



## Efficiency of Three PPRV Vaccines Commercially Available in Turkey

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**Abstract:** The efficiencies of three different commercially available Peste des petits ruminants virus (PPRV) vaccines were evaluated on the basis of the antibody response surveyed by competitive enzyme-linked immunosorbent assay (c-ELISA) and the results were confirmed by virus neutralization test (VNT) in sheep and goats. The study was conducted during the period from June 2010 to January 2011 to determine the antibody response against three different commercially available homologous PPR (Nigeria 75/1) vaccines. A total of 150 sheep and 50 goats of both genders were divided into three groups, as Group 1, 2 and 3. Each group was inoculated one out of three vaccines. A total of three samples of were collected three times from all the groups at pre- (day zero) and post-vaccination (18<sup>th</sup> days and 8<sup>th</sup> months). The results indicate that treated goats developed antibodies after the vaccination, while the most of sheep did not have any antibodies for PPRV.

**Key words:** Goat, PPRV, Sheep, Turkey, Vaccine.

### Türkiye`de Ticari Olarak Kullanılan Üç Farklı PPRV Aşısının Etkinliği

**Özet:** Koyun ve keçilerde ticari üç farklı küçük ruminant vebası virus (PPRV) aşısının etkinliği antikor cevabına dayalı bir metot olan competitive enzyme-linked immunosorbent assay (c-ELISA) ile kontrol edildi ve sonuçlar virus nötralizasyon testi (VNT) ile doğrulandı. Söz konusu çalışma, Haziran 2010 ile Ocak 2011 tarihleri arasında yapıldı. Her iki cinsiyeti içeren 150 koyun ve 50 keçiden oluşan çalışma grubu, Grup 1, 2 ve 3 olmak üzere üç gruba ayrıldı. Her gruba farklı bir aşı uygulaması yapıldı. Serum örnekleri aşılama öncesi (0. gün) ve aşılama sonrasında (18. gün ve 8. ay) olmak üzere toplam üç kez alındı. Araştırma sonuçlarına göre, keçilerde PPRV'ye karşı antikor cevabı şekillenirken, koyunların çoğunda antikor cevabınının şekillenmediği tespit edildi.

**Anahtar kelimeler:** Keçi, PPRV, Koyun, Türkiye, Aşı.

## INTRODUCTION

**P**este des petits ruminants (PPR) disease is a severe fast spreading disease of mainly domestic small ruminants caused by PPR virus (PPRV) that belongs to morbillivirus genus of *Paramyxoviridae* family. Highly pathogenic viruses such as rinderpest, canine distemper, measles and marine mammal viruses are closely related members of the genus (Barrett et al., 2005). The disease is characterized by sudden onset of depression, fever, discharges from the eyes and nostrils, sores in the mouth, disturbed breathing and cough, foul smelling diarrhoea and death (Diallo, 1988; Albayrak and Alkan, 2009; Ozmen et al., 2009).

The disease is considered as one of the major threats to small ruminant production in its endemic regions (Anonymous, 2009). Until the mid-1980s, PPR was regarded as a disease of West African countries. From that period onwards, the known distribution of PPR has progressed towards the Middle East, Iran, the Indian sub-continent, Turkey and, recently some countries in Central Asia (Kwiątek et al., 2007; Albayrak and Alkan, 2009).

As there is no suitable antiviral therapeutic schedule to protect the animals against this disease, vaccination is the only option for controlling the PPR. The usual practice in Turkey is to use homologous vaccines. The main disadvantage of this vaccine, like other Morbillivirus vaccines, is its poor thermal stability (Siddique et al., 2006). A campaign for PPR vaccination commenced, by Turkish government, in 2010, but some of the sheep have been diagnosed with PPR, even though they had been vaccinated previously. This brought up the question of the efficiency of vaccines. Official Veterinary staff performed all the vaccine administrations (unpublished data). The study was conducted in Samsun, a city in Blacksea region (41° 17' N, 36° 20' E). Previous studies have showed that the disease is commonly seen in this region (Albayrak and Alkan, 2009).

Therefore, the aim of this study was to investigate the efficiency of three commercial PPR vaccines in goats and sheep flocks in northern Turkey.

## MATERIALS and METHODS

### Animals

In this study, local flocks, consisting of both sheep and goats, were used. Overall, 200 animals were involved in the study (150 sheep and 50 goats). The mean age of the animals at the beginning of the study was 36 months (min.-max.=1-5 years). The breeds of sheep were the local Karayaka breed, while goats were Saanen and hair goat breeds.

### Immunisation of Animals

The vaccination of animals was carried out on a private farm in a village of Samsun, Turkey. The immune response of PPR vaccine was studied on 150 sheep and 50 goats on the 18<sup>th</sup> day. However, due to the reasoning of farm owner selling out some of the animals during the period between the first and second studies, the second study was performed on a lesser sheep population (n=73). Both sheep and goats were divided into three groups according to the vaccine types used. Group 2 and 3 contained 50 sheep and 20 goats while group 1 contained 50 sheep and 10 goats. The flocks of sheep and goat were vaccinated at different times. All the flocks were vaccinated with same serial, but different vial of vaccine given at the same time. There are only three PPR vaccines commercially available in Turkey. All the three vaccines are lyophilised vaccines containing attenuated PPR vaccine strain Nig 75/1 grown on the Vero cell line. While the vaccine used for the 1<sup>st</sup> and 2<sup>nd</sup> group had a minimum of 10<sup>2.5</sup> TCID<sub>50</sub> (tissue culture infected dose) of the virus for each dose, the vaccine given in the 3<sup>rd</sup> group had it at 10<sup>3.0</sup> TCID<sub>50</sub> dose. All the three vaccines contained 10 % sucrose and 5 %

lactalbumin hydrolysate. Blood samples of each animal were collected prior to the vaccination and on the 18<sup>th</sup> day after vaccination and lesser number of animals were also sampled on the 8<sup>th</sup> month after vaccination. Blood samples were taken from the *V. jugularis*. Blood tubes (without EDTA) were centrifuged at 3,000 × *g* for 10 min, and the samples were transferred into sterile tubes and stored at –20 °C until being used.

### C-ELISA

Samples were examined for hemagglutinin protein (H) of PPRV using specific PPRV monoclonal antibody-based c-ELISA (Biological Diagnostic, UK). The ELISA was performed according to the manufacturer's instructions, as described elsewhere (Anderson et al., 1991). Briefly, we used 50 ml of each reagent in each step of the test. Incubation was performed at 37 °C for 1 h on an orbital shaker (Heidolph Titramax, Kelheim, Germany) in each step, and the plates were washed three times after each. Microtiter plates (Nunc, Roskilde, Denmark) were coated with 1/100 dilutions of PPRV antigens in phosphate-buffered saline (pH 7.4). Then, 1:5 dilutions were made and control sera added. The dilution was made by adding 10 ml of serum following the addition of 40 ml of blocking buffer (0.1 % v/v Tween-20 and 0.3 % v/v negative anti-PPRV serum). Afterwards, 50 ml of a 1/100 dilution of the reconstituted pre-titrated monoclonal antibodies (Mab) was added. Mab control wells received 50 ml at the same concentration in addition to 50 ml blocking buffer. Conjugate control wells received 100 ml blocking buffer and 50 ml of 1/1000 dilution of rabbit anti-mouse horseradish peroxidase conjugate. Freshly prepared orthophenylenediamine (OPD) containing 0.004 % v/v hydrogen peroxide was added, and plates were incubated at room temperature for 10 min before the reaction was terminated by adding 50 ml 1M sulphuric acid. The plates were read by a spectrophotometer (Biotech ELX50, CA, USA) at 492 nm. Optical Density (OD) values were calculated to

determine percentage inhibition of a given Mab using the formula, as follows: % inhibition=100 X (OD of sample/OD of control).

### Virus Neutralisation Test (VNT)

A field isolate of PPRV (Lineage 4) was obtained from the Virology Department of the Faculty of Veterinary Medicine, Ankara University (GenBank no. AF384687.1); a Vero cell line maintained with Dulbecco's Modified Essential Medium containing 5 % fetal calf serum was used for virus propagation and neutralisation tests. The VNT was performed in 96-well plates. All sera samples were mixed in equal volumes of 100 TCID<sub>50</sub> dilution of PPRV in four wells for each sample and were incubated for one h at 37 °C. Then, the cell suspension was added into each well. The plates were observed daily for up to five days for morphological changes using an inverted microscope (Olympus CKX 41, Japan). Positive control serum was PPR antiserum (CIRAD-EMVT, Montpellier, France).

## RESULTS

In sheep, in all three groups, few sheep had antibodies for PPRV prior to vaccination [2/50 in group 1 and 3, 1/50 in group 2]. On the 18<sup>th</sup> day post-vaccination, antibodies were detected from only five additional animals in the 1<sup>st</sup> group. In the 2<sup>nd</sup> group however, this number was only two, while 19 animals had antibodies in the 3<sup>rd</sup> group. At the 8<sup>th</sup> month post-vaccination, six out of 28 sheep in the 1<sup>st</sup> group and four out of 22 sheep in the 2<sup>nd</sup> group had antibodies and, as expected, the highest number of animals with the antibodies was in the 3<sup>rd</sup> group, with 10 out of 23.

In goats, only one goat had antibodies prior to the vaccination in the 3<sup>rd</sup> group. However, on the 18<sup>th</sup> day post-vaccination, all goats developed antibodies in the 2<sup>nd</sup> and 3<sup>rd</sup> groups, while seven out of 10 goats had antibodies in the 1<sup>st</sup> group. At the 8<sup>th</sup> month post-vaccination, antibodies were detected

from all goats from the 2<sup>nd</sup> and 3<sup>rd</sup> groups, as expected, and in the 1<sup>st</sup> group only one goat did not have antibodies (Table 1). All positive serum samples were double-checked and confirmed by the VNT.

## DISCUSSION

Many serologic test methods have been used for the diagnosis of PPR, such as c-ELISA and VNT (Gür and Albayrak, 2010). The c-ELISA has a higher specificity (98.4 %) and sensitivity (92.4 %) than the VNT. The sensitivity of c-ELISA for PPRV infection increases up to 95.4 %, if the target population is not vaccinated (Singh et al., 2004).

The results of this study clearly indicated that the three homologous PPRV vaccines commercially used in Turkey do not constitute antibody response in all sheep, except for goat flocks. Similar studies have been conducted previously on the efficiency of the PPRV vaccines that were the counterparts of

vaccines used in our study. However, the results of those studies are not consistent with the present results. For instance, a study from Pakistan showed that sheep and goats both had 100 % of antibody response to the vaccine (Intizar et al., 2009). According to another study from India, the PPR vaccine could have protected its potency for 14 days even though it was kept in room temperature and it could still have caused antibody response in goats (Siddique et al., 2006). Our study revealed some possibilities such that there could be noticeable differences in protective potential of the vaccines from different serials and vials. Moreover, differences between the antibody response in sheep and goats introduced the idea of a further study warranted in this area. Undoubtedly, the result of seroprevalance studies might be influenced by many other factors, such as the number of animals sampled, their age, time of sampling, conditions of care and feeding, individual differences and so on.

**Table 1.** The proportion of the number of animals with antibody response against the number of vaccinated animals  
**Tablo 1.** Antikor cevabı şekillenen hayvanların aşılanan hayvanlara oranı

	Group 1			Group 2			Group 3		
	Day 0 (%)	Day 18 (%)	8 <sup>th</sup> month (%)	Day 0 (%)	Day 18 (%)	8 <sup>th</sup> month (%)	Day 0 (%)	Day 18 (%)	8 <sup>th</sup> month (%)
Goats	0/10 (-)	7/10 (70.00)	9/10 (90)	0/20 (-)	20/20 (100)	20/20 (100)	1/20 (5.00)	20/20 (100)	20/20 (100)
Sheep	2/50 (4.00)	7/50 (14)	6/28 (21.42)	1/50 (2.00)	2/50 (4.00)	4/22 (18.18)	2/50 (4.00)	19/50 (38)	10/23 (43.47)
Total	2/60 (3.33)	14/60 (23.33)	15/38 (39.47)	1/70 (1.42)	22/70 (31.42)	24/42 (57.14)	3/70 (4.28)	39/70 (55.71)	30/43 (69.76)

Although the vaccines evaluated in this study had higher immunising doses ( $10^{2.5}$  and  $10^{3.0}$  TCID<sub>50</sub>) than the minimum effective dose for PPRV vaccine ( $10^{0.8}$  TCID<sub>50</sub>), the present trials did not lead to efficient antibody response in sheep flocks.

As with all members of the family *Paramyxoviridae*, PPRV is highly heat-sensitive and this is a serious drawback to the efficient use of the live attenuated vaccine in endemic areas, having hot climatic environments. In addition, these regions

usually have poor infrastructures and it is thus difficult to maintain a cold chain to ensure the preservation of vaccine potency. Our vaccine trials were carried out in goat flock in April and in sheep flock in June. Due to seasonal differences, the results of this study may have been affected, more or less, by the climatic conditions. Because of heat-sensitive feature of lyophilised PPR live vaccines, it could be inactivated during the vaccination. This shortcoming nature of the vaccine was overcome by

Worwall et al. (2001) through the development of a thermo-tolerant vaccine, freeze-dried in the presence of a cryoprotectant containing trehalose. Under these production conditions, the vaccine could remain stable at 45 °C for 14 days with a minimal level of loss of its potency. The PPR control will benefit greatly from the use of this thermostable form of the attenuated vaccine. Recently, a similar vaccine has been produced in India, using a local strain of PPRV (Sarkar et al., 2003). Moreover, our results indicate that, PPR vaccines commercially available in the market have not being adequately controlled by responsible institutions in Turkey. Therefore, desired vaccination results against the PPR could not be achieved for the time being.

Additionally, there have been several studies on developing new PPRV vaccines. It was recently reported that the hemagglutinin (H) and fusion (F) protein genes of several morbilliviruses could be expressed in various vector systems and they might be used as effective sub-unit vaccines (Diallo et al., 2007). Furthermore, a new study is in progress on a DIVA vaccine (Baron et al., 2011), which would be a further advantage for the endemic areas like our country. As highlighted by Diallo et al. (2007), the current vaccination against the rinderpest virus in cattle has been ceased that makes the PPRV infection even more serious, due to the fact that PPR could also infect this species.

Conclusively, the results of our study dictate clearly that the control of commercial vaccines and vaccination strategy have to be evaluated by official Veterinary authorities immediately to combat the PPR infection, since it was first reported 15 years ago in Turkey, yet threatening severely the population of small ruminants concerned.

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