Preparation of Hyperimmune Serum against Peste Des Petits Ruminants to Be Used in Emergency Cases

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Abstract
Hyperimmune serum against peste des petites ruminants virus was successfully prepared in horses where it was found to have specific peste des petites ruminants virus (PPRV) neutralizing antibodies titer of 1024/ml as determined by serum neutralization test (SNT). Quality control testing of such serum revealed that it was free from bacterial, fungal and mycoplasma contaminants as tested on specific media and safe when inoculated in sheep. Passive induced immunity in sheep was persisted with a protective level up to 5 weeks post inoculation as determined by SNT and ELISA. On the other side, inoculation of PPR vaccine with antisera in sheep showed similar findings with slight rise in antibody titer. Depending on the obtained results, it could be concluded that horse anti-PPR serum is of a significant importance and can help to protect and control PPR infections especially in case of outbreaks that need rapid management.

Key words: PPR – Hyperimmune serum – Horse – Sheep.

Introduction

Peste des petites ruminant (PPR) disease is an acute contagious disease caused by a Morbillivirus in the family Paramyxoviridae. It affects mainly sheep and goats and occasionally small ruminants in the wild life. PPR occurs in Africa in countries lying between the Equator and the Sahara, in the Arabian Peninsula, throughout most of the Near East and Middle East, and in South-West Asia. The clinical symptoms of this disease resemble rinderpest in cattle. It is usually acute and characterized by serous ocular and nasal discharges. PPR is characterized by severe pyrexia, erosive lesions on different mucous membranes particularly in mouth, diarrhea and pneumonia. At necropsy, characteristic zebra markings may occur in the large intestine, but are not a consistent finding. Lesions also occur in the lungs showing congestion or bronchopneumonia when associated with bacterial infection. The morbidity rate can be up to 100% with a mortality rate up to 100% in severe cases. However, this may not exceed 50% during milder outbreaks ***(OIE, 2008).***

Preventing disease after exposure to a biological agent is partially a function of the immunity of the exposed individual. Unlike vaccines, which require time to induce protective immunity, it depends on the host's ability to mount an immune response ***(Casadevall, 2002).*** Antibodies, also known as immunoglobulins are proteins that are used by the immune system to identify and neutralize foreign structures, such as bacteria and viruses. Because of versatility of antibodies, antibody based therapies may be developed against any pathogen. The serum therapy was firstly described in 1890. In the next years, antibodies were largely produced and used to control a wide range of infectious disease ***(Wang et al., 2010)***

Abu Yousuf et al., (2015) described a treatment technology called "Antibiotic Combined Hyperimmune Serum Therapy (ACHST)" for the treatment of PPR disease; they got 93.23 % success in infected goats. Hyperimmune serum and antibiotics combined with dexamethasone and metronidazole could be used to save the life of the infected goats.

So, this study aimed to prepare anti-PPRV hyperimmune serum in horses with evaluation of its efficacy to be used in emergency cases of PPR infection in sheep and goats.

Material and Methods

1. Animals:

1.1. Horses:

Three local breed male healthy horses of about 3- 5 years old; free from external and internal parasites; were used for preparation of anti-PPR hyper-immune serum.

1.2. Sheep

Twenty one local breed sheep of about 10- 12 months old; free from external and internal parasites and free from PPR antibodies as tested by SNT were used for testing prepared PPR antisera and vaccine.

2. PPR vaccine:

A locally live attenuated cell culture PPR vaccine, was prepared according to ***OIE (2008)*** in the Department of Rinder Pest Vaccine Research, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, it was used for preparation of antiserum in horses as well as for qualitative and quantitative antibody titration post vaccination.

3. Preparation of anti-PPRV hyperimmune serum according to the method described by Atanasin and Lepine, (1973).:

It was prepared in horses by subcutaneous (S/C) injection of multiplied doses of PPR vaccine (10^2; 10^3; 10^4,10^5 and 10^6 /horse) one week intervals up to 5 weeks.

Serum samples were obtained from all horses weekly after one week from 1st injection for 6 weeks.

4. Experimental design:

The twenty one sheep were divided into three groups as follow:

*__Group (1)__ included 9 sheep divided into three subgroups (3 sheep/ each) including subgroup 1-a, 1-b and 1-c were inoculated intravenously with 5, 10 and 15 ml of prepared anti-PPR hyper-immune serum / animal, respectively.

*__Group (2)__ contains 9 sheep divided into three subgroups (3 sheep/ each) including subgroup 2-a, 2-b and 2-c. such subgroups were vaccinated subcutaneously with PPR vaccine using the field dose (10^3 $TCID_{50}$/ animal) simultaneously with intravenous injection of 5, 10 and 15 ml of the prepared anti-PPRV hyper-immune serum, respectively.

***Group (3)** the rest 3 sheep were injected intravenous with 10 ml of normal saline and kept as non-immunized control.

5. Evaluation of the prepared antisera*:*

The prepared antisera were subjected to the quality control tests (freedom of foreign contaminants; safety and potency) according to ***OIE (2008).***

5.1. Serum neutralization test (SNT):

SNT was carried out using the micro-titer technique according to ***OIE (2008)*** to estimate the PPRV-neutralizing antibody titers in sera of immunized horses and sheep. The titer of PPRV serum neutralizing antibody titer was calculated as the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100 $TCID_{50}$ of PPRV according to ***Singh and Elcicy (1967).***

5.2. Indirect Enzyme linked immunosorbent Assay (ELISA):

PPRV antigen for ELISA was prepared according to standard operating procedures ***(FAO, 1994)*** and used for performing indirect ELISA using rabbit Anti-sheep IgG Peroxidase Conjugate supplied by (KPL), Gaithersburg, MD, USA.

Results

1-Titration of Prepared anti-PPRV hyperimmune serum:

Table (1) illustrated the titer of prepared anti-PPRV hyperimmune serum in horses as it showed a gradual increase in the antibody titer after the 1^{st} week of immunization as it started with 32 units and reached to 1024 units two weeks after the last injection (Table 1).

2. Quality control testing of the prepared anti-serum:

The prepared horse anti-PPRV hyperimmune serum was free from foreign contaminants (aerobic and anaerobic bacteria; fungi and mycoplasma) and safe when inoculated in sheep showing no significant local or systemic reactions or deaths.

3. Potency of the prepared PPR antisera in sheep:

1-Duration of induced passive immunity in sheep induced by the

prepared anti-PPRV hyper-immune serum:

Monitoring of passive PPR immunity induced in sheep as measured by SNT revealed that inoculation of 5, 10 and 15 ml of the prepared anti-PPR serum provided sheep with PPR geometric mean neutralizing antibody titers of 64; 53.3 and 85.3 respectively at the first day post immunization and remained stable to the 2nd week then began to decrease gradually by the 3rd week as it gave titers 26.6, 32 and 32 respectively then reached the lowest titers at the 5th week as it gave titers 4.6, 6.6 and 5.3 respectively as shown in table (2).

ELISA results **(Table 3)** parallel to those of SNT recorded the mean values of 1.373; 1.893 and 2.865 at the 1st day post immunization in sheep inoculated by 5, 10 and 15 ml of the prepared serum respectively then began to decrease by the 2nd week to reach their lowest value (0.422; 0.647 and 0.060 respectively) by the 5th week later.

Sheep received anti-PPR serum and vaccine exhibited serum neutralizing antibody titers began with a value of 32 by the 1st day in all sheep groups recording the highest levels (64) by the 2nd week then began to decline by the 4th week reaching the lowest level (16) by the 5th week post immunization **(table 4)**.

ELISA showed that sheep inoculated with 5, 10 and 15 ml of anti-PPR serum with the vaccine exhibited titers of 0.065 for all subgroups at the 1st day then increased to 2.022; 1.119 and 2.633 respectively at the 3rd week then declined to 1.417; 0.770 and 2.296 by the 5th week as tabulated in table (5).

Table (1): *PPR serum neutralizing antibody titer in immunized horse serum*

weeks post immunization	PPR SN antibody titer* in serum		
	Horse 1	Horse 2	Horse 3
Pre-immunization	0	0	0
1	32	32	64
2	64	64	128
3	128	128	128
4	256	256	256
5	512	512	512
6	1024	1024	1024

* **PPR antibody titer =** the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100 $TCID_{50}$ of PPRV.

Table (2): *Duration of induced passive immunity in sheep inoculated with the prepared anti-PPRV hyperimmune serum as measured by SNT*

Sheep groups	Sheep No.	PPR SN Antibody titer* / Days and weeks post inoculation							
		0	1DPI**	4DPI	1WPI***	2WPI	3WPI	4WPI	5WPI
Subgroup 1-a received 5 ml	1	0	64	64	64	64	32	16	8
	2	0	64	64	64	64	32	8	4
	3	0	64	64	64	64	16	8	2
GMT****		0	64	64	64	64	26.6	10.6	4.6
Subgroup 1-b received 10 ml	4	0	32	64	64	64	32	16	8
	5	0	64	64	64	64	32	16	8
	6	0	64	64	64	32	32	16	4
GMT****		0	53.3	64	64	53.3	32	16	6.6
Subgroup 1-c received 15 ml	7	0	128	128	128	64	32	16	8
	8	0	64	64	64	64	32	16	4
	9	0	64	64	64	32	32	8	4
GMT****		0	85.3	85.3	85.3	53.3	32	13.3	5.3

* PPR antibody titer = the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100 $TCID_{50}$ of PPRV.
**DPI= Days Post Immunization ***WPI= Week Post Immunization
GMT** = Geometric mean titer**

Table (3): *Duration of induced passive immunity in sheep using the prepared anti-PPRV hyperimmune serum as measured by ELISA*

Sheep groups	Sheep No.	PPR ELISA titer* / Days and weeks post inoculation					
		1DPI**	4DPI	2WPI***	3WPI	4WPI	5WPI
Subgroup 1-a received 5 ml	1	1.643	1.234	1.229	0.962	0.060	0.570
	2	1.156	1.172	1.167	0.855	0.060	1.047
	3	1.320	1.070	1.135	0.832	0.060	0.650
GMT****		1.373	1.159	1.177	0.883	0.060	0.422
Subgroup 1-b received 10 ml	4	2.121	1.246	1.643	1.046	0.763	0.443
	5	1.520	1.229	1.156	1.238	0.985	0.848
	6	2.050	1.100	1.320	1.114	0.870	0.650
GMT****		1.893	1.192	1.373	1.333	0.873	0.647
Subgroup 1-c received 15 ml	7	3.261	3.337	1.450	1.169	0.860	0.060
	8	2.868	2.560	2.987	1.331	0.764	0.060
	9	2.465	2.234	1.870	1.230	0.660	0.060
GMT****		2.865	2.710	2.102	1.243	0.761	0.060

N.B.: Control positive: 0.559 control negative: 0.350
*PPR-ELISA titer= expressed as optical density (OD) reading

**DPI= Days Post Immunization ***WPI= Week Post Immunization

GMT** = Geometric mean titer**

Table (4): *PPR serum neutralizing antibody titer in sheep receiving the prepared anti-PPRV hyperimmune serum with PPR vaccine as measured by SNT*

Sheep Groups	Sheep No.	PPR SN antibody titer* / Days and weeks post inoculation							
		0	1DPI**	4DPI	1WPI***	2WPI	3WPI	4WPI	5WPI
Subgroup 2-a received 5 ml with PPR vaccine	1	0	32	32	32	64	64	32	16
	2	0	32	32	64	64	64	32	16
	3	0	32	32	64	64	64	32	16
GMT****		0	32	32	64	64	64	32	16
Subgroup 2-b received 10 ml with PPR vaccine	4	0	32	32	32	64	64	32	16
	5	0	32	32	32	64	64	32	16
	6	0	32	32	32	64	64	32	16
GMT****		0	32	32	32	64	64	32	16
Subgroup 2-c received 15 ml with PPR vaccine	7	0	32	32	32	64	64	32	32
	8	0	32	32	32	64	64	32	32
	9	0	32	32	32	64	64	32	32
GMT****		0	32	32	32	64	64	32	32

* PPR antibody titer = the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100 $TCID_{50}$ of PPRV.

**DPI= Days Post Immunization ***WPI= Week Post Immunization

GMT** = Geometric mean titer

Table (5): *PPR serum ELISA antibody titer in sheep receiving the prepared anti-PPRV hyperimmune serum with PPR vaccine*

Sheep groups	SheepNo.	PPR-ELISA titer* / Days and weeks post inoculation					
		1DPI*	4DPI	2WPI**	3WPI	4WPI	5WPI
Subgroup 2-a received 5 ml with PPR vaccine	1	0.065	0.856	1.741	2.089	1.936	1.507
	2	0.065	0.783	1.650	1.989	1.785	1.372
	3	0.065	0.783	1.650	1.989	1.785	1.372
GMT****		0.065	0.807	1.680	2.022	1.835	1.417
Subgroup 2-a received 10 ml with PPR vaccine	4	0.065	0.763	0.907	1.180	0.877	0.856
	5	0.065	0.732	0.867	1.089	0.760	0.727
	6	0.065	0.732	0.867	1.089	0.760	0.727
GMT****		0.065	0.742	0.883	1.119	0.799	0.770
subgroup 2-a received 15 ml with PPR vaccine	7	0.065	0.985	1.360	2.765	3.365	2.375
	8	0.065	0.970	1.254	2.567	3.145	2.257
	9	0.065	0.970	1.254	2.567	3.145	2.257
GMT****		0.065	0.975	1.289	2.633	3.218	2.296

N.B.: Control positive: 0.559 control negative: 0.350

*PPR-ELISA titer= expressed as optical density (OD) reading
**DPI= Days Post Immunization ***WPI= Week Post Immunization
GMT**** = Geometric mean titer

Discussion

Antiserum is a serum containing antibody (ies) specific for one or more antigens obtained from an animal immunized either by injection of antigen or by infection with microorganisms containing antigen ***(Anon, 2012)***. It is used to confer passive immunity to that disease. Antisera do not provoke the production of antibodies. Serum that contains IgG against specified antigens could be used therapeutically. The present obtained results proved the success of preparation of anti-PPR serum in horses with high antibody titers (1024) (Table-1). In this respect, it was stated that polyclonal serum that contains demonstrable antibody or antibodies specific for one (monovalent or specific antiserum) or more (polyvalent antiserum) antigens; may be prepared from the blood of animals inoculated with an antigenic material or from the blood of animals and people who have been stimulated by natural contact with an antigen (as in those who recover from an attack of disease) in agreement with ***Anon (2012).*** Also ***Mupapa et al. (1999)*** stated that antiserum is blood serum containing polyclonal antibodies and is used to pass on passive immunity to many diseases. Antibodies in the antiserum bind the infectious agent or antigen. The immune system then recognizes foreign agents bound to antibodies and triggers a more robust immune response. The use of antiserum is particularly effective against pathogens which are capable of evading the immune system in the unstimulated state but which are not robust enough to evade the stimulated immune system. The existence of antibodies to the agent therefore depends on an initial "lucky survivor" whose immune system by chance discovered a counteragent to the pathogen, or a "host species" which carries the pathogen, but does not suffer from its effects. Further stocks of antiserum can then be produced from the initial donor or from a donor organism that is inoculated with the pathogen and cured by some stock of preexisting antiserum.

The present obtained results revealed that the prepared horse anti-PPR serum is free from foreign contaminants (aerobic and anaerobic bacteria; fungi and mycoplasma); safe inducing no local or systemic reactions in inoculated sheep and highly potent (with a titer of 1024 by SNT) as shown in table (1). These findings agree with the recommendations of *OIE (2012)* concerning the purity

and safety of veterinary biologics. Regarding the obtained PPR antibody titer in the prepared serum (1024); similar results were obtained by ***Appiah (1982); Diallo et al., (1989)*** and ***Mouaze et al., (1998)*** who concluded that preparation of PPR antiserum in large hosts (as goat and horses) is preferable producing larger amounts than those obtained from rabbits with similar suggestion of ***Khodeir and Daoud (2008)*** who used horses for preparation of rabies antiserum for post exposure passive immunization of farm animals.

Following up the levels of PPR antibodies in passively immunized sheep; SNT revealed that inoculation of 5, 10 and 15ml of the prepared anti-PPR serum inducing PPR mean neutralizing antibody titers of 64; 53.3 and 85.3 respectively by the first day post immunization and remained stable to the 2nd week then began to decrease gradually by the 3rd week to reach the lowest titers (4.6; 6.3 and 5.3 respectively) as shown in table (2). Also ELISA results (table-3) recorded the mean values of 1.373; 1.893 and 2.865 by the 1st day post immunization then began to decrease by the 2nd week post immunization to reach their lowest value (0.422; 0.647 and 0.060 respectively) by the 5th week later. Such antibody titers were found to be protective for sheep against PPR infection as reported by ***Taylor (1979)*** and ***Khodeir and Mouaz (1998).*** In this respect, ***Adu and Joannis (1984)*** showed that administration of hyperimmune serum to animals incubating the disease as in the early stage results in protection and recovery and ***Ihemelanadue et al. (1985)*** who used PPR hyperimmune serum in the control of PPR.

On the other side, it was found that sheep received anti-PPR serum and vaccine exhibited specific PPR antibodies began with a value of 32 by the 1st day post immunization in all sheep groups recorded the highest levels (64) by the 2nd week then began to decline by the 4th week reaching the lowest level (16) by the 5th week post immunization (table-4) with parallel ELISA results which showed titers of 0.065 at the 1st day post immunization recorded peak values of 2.022; 1.119 and 2.633 respectively by the 3rd week post immunization then declined to 1.417; 0.770 and 2.296 by the 5th week later (table -5). These findings could be explained by those of ***Taylor (1979)*** who assumed that small ruminants vaccinated with rinderpest vaccine and hyperimmune serum simultaneously would develop a durable immunity without triggering off clinical disease and ***Adu and Joannis (1984)*** used serum-virus simultaneous method for overcome PPR infection in sheep. Also it is well known that passive immunity lasts for few days or weeks.

Depending on the obtained results, it could be concluded that horse anti-PPR serum is of a significant importance and can help to protect and control PPR infections especially in case of outbreaks that need rapid management.

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تحضير مصل عالي العيارية ضد طاعون المجترات الصغيرة لإستخدامه فى الحالات الاضطراريه

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الملخص العربي

تم خلال هذا العمل تحضير مصل مناعى عالى العيارية ضد طاعون المجترات الصغيرة فى الخيول بمعيار 1024 وحدة طبقا لاختبار المصل المتعادل وقد وجد أن هذا المصل خالى من الملوثات البكتيرية والفطرية والميكوبلازما و ذو أمان حيوى عند حقنه فى الأغنام.

ولاختبار كفاءة هذا المصل المناعية تم حقن مجموعة من الأغنام و تم قياس المناعة السلبية باستخدام اختباري المصل المتعادل و الاليزا و وجد استمرار تواجد الأجسام المناعية لمدة تصل الي خمسة أسابيع. علي الجانب الأخر تم حقن مجموعة من الأغنام المصل المحضر و لقاح طاعون المجترات الصغيرة في نفس الوقت و قد أعطت نتائج مماثلة للمجموعة السابقة مع ارتفاع بسيط في تركيز الأجسام المناعية. و بناء علي ذلك فإن المصل المناعى عالى العيارية المحضر في الخيول ضد طاعون المجترات الصغيرة ذو أهمية فعالة و يمكن استخدامه في الوقاية و السيطرة علي مرض طاعون المجترات الصغيرة خاصة في حالات الوباء التي تحتاج تدخل سريع.