously for 1 hour at 22-24°C and centrifuged at 3,000 rpm for 15 minutes at 4°C. After decanting the supernatant, precipitate was dissolved in 10ml of 0.15M PBS (PH 7.2) and dialyzed against the same buffer for 3 days at 4° C. Afterwards, the preparation was filtered through 0.45µm Millipore filter and the filtrate was stored at -80° C until use.

Determination of antibody level

Antibody level in goat was monitored by indirect enzyme linked immuno assay (EIA) [6]. In brief, 96-well microtitre plate (Nunc-Immuno U) was coated overnight at 4°C with 100µl of 1µg/ml of Russell's viper venom in 0.05 M carbonate buffer, pH 9.6 in moist chamber. After five washings with PBS/Tween 20, remaining unbound free binding sites were blocked with 3% BSA-PBS for one hour at 37˚C. The plate was washed five times with PBS-Tween and 100µl of goat sera were added and incubated at 37˚C for one hour. The plate was washed again in PBS/Tween 20 and 100µl of 1: 2000 dilution of peroxidase conjugated rabbit anti-goat IgG (Dakkopatt) was added and incubated for one hour at 37˚C. The wells were then washed and the final reaction revealed by adding 100 µl of substrate containing 0.03% H_2O_2 and 2.5mg/ml dihydrochloride O-phenylene-diamine diluted in 0.1M pH 5.0 citric acid buffer solution. The reaction was interrupted after 15-minute incubation at room temperature by adding 50µl/well of 2.5M H_2SO_4. The reaction was read in EIA reader (Dynatech Lab) at 490 nm wavelength.

Determination of protein concentration of goat antivenom

Protein concentration of the goat antivenom was determined by measurement of absorbance at 280nm.

RESULT

Antibody response in goat following immunization

Antibody reached its peak at 24 wks after first immunization. Boosting at 4 weeks interval from 24 weeks onwards resulted in maintenance of its peak throughout the study which lasted for 2 years (Fig. 1).

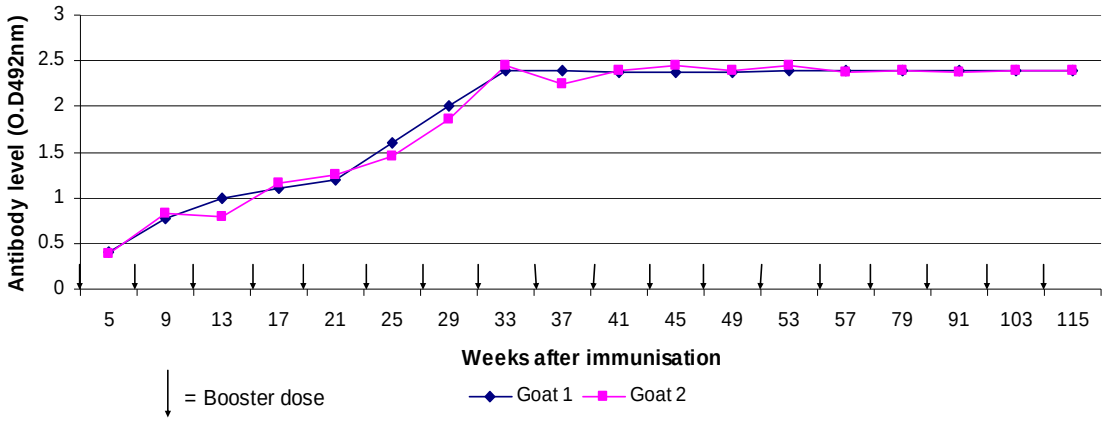

Fig.1. Pattern of antibody response following immunizations with Russell's viper venom in goats

Goats were immunized with 2 mg Russell's viper venom with adjuvant at monthly interval and antibody levels were determined on sera collected at 8 days following each immunization. Each point represents mean of three determinations.

Reactions following immunization with Russell's viper venom

Local induration measuring 1 cm or greater diameter lasted for 5 days was observed after the first, second and third injections. Local swelling and upset in general constitutional symptoms was not observed. No reactions were observed in subsequent boostings.

LD_{50} of the venom and ED_{50} neutralising efficacy of the antivenom

The intravenous LD_{50} of Russell's viper venom was 4.8 µg/mouse (95% confidence limits, 4.32-5.28 µg/mouse). The ED_{50} of the pooled sulphate precipitated goat anti-venom (6, 12, 18 month) were 15µl, 15.8µl and 16.2µl respectively (mean 15.6µl) (13.48-18.08µl) (95% confidence limits).

DISCUSSION

Antivenom is used for treating specific snake bites throughout the country. Anti-venom could be rasied in a variety of hosts: horses [7], sheep [8], goats [9-10], rabbits [11] and laying chicken [12]. Effectiveness of antivenom depends on antibody titre, specificity and degree of neutralization. According to Russell *et.al.* [9], antivenom raised in goat was found to be safe, efficacious and produced significant amount of antiserum. Despite these advantages, there was no attempt to produce antivenom in goat yet. The study highlights that goat can be used for raising antivenom as an alternative to conventional host, horses. A long lasting high titered antibody response with good venom neutralizing efficacy was achieved in goat using a minute amount of venom, 2 mg / goat. Large amount of venom up to 1200 mg / injection is

required in hyperimmunization of horses [7]. Although serum yield from each bleed in goat is less than horses, they are cheaper to maintain than horses and are suitable for tropical climate.

The ED_{50} of sulphate precipitated goat antivenom was 15.6µl (1 ml neutralize 0.307 mg of Russell's viper venom) and is comparable to that of current Russell's viper antivenom (Batch no: C98011. expiry 4/2001) 12µl (1 ml neutralize 0.383 mg of RVV) [13].However, high neutralization efficacy of antivenom could be achieved by further concentrating the antivenom.

We demonstrated that goat immunized with a low dose of venom produced high titer antibody with venom neutralising capability. Owing to its efficacy and simplicity, this method can be used in raising other antivenoms. In conclusion, the goat antivenom has comparable venom neutralizing efficacy to that of current Russell's viper antivenom manufactured by Myanmar Pharmaceutical Factory and this technology could be used in addition to current antivenom production in horses in order to step up antivenom production.

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