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# RADIOIMMUNOASSAY FOR DETECTION OF ANTI-RINDERPEST ANTIBODIES IN CATTLES

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## SUMMARY

Liquid Phase Radioimmunoassay (RIA) was developed to detect and measure anti-rinderpest immunoglobulins in field animals sera, within two hours. Rinderpest virus adapted on Vero cell line culture and antigen purified by treatment with Triton-Gentron 13-Butanol, and, labeled with I isotope, using chloramin T iodination method.

Comparative studies for detecting anti-rinderpest immunoglobulin in 80 calves sera samples, using the developed assay in parallel with virus neutralization test (VNT). The study showed 58.75 % agreement between the two methods. However, 71 % of seven months old, non-vaccinated calves showed anti-rinderpest antibodies in their sera, also 81 % of 10 months old vaccinated calves were developed antibodies in their blood.

These results demonstrate the development of sensitive, specific and rapid quantitative / qualitative radioimmunoassay, necessary for screening the development of immunity against rinderpest in cattle.

## INTRODUCTION

Rinderpest virus continues to pose a threat to livestock in Iraq, and many other countries. The eradication depends a large extent on the rapid diagnosis of sporadic cases coupled with the development of immune belts, and restriction on the movement of animals as a means of assessing the immune status before and after vaccination programs and to identify susceptible animals that required immunization, it is necessary to have a quick and simple method for measuring immune status of the animals.

The virus neutralization test (VNT) has been the standard technique for the demonstration the resistance or susceptibility of livestock to rinderpest virus, nevertheless, its elaborate and time consuming. Trials were made to replace VNT with measles haemagglutination inhibition test (MHT) and enzyme immunoassay. MHT has been rejected due to considerable disagreement between the result with VNT (10 & 11).

Enzyme linked immunosorbant assay (ELISA) (1, 2 & 3) were applied for detection of antibodies to rinderpest virus in sera of cattle. They have demonstrated low background readings, but still the work need more confirmation. In 1983 Anderson et al (4) in a further study to compare the efficacy of ELISA and VNT in field serum samples of cattle from different countries as well to study the rang of background levels in order to discriminate between positive and negative ELISA technique results. They considered serum samples showing twice the man value were taken as positive for the presence of antibodies to rinderpest virus.

The aim of this study was to develop liquid phase radioimmunoassay for detection of anti-rinderpest antibodies in field sera samples.

## MATERIALS AND METHODS

Rinderpest virus – Kabete " O " strain was obtained from Al-Kindei Veterinary vaccines production Co. Baghdad – Iraq, which was being in primary bovine Kidney Cell culture. The virus then serially passaged for four times in Vero Cell line culture supplemented with culture medium contained lactalbumin hydrolysit faetal calve serum (FCS) sodium bicarbonate and antibiotics.

### Purification of Rinderpest virus antigen

Confluent monolayers of Vero cells cultures were inoculated with $10^5$ TCID50 of seed virus. The cultures were maintained with culture medium containing 1 % FCS, unit 85 % of the cells show cytopathological effects (CPE), than freezed and thawed for three times before clarifying the culture by centrifugation at 5000 rpm for 10 minutes at 4 c. and supernatant stored at 70 °C until used.

Purification was performed according to (5) briefly. The supernatant treated with tenth volume of 20 % Triton x-100 for 20 minutes at room temperature, then mixed vigorously with Gentron-13 for 10 minutes at 25 °C, then after, centrifuged at 1000 Xg for 15 minutes, the aqueous phase collected and treated with pre-chilled N-butanol for 10 minutes at 25 °C followed by 1000 Xg centrifugation for 15 minutes. The three layers formed were collected separately, and protein concentrations were determined at 280 nm by schemat-zo-210 spectrophotometer. The preparation named RP-TGB.

### Preparation of Rabit anti-Rinderpest hyperimmune serum

Adult rabbits were inoculated with RP-TGB, and booster doses were given two weeks later. Blood samples were

collected after three weeks of the boosting doses. Antibody rise was evaluated by gel immunodiffusion test.

**Labeling RP-TGB with Iodine 125 Isotope ($I^{125}$)**

Chloramine – T iodination procedure with modification was utilized for labeling RP-TGB with $I^{125}$ isotope. Briefly ; 50/µg of Rp-TGB in 10/µl of 0.01 M PB-0.15 M NaCl was mixed with 10/µl of $I^{125}$ isotope containing 1 m Ci and then 10/µl of 5 mg/,ml of chloramine – T was added to the mixture. After 30 seconds of reaction, 10/µl of 12 mg/ml of metabisulfate was added to terminate the reaction. The final preparation was stabilized by 500 / µl of 2 % human serum albumin in buffer (PBS).

Radioactivity of the prepared labeled RP-TGB was measured before treatment with Amber lit resin (1 gm concentration) and after column chromatography purification (Sepharose 4 B, FR = 1 m/ 10 min.). Radioactivity curve of the fractions was plotted together and its immuno-radioactivity was tested. The final preparation named tracer RP – $I^{125}$.

**Liquid – phase Radio Immuno-assay for anti – Rinderpest virus Antibody detection :**

After thourgly mixing 100/µl of tracer RP – $I^{125}$ with 100 / µl of either standard controls antisera or animals sera samples, the mixtures were incubated at 37 °C for 2 hours, then 200/µl of 10 % of PEG-6000 (polyethylene glycol-6000) in PBS were added and the mixture incubated at 37 °C for 30 minutes. The mixture then centrifuged at 2500 rpm/15 minutes and supernatants decanted and tubes plotted.

Immuno-radioactivity binding between tracer RP – $I^{125}$ and specific anti-rinderpest immunoglobulin in standards and

test samples were measured, utilizing gamma counter at one minute time / tube.

The developed assay was utilized for detecting anti-rinderpest antibodies in 80 sera samples of vaccinated and unvaccinated calves.

**Counting antibody titer :**

Counting of binding percentage of antibody and tracer RP – $I^{125}$ depends on the total count of tracer RP-$I^{125}$ and the immuno-radioactivity of the antigen-antibody complex (tracer RP – $I^{125}$ plus antibody).

Binding curve of the standard plotted according to the binding percentage and the concentrations of the standard, horizontal line then draw from the binding axis to meat the curve line, and from the meeting point, vertical line dropped on the axis of concentration. Meeting point represents the antibody titer.

**Elimination of Non – specific binding :**

To overcome non-specific binding caused by 10 % PEG, 6000, PBS and/or tracer RP – $I^{125}$ trials were performed by adding certain animals protein to the dilute buffer such as human serum albumin, or horse serum at 2, 4, 8 and 10 %. The buffer (PBs) was used for diluting standard and test materials.

**Discrimination between positive and negative animals :**

To discriminate between positive and negative test materials value of binding activity revealed by testing foetal calf serum (FCS) which was considered as the negative control and the base line for calculation titers of the test samples from

animals of the field. Newborn calf serum was included in the experiment for comparison.

**Competitive radio-immunoassay for Rinderpest virus antigen Detection (C-RIA)**

Equal volumes of tracer RP – $I^{125}$ and test specimens (100/µl of each) were well mixed and then 100/µl of Rbanti – RP hyperimmune serum, were added. The mixture then incubated at 37 °C / 2 hours, followed by the addition of 200/±l of 10 % PEG-6000. After 30 minutes incubation at 37 °C. The mixture was centrifuged for 15 minutes at 2500 rpm and supernatants decanted. The immuno-radioactivity of the precipitate was measured at one minute time for tube by gamma counter.

Top counting meaning highest binding between tracer RP – $I^{125}$ and Rb anti-RP antisera. Antigen quantities in test specimen was calculated according to percentage of binding and the standard antigen concentration curve.

## RESULTS

**Virus propagation :**

Infected Vero Cell Culture with RPV of passage four demonstrate about 80 % CPE at day-7- post. Inoculation, and characterized by formation of synciatial and rounded cells. The obtained virus had $10^{6.2}$ TCID50 titer.

**Antigen Purification :**

The applied purification method gave best pure and highest concentration of antigen in the bottom layer as determined by the established competitive-RIA (Fig.1). Control

negative antigen gave 27.9 % + -1.8 binding activity. Whereas, the binding activity revealed by the three layers from top to bottom were 4.7 % + -0.8, 4.5 % + -0.5 and 2.7 % + -0.2 respectively.

**Tracer RP – $I^{125}$ preparation and its shelf life :**

Utilization Amber lits resin to remove the free RP – $I^{125}$ together with the column chromatography fractionation gave very clear peak of labeled RP – $I^{125}$ (Fig.2) and the preparation binding activity did not effected significantly after four weeks of storage at +4 °C.

**Elimination of Non – specific binding :**

We find that using PBS of pH 7.2 only as a diluent buffer for either standard or test specimen, will effect greatly the results, since standard curve loose its linearity as well as higher non-specific binding activity was demonstrated at higher dilutions.

These problems were overcome greatly when human serum albumin was added to the PBS at 4 % concentration. (Fig.3).

**Assessments of sera samples of vaccinated and unvaccinated calves :**

The results of the application of the developed assay (RIA) for detection RP-antibodies in 46 sera samples of vaccinated ten months old calves (bleed 7-days post-vaccination), compared with VNT, demonstrate that 29 % of them have no detectable antibodies, however 71 % of them have detectable antibodies when tested by RIA. Moreover, results of testing 33 sera samples of seven months unvaccinated calves,

showed that only 19 % of them have antibodies as measured by RIA and VNT, whereas, RIA demonstrate 81 % of the samples have detectable specific RP-antibodies.

Foetal calf serum which used as standard negative control included in each test gave binding activity of 3.2±0.35 (Fig.3).

## DISCUSSION

Vero cell line culture supplemented with 1% FCS for RPV antigen preparation used in this study is attempted for the first time in RIA technique. In 1984 Nawroz, (6) used Vero cell line culture supplemented with 2 % PEG treated goat serum for RPV antigen preparation used for ELISA technique to detect RPV antibodies. Earlier worker in this field (1,2) used primary bovine kidney (BK) culture supplemented with bovine serum for the production of RPV antigen for the use in ELISA. Because of th extensive non-specific background reading, they strongly recommended the use of RPV antigen prepared in other heterologous system such as vero cell line.

Application of TGB purification method modified by (5) for the preparation of RP-antigen has several advantages over using PEG-6000 or ultracentrifugation techniques, to get off the traces of IgG which can be precipitated with the antigen in those two techniques. This traces of IgG can bring up the non-specific background which noticed by other authors (2,3).

The assay established (Liquid phase RIA) in this study characterized by its simplicity, reliability, reproducibility and time saving, compared to the conventional techniques routinely used in this field.

Reduction in time and the simplicity of the assay, has great advantage in controlling the spread of the disease as well it greatly helpful in vaccination program.

The established assay have great sensitivity in detection very traces of RPV antibodies in field sera samples of weaned and/or recently vaccinated calves. Discrimination between positive and negative animal sera samples to RPV-antibodies was depended on the value of binding activity obtained from using foetal calf serum (FCS) as negative control to determine the base line, above which was considered positive results.

The problems with the other studies are the authors considered an arbitrary values for base line, which may cause a bias discrimination between animals have immunity to rinderpest virus or they are free of it (4,6).

In a study on transfer and persistence of maternally derived antibody levels in calves, (7), observed that the calves at birth did not show the evidence of rinderpest antibody before suckling but received neutralizing antibodies from the colostrum, whenever the dam were primarily immunized against rinderpest. The maternal antibody level in calves decline gradually and were invariably absent around ten months, although the half life of this type of antibody was one year.

Rowe et al (8) recorded that calves in the age 9-11 months contained maternal antibodies. These results are almost in parallel with our findings. The absence of any demonstrable maternal antibodies level in 29 % of calves under the study could be due to either gradual deline due to several factors such as, age progress, absence or low level of colostrum antibodies, or due to non-responded to the vaccination, since maternal antibody present (9).

The results obtained in this study, demonstrate the superiority of RIA in detection RPV antibodies compared to the result demonstrated by VNT. No studies have been done in this field have used RIA, but comparative studies using ELISA and VNT, showed a limited correlation between their results (4,6).

In conclusion an improved liquid phase RIA technique has been standardized for the assay of rinderpest virus antibodies in cattle sera. The improvement included :

a- The use of heterologous system for the production of RPV antigen.

b- Selective human serum to reduce the non-specific finding.

c- Highly accurate results in detecting very traces antibodies.

d- Simple, specific, sensitive and time saving assay.

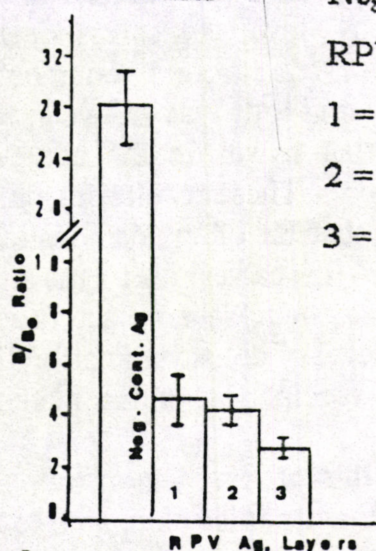


Fig.1: Rinder-Pest Virus Antigen Purification

Neg. conf. Ag = negative control antigen.

RPV Ag Layer = Rinderpest virus antigen laye

1 = Top Layer.

2 = Med. Layer.

3 = Bottom Layer.

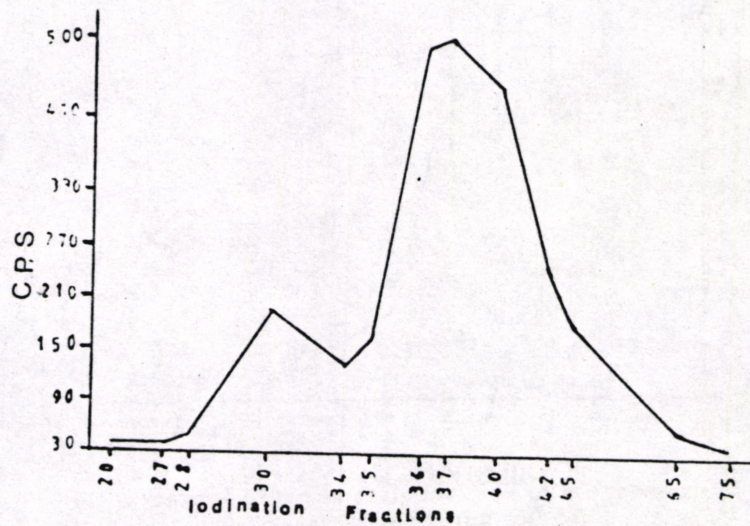


Fig .2: Iodination of Rinder-Pest Virus Antigen Using $NaI^{125}$

CPS = Count per Seconds

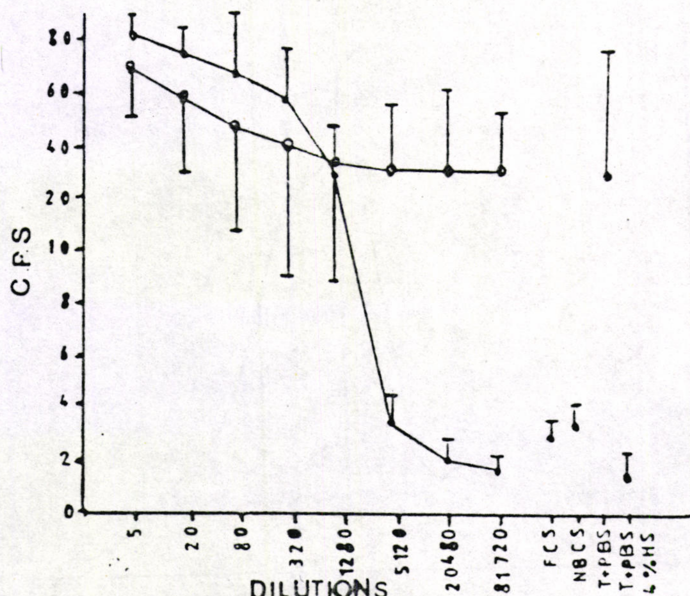


Fig.3: ELIMINATION OF NON-SPECIFIC BINDING & DETERMINATION THE CUT-OFF LINE

CPS = Count per Seconds

FCS = Fetal calf serum.

NBCS = newborn calf serum.

T + PBS = Test serum + PBS.

T + PBS of

4 % HS = Test serum + containing 4 % human serum albumin.

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# مقايسة مناعية إشعاعية للكشف عن الأجسام المناعية المضادة لمرض الطاعون البقري

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الخلاصة :

لقد استحدثت مقايسة مناعية إشعاعية ذات الطور السائل لقياس وكشف الأجسام المناعية المضادة لفايروس الطاعون البقري في مصول حيوانات الحقل وخلال ساعتين. لقد طبع فايروس الطاعون البقري على خلايا الفيرو للزرع النسيجي وتم تنقية المستضد باستخدام الترايتون – جنترون ١٣ – بيوتانول. وسم المستضد باليود المشع ١٢٥ باستخدام كلورامين – تي.

أجريت دراسة مقارنة بين الطريقة المستحدثة والطرق التقليدية ( التعادل المصلي ) باستخدام ٨٠ نموذجا مصليا أخذ من عجول ذات أعمار ٧ و ١٠ أشهر ، كان بعضها ملقحا والآخر غير ملقح. أظهرت الدراسة تطابقا في النتائج بنسبة ٥٨,٧٥ % بين الطريقتين. لقد أظهرت الطريقة المستحدثة وجود الأجسام المناعية في ٧١ % من العجول ذات العمر ٧ أشهر غير الملقحة وفي ٨١ % من العجول ذات العمر ١٠ أشهر والملقحة.

ان نتائج الدراسة تشير إلى مقايسة المناعة الاشعاعية المستحدثة ذات حساسية ونوعية عالية إضافة إلى سرعة الاداء في كالشف النوعي أو الكمي للأجسام المناعية ويمكن استخدامها لعمليات المسح الواسع للتأكد من نجاح عمليات التلقيح ضد المرض.