GENOMIC COMPARATIVE STUDIES ON RINDERPEST, PPR AND MEASLES VIRUSES CONCERNING WITH THEIR ANTIGENIC PERFORMANCIES

By

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ABSTRACT

Rinderpest antibodies in sera of vaccinated cattle were detected with rinderpest (RP) and peste des petits ruminants (PPR) viruses but not with canine distemper (CD) virus. While, PPR antibodies in immune sheep sera were recognized with the three viruses. But, canine distemper antibodies in sera of immune dogs were detected only with canine distemper and PPR viruses. On reviewing the published nucleotide and deduced amino acid sequences of H, F and NC genes of the three aforementioned morbilli-viruses, it has been noticed that a closer degree of similarity was found between RP and PPR viruses than between RP and CD viruses. This gives a clue for more sharing consensus sequences between RP and PPR that might explain their closer antigenic relationship and however, the degree of homology among the three viruses are relatively low especially on the H-gene level as it carries the characteristic epitopes determining host specificity for each virus. These studies might be useful in terms of immunization and sero-surveillance purposes.

INTRODUCTION

The paramyxoviridae family was classified in 1993 into two subfamilies, paramyxovirinae and pneumovirinae. The first one includes the general of parainfluenza, rubella and morbilli viruses. The last genus contains measles, dolphin morbillivirus, canine distemper (CD), Peste des Petits Ruminants (PPR), rinderpest (RP) and phosine distemper virus (**Collier *et al.*, 1998 and Field' s *et al.*, 1996**).

Many workers have suggested cross-antigenic relationship among members of genus morbilli (**Max *et al.*, 1981**). On the basis of the nucleic acid and protein sequences of the all morbillivirus nucleoproteins, 2 major subgroups were detected, one included canine distemper and phosine distemper and the other subgroup harboured measles, rinderpest and PPR viruses (**Diallo *et al.*, 1994**). Rinderpest and PPR were proved to be more closely antigenically related than the other members of the genus (**Losos, 1986, Limo and Yilma, 1990**).

However, monoclonal antibodies directed against non-overlapping antigenic domains on the nucleocapsid (N) could be successfully used in immunocapture ELISA to differentiate between RP and PPR viruses in the field samples (**Libeau *et al.*, 1994**). The amino acid homology of CDV-H protein was 38% similar to that of rinderpest virus but on the nucleotide level, the percent was relatively higher (52%) (**Lefever *et al.*, 1991**).

Our herein studies were devoted for studying the cross antigenic relationship among some members of morbilliviruses (RP, PPR and CD) that are of prime veterinary importance. These studies might have extreme significance in the respect of epidemiological investigations as well as the sero-surveillance of such diseases. Our studies were also augmented with some comparative molecular biological reviews on the sequence of some cognate genes of the above mentioned viruses so as to give a reasonable elucidation to the entity and the nature of that relationship.

MATERIAL AND METHODS

1. Animals:

1.1. Cattle:

Eight cross-breed Freizian cattle one to one and half years old were used in these studies. Two of them were kept as non-inoculated controls, while the rest six animals were subcutaneously inoculated each with one ml containing 3 $\log_{10}$ $TCID_{50}$ of rinderpest vaccine.

1.2. Sheep:

One year age twelve Barki sheep were divided into two groups. The first was made of four ones devoted as non-vaccinated controls. Whereas the rest eight were subjected to S/C inoculation with 3 $\log_{10}$ $TCID_{50}$/ml of PPR vaccine.

1.3. Dogs:

Twelve dogs of 6 months of age were vaccinated with canine distemper vaccine, each one received 3 $\log_{10}$ $TCID_{50}$/ml subcutaneously. Meanwhile, 4 animals were kept as non-inoculated controls.

All animals used to fulfil our experiments were negative to RP, PPR and CD viruses. They were kept in mosquito proof-double doored stables.

2. Virus vaccines:

2.1. Tissue culture rinderpest virus vaccine (TCRPV):

TCRPV vaccine was propagated on Vero cells in accordance with the method of **Osman *et al.*, (1990)**. The vaccine had a titre of 6 log10 $TCID_{50}$/ml. It was stored at -20°C till used.

2.2. Tissue culture Peste des Petits Ruminants vaccine:

Vero cells adapted PPR vaccine was prepared from the isolated Egyptian strain designated as Egypt-87 (**Khodeir and Mouaz, 1998**). The virus had a titre of 5 log10 $TCID_{50}$/ml. The vaccine was stored at -20°C.

2.3. Tissue culture canine distemper virus vaccine:

A living attenuated CD virus was prepared on Vero cells according to the method devised by (**Guirguis, 1991**). The vaccine titre was 4 log10 $TCID_{50}$/ml and stored at -20°C till used.

All virus vaccines used either in animal inoculation or in serological assays were prepared in Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt.

3. Virus neutralization test (VNT):

Both screening and quantitative SNT were performed in our herein studies. SNT macro-techniques (tube method) following (**Singh *et al.*, 1967**) was used to determine the antibodies raised in sera of cattle, sheep and dogs as a result of inoculating them with RP, PPR and CD viruses. The end point neutralizing antibody titres were expressed as the reciprocal of the final dilution of serum inhibiting the CPE of 100-200 $TCID_{50}$ of the relevant virus.

4.1. PC/Gene Computer Program:

PC/gene release 6.6 (February, 1991) from Intalligeneyics contains over 70 programs for the analysis of protein and nucleic acids and management of sequence data.

4.2. Internet, EMBL and GenBank;

Internet (the World's largest computer network) has many facilities concerning the use of database and transfer files. EMBL is the European Molecular Biology Laboratory in Heidelberg, Germany. It has an important collections of molecular biology computer databases including the Gen Bank nucleotide and protein sequences.

RESULTS

I. Detection of RP-antibodies in sera of vaccinated cattle using RP, PPR and CD viruses:

Three weeks post vaccination of cattle with TCRPV-TC vaccine, their sera were tested by using VNT against RP, PPR and CD viruses. It has been found that the SN antibody titre detected by RP virus was the highest one (128), while in case of PPR, the titre was (4), but in case of CD no antibody titre was detected (Table 1).

II. Detection of PPR antibodies in case of vaccinated sheep using PPR, RP and CD viruses:

The PPR-VN antibody titres in sera of vaccinated sheep with PPR virus were estimated three weeks post vaccination against PPR, RP and CD viruses. They were 64, 8 and 2, respectively (Table 1).

III. Deteetion of CD antibodies in sera of vaccinated dogs using CD, PPR and RP viruses:

CD neutralizing antibody titres in sera of vaccinated dogs three weeks post-vaccination were 128, 2 and 0, respectively (Table 1).

IV. The nucleotide (Nu) and the amino acid (A. A.) homology among the H-Gene of RP, PPR and CD viruses using that of RP as standard sequence:

The sequence homology between H-gene of rinderpest to that of PPR was 50.74% while to that of CD virus was 37.34% on the nucleotide level. While, on the amino acid level they were 73.71% and 68.38%, respectively.

V. The nucleotide and amino acid homology among F-gene of PPR and CD viruses:

The F-gene nucleotide homology between RP and PPR was 63.63% while it was 55.2% between RP and CD viruses.

On the other hand, the homology percent on the amino acid level between RP and PPR was 63.84% and between RP and CD was 59.56%.

VI. The nucleotide and amino acid homology among NC-gene of RP, PPR and CD viruses:

On the nucleotide level, the homology percent between RP and PPR viruses was 69.67% and between RP and CD viruses was 65.01% whereas on the amino acid level, the homology percent between RP and PPR was 73.71% and between RP and CD viruses was 68.38%.

Table (1): Results of serum neutralization test of cattle, sheep and dogs sera.

Samples⇨	Cattle		Sheep		Dogs	
Virus⇩	Vaccinated	Control	Vaccinated	Control	Vaccinated	Control
RPV	128*	0	8	0	0	0
PPRV	4	0	64	0	2	0
CDV	0	0	2	0	128	0

* The reciproeal of the highest dilution that inhibit the CPE.

DISCUSSION

Rinderpest, peste de petits ruminants and canine distemper are still rampant maladies in some of African and Asian countries and so far, they cause catastrophic losses among cattle interprises, sheep and goat populations and pet animals respectively. Their etiological agents are viruses belong to genus morbillivirus under the subfamily paramyxovirnae (**Diallo *et al.*, 1987**). Many workers reported on the antigenic relationship among the three viruses and here we tried to identify the nature and the degree of this relationship.

Our studies have revealed that RP neutralizing antibodies in sera of vaccinated cattle could be detected with RP and PPR viruses with titres of 128

and 4, respectively. While, it could not be detected with CD virus. Whereas PPR neutralizing antibodies in immune sheep sera were traced with PPR, RP and CD viruses with titres of 64, 8 and only 2, respectively. CD neutralizing antibodies in positive dog sera could be captured only by CD and PPR viruses with titres of 128 and 2, respectively. The same findings were previously obtained by (**Obi *et al.*, 1984; Losos, 1986 and El-Dakhly, 1999**) whom stressed on the potentiality of rinderpest and PPR viruses to recognize their homologous as well as their heterologous antibodies.

Further genetic speculations were devoted for elucidation the molecular identities among the three viruses so as to give a clue for the entity of the antigenic relationship between them. On the level of H-gene, the homology degree on the nucleotide and amino acid aspects between RP and PPR was higher than that between RP and CD viruses. Correspondingly, the homology between RP and PPR were relatively higher than that between RP and CD viruses either on the F and NC-genes. This might give a reasonable explanation on the higher antigenic relatedness between RP and PPR viruses over between RP and CD viruses. These notions come in close agreement with those given by (**Max *et al.*, 1981; Hamdy *et al.*, 1975 and Taylor and Abegunde, 1979**). However, the degree of homology was very low in case of H-gene because it is considered as a species specific gene since it carries the neutralizing epitopes determining the antigenicity and virulence.

Coincident data that are identicals to ours were also assessed by (**Kamata *et al.*, 1991; Kovamees *et al.*, 1991 and Lefever *et al.*, 1991**). Some opinions adopted the idea of categorizing the morbilliviruses into distemper virus group (canine and focine) and the other one comprising rinderpest, measles and PPR viruses (**Diallo *et al.*, 1994**). Thus, it could be concluded that RP and PPR viruses have closer antigenic relationship than RP and CD viruses. This could be exploited in using RP in immunization as well as sero-surveillance purposes even for PPR among sheep and goats and it could be utilized in devising the subunit vaccines.

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الملخص العربى

دراسة مقارنة لجينوم فيروس الطاعون البقرى وفيروس طاعون المجترات الصغيرة وفيروس حصبة الكلاب وأداءهم الأنتيجينى

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أمكن التعرف على الأجسام المناعية لفيروس الطاعون البقرى فى أمصال الأبقار وذلك باستخدام كلاً من فيروس الطاعون البقرى وفيروس طاعون المجترات الصغيرة ولكن لم يستدل عليها باستخدام فيروس حصبة الكلاب. بينما تم تبين الأجسام المناعية لفيروس طاعون المجترات الصغيرة فى الأمصال المناعية للأغنام وذلك باستخدام الفيروسات الثلاثة ولكن فى حالة الأجسام المناعية لفيروس حصبة الكلاب فقد تم الكشف عنها باستخدام فيروس حصبة الكلاب وفيروس طاعون المجترات الصغيرة. وعند عمل مقارنة على التسلسل النيوكليوتيدى والأمينى لجينات H , F, NC للفيروسات الثلاثة وجد ان درجة التشابه بين فيروس الطاعون البقرى وطاعون المجترات الصغيرة أعلى من نظيرتها بين فيروس الطاعون البقرى وحصبة الكلاب وهذا يمكن ان يعطى دلالة على وجود مناطق متشابهة أكثر بين فيروس الطاعون البقرى وطاعون المجترات الصغيرة عن نظائرها بين الطاعون البقرى وحصبة الكلاب وهذا يدل على درجة قرابة أعلى بين الفيروسين الأولين على الأخرين. ولكن وجد ان درجة التشابه بين الفيروسات الثلاثة على مستوى الجين (H) كان ضئيلاً وذلك لأن هذا الجين هو المسئول عن تحديد العائل لكل فيروس وإحداث الحالات المرضية.