Veterinary Serum and Vaccine Research Abbasia Cairo,
Agricultural Research Center, Ministry of Agriculture.
Director: Prof. Dr. S. Salama.

STUDIES ON THE METHOD OF PREPARATION OF RINDERPEST HYPERIMMUNE SERUM IN CALVES

(With One Table)

By

Z. MOHAMED and S.M. HAFEZ

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دراسات على تحضير المصل فوق المناعي ضد الطاعون البقرى في العجول

زين العابدين عبده ، سمير حافظ

تم دراسة طريقة تحضير المصل فوق المناعي ضد الطاعون البقرى في العجول ، تم تحصين العجول مبدئيا بالنقاح النسيجي لفيروس الطاعون البقرى. تم حقن العجول بثلاث جرعات متتالية من عترة الفيروس الضارى للطاعون البقرى المضاف اليها المنشطات الغير نوعية لرفع المناعة في الجسم بفارق أسبوع بين كل جرعة أخرى وذلك بعد ثلاثون يوما من التحصين المبدئي بالنقاح النسيجي للطاعون البقرى . ولقد أعطت التجارب على تحضير المصل فوق المناعي نتائج مرضية استعملت في تشخيص الطاعون البقرى بواسطة تجربة التخلل المناعي .

SUMMARY

The procedures for the preparation of rinderpest hyperimmune sera in calves were studied. Calves were first immunized with rinderpest tissue culture virus -vaccine. 30 days following immunization the calves recevied three booster injections at weekly intervals with virulent rinderpest virus emulsion mixed with an oil adjuvent. A satisfactory hyperimmune- serum was obtained and used in the diagnosis of rinderpest, by using the immunodiffusion test.

INTRODUCTION

Having realized the importance of potent rinderpest hyperimmune serum to control an invasion of thedisease, WHITE (1958 b & 1962) reported that, the sera of convalescent animals from rinderpest rarely contain precipitins. SCOTT (1962 a) Hyperimmunized cattl by the technique evolved by NAKAMURA (1931). Subsequent injections increased the potency of sera but the potency was never as great as the potency of stock rinderpest-hyperimmune-serum prepared in rabbits.

Two minor disadvantages of ox serum in agar gel diffusion tests were the development of a dense haze around the serum well and the frequency of non specific bands of precipitation that occurred when theserum was diffused against antigens prepared from pig and rabbits.

PROVOST et al. (1963), also tried a serum prepared in hens, using infected goat tissues as the source of virus. Specific precipitins were produced with many preciptation lines developing and the technique was not recomneded. The present

study was conducted to prepare a potent hyperimmune serum used in the immunodiffusion test which is a very simple and prompt means for the diagnosis of rinderpest cases in outbreak.

MATERIAL and METHODS

Material :

1. Virus :

A- Rinderpest virulent virus: was an Etyptian strain beeing maintained by passage in cattle, since 1903, using spleen, lynph- nodes, and defibrinated blood form infected into susceptible animals (ATA and SINGH, 1967).

B- Rinderpest - vaccine strain "Kabeto O strain": Obtained from Dr. Nakamura and Miyamoto 1933. It was passaged for 99 times in primary bovine kidney cultures. Furthermore three passages were made in primary boving kidney cultures in Egypt. The virus was stored at 70°C in vials each contouning 10 ml (SINGH et al., 1967).

2. Animals :

Two local breed calves (Balady) 1-1.5 years - old, of about 150-200 Kg. were tested for their susceptibility to rinderpest - virus infections by using the tissue culture serum neutralization test (screening test) PLOWRIGHT and FERRIS, 1959).

Methods :

Table (1): the procedures for rinderpest hyperimmunization of calves.

Days of inoculation	Schedule of inoculation and bleeding	** AG Pt Results
0	1 ml rinderpest-virus culture vaccine S/c containing 10 ID_{50}/ml.	* - ve
30	1/m (100 ml) 50 ml's virulent rinderpest virus (spleen and lymph-node) with 50 ml in-complete Fruend adjuvent (7.5 ml A rlacieol : 42.5 ml's Bayol-F.).	- ve
37	1/ml (100 ml's) 50 ml's virulent emulsion with 50 ml's incomplete Fruend adjuvent.	diffus line
44	1/m (100 ml's) 50 ml's virulent emulsion with 50 ml's incomplete Fruend adjuvent.	+ + Ve
51	Collect 50 ml's blood for testing	sharp line

* : - Ve = negative. **: AG PT= agar - gel diffusion test. S/C subcut 1/M Imtramuscular.

2- Immunodiffusion technique :

Agar plates for immunodiffusion were prepared in 10 cm. petri dishes, each containing 25 ml of diffusion medium consisting of 1.5 percent Purified agar (Difco) dissolved in 0.85% Saline (PH 7.2) and 0.1% Sodium merthiolate. Six wells were cut

around the central well of 6 mm diameter, 2 mm apart from each other. The supernatent fluids of homogenised lymph nodes from mormal and infected catle were used as the negative and positive antigens respectively. the antigen was placed in the central well and tested sera in the surrounding wells. the plates were incubated at room temperature for 1-2 days before reading the results.

RESULTS

Pre- inoculation sera of all calves used in this experiment did not form any visible precipitine lines with the antigens prepared from normal control or infected tissues.

The calves immunized with rinderpest - tissue - culture vaccine, were bled on day 30 but non of the sera - produced visible lines with the infected rinderpest tissue.

Additional 3 booster injections with virulent rinderpest-virus (spleen, and lymph-nodes), oil adjuvent mixture were given intramuscularly on day 30, 44 sera collected on day 37 and 44 were not satisfactory in immunodiffusion test.

Only on day 51 the sera collected from the two calves and tested by immuno-diffusion test produced visible sharp line. With infected rinderpest tissues (lynph-nodes and spleen). It was used as screening method for more than 150 samples during the outbreak of rinderpest in Egypt during 1982 (THANAA et al., 1983).

DISCUSSION

This study aimed to investigate the probable factors which could have a role in the impotency of hyperimmune serum. The effect of oil adjuvent when added to the virulent emulsion and the effect of the dose on the frequency of inoculation were studied.

Results of this study using NEAHI* method with the limited mumbers of calves indicated that an oil - adjuvent when mixed with virulent emulsion of rinderpest tissues (lymph-node and spleen) and with the frequency of inoculation, enhanced antibody responses in inoculated calves. This was indicated by a definte sharp pricipi-tin line and this overcome the disadvantages which occurred in the technique of NAKAMURA (1931) and used by SCOTT (1962), who reported that, two minor dis-advantages of hyperimmune ox serum in agar-gel diffusion test were the development of a dense haze around the serum well and the frequency of nonspecific pricipitin bands occurred when the serum was diffused against antigens prepered from pigs and rabbits. PROVOSET et al. (1963) also tried a serum prepared in hens, using in-fected goat tissues as the source ov virus. Specific precipitation lines developed and the technique was not recomneded.

The rinderpest hyperimmune calves sera prepared by the NEAHI method have been routinely used in dignostic work by immunodiffusion test, the results were satis-factory.

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