

Evaluation of immune response of small ruminant flocks to ovine Pasteurellosis and Peste Des Petits Ruminants vaccines in North Shewa, Ethiopia

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Abstract

The present study was conducted to evaluate the flock immune response of small ruminants to ovine pasteurellosis Bio type A and Peste des petits ruminants (PPR) vaccines and to determine their effects on the morbidity and mortality of animals due to ovine pasteurellosis and PPR from October 2017 to November 2019 in selected districts of the north Shewa Zone, Ethiopia. A total of 553 serum samples were collected and examined by using the Competitive Enzyme-Linked Immunosorbent Assay and an indirect haemagglutination inhibition test for PPR and ovine pasteurellosis antibodies, respectively. Based on the results, the number of animals with an antibody titer of 1:10 after 28 days and six months of vaccinations of *P. multocida* Biotype A was 161 (93.1%) and 152 (81.7%), respectively. Also, the positive antibody level (i.e., percent of colour inhibition PI > 50) after 28 days and 12 months of PPRV vaccinations was 82 (86.3%) and 69 (69.7%), respectively. There was a statistical difference ($p < 0.001$) between the antibody levels after 28 days and six months of vaccination against ovine pasteurellosis and after 28 days and 12 months of vaccination ($p < 0.01$) for PPRV vaccines. During three consecutive years of intervention, out of a total of 2350 study animals, 500 cases (21.3%) showed respiratory signs, out of which 45 (1.9%) died. The PPR and ovine pasteurellosis vaccines applied in the field were effective in developing antibodies above the threshold herd immunity level of 80% and 70% respectively. Periodic vaccination against ovine pasteurellosis and PPR according to the epidemiology of the diseases results in a significant reduction in morbidity and mortality of small ruminants. Therefore, the current ongoing efforts to control ovine pasteurellosis and PPR of small ruminants through vaccination should be encouraged and strict sero-surveillance and monitoring of these diseases should be done side by side.

Keywords: Immune response, Ovine Pasteurellosis, Peste Des Petits Ruminants, Vaccination

Introduction

Respiratory disease in small ruminants is complex, occurring as a result of host interaction with various infectious agents under the influence of environmental factors (Lacasta et al., 2008; Brogden et al., 1998). Various etiological agents contribute to the respiratory disease complex. Though one agent may be the primary invader, most respiratory infections are complicated by the presence of secondary or opportunistic organisms, and mixed infections are also a common phenomenon (Kumar et al., 2013; Scott, 2011; Garedew et al., 2010; Kumar et al., 2000).

Ovine pasteurellosis is a respiratory infection caused by *Mannheimia haemolytica*, which exists in two biotypes, A and T. These biotypes are further divided into serotypes based on their surface antigen. It has two main disease types in sheep and goats: pneumonic pasteurellosis and septicaemic pasteurellosis (Rad et al., 2011; Donachie, 2000). Biotype A is particularly associated with pneumonic pasteurellosis in sheep, whereas biotype T causes septicaemic pasteurellosis in lambs (Gilmour et al., 1991; Gilmour and Gilmour, 1989). All serotypes can be involved in pneumonic pasteurellosis in sheep, but serotype A2 is the most commonly isolated serotype from cases of ovine pneumonic pasteurellosis in the United Kingdom, Germany, and the United States (Davies et al., 1997).

A Peste des petits ruminant (PPR) is one of the diseases of major economic importance and imposes a significant constraint upon sheep and goat production owing to its high mortality rate. In the worst situations, PPR-related morbidity can reach 100%, with a mortality rate that can reach 90% (OIE, 2020). However, reported morbidity and mortality rates have varied between 90–100% and 50–100%, respectively (OIE, 2009). It is an acute, highly contagious, and frequently fatal disease of sheep and goats caused by the PPR virus (PPRV), a member of the genus *Morbillivirus* of the family *Paramyxoviridae* (Zahur et al., 2009).

The respiratory disease complex, mainly in the form of pneumonia, has been noted to be the most prominent infectious cause of mortality both on-farm and on-station in Ethiopian highland sheep (Mukassa-Mugerwa et al., 2000; Bekele et al., 1992). A few studies were conducted to investigate the underlying causes of the respiratory disease complex (Fentie and Zerihun, 2012; Garedew et al., 2010; Ayelet et al., 2004; Tibbo et al., 2001; Bekele et al., 1992), involving multiple agents such as bacteria (*Pasteurella*, *Mannheimia*, *Chlamydia*, *Mycoplasma* species, etc.), viruses (PPR, Parainfluenza-3 virus, Maedi-visna, etc.) and lungworms (*Dictyocaulus fillaria*, *Muelleris capillaries* and *Protostrongylus refuscence*). The predisposing factors are mainly environmental stresses, including weather fluctuations and

feed shortages, usually complicated by inadequate management and husbandry practices (Bekele et al., 1992).

The participatory epidemiological survey conducted by ILRI systematically described the impact of respiratory diseases on small ruminants in Ethiopia in much more detail (Alemu et al., 2019; Wieland et al., 2016), showing that farmers consider respiratory diseases the highest priority among small ruminant diseases. Ovine Pasteurellosis and PPR were identified as priority diseases that must be controlled due to their economic implications (OIE, 2020; OIE and FAO 2015; Mohamed and Abdelsalam, 2008).

During the past decades, PPR was reported in different localities in Ethiopia with different prevalence's: 46.53% in goats of the southern parts of the Tigray Region (Afera et al., 2014); 28.1% in unvaccinated small ruminants of the Eastern Amhara Region bordering Afar (Biruk, 2014); 48.43% in East Shewa and Arsi Zones, Oromia Region (Getachew et al., 2017); and Gebresilassie et al. (2021) reported a 12.9% prevalence in Dello Mena and Madda Walabu Districts of Bale Zone, South Eastern Ethiopia.

Various management measures to prevent or treat respiratory diseases are important, including the use of vaccines and antibiotics. The most economical and feasible control method for these diseases in developing countries like Ethiopia is the use of vaccines (Jones et al., 2016; Maru et al., 2013). Despite biannual vaccination against ovine Pasteurellosis with a monovalent vaccine (inactivated *P. multocida* biotype A) and annual vaccination against PPR, there are high rates of mortality and morbidity associated with respiratory problems, and farmers and animal health experts have complaints, particularly about the effectiveness of the ovine Pasteurellosis vaccine in community-based breeding programs and small ruminant health monitoring villages in North Shewa. Therefore, the aims of this study were to evaluate the immune responses of small ruminant flocks to ovine Pasteurellosis Bio type A and Peste des petits ruminants (PPR) vaccines (thermo-labile live attenuated) and to determine their effects on the morbidity and mortality of animals.

Materials and methods

Study areas

The study was conducted from October 2017 to November 2019 at on-farm (Efratana-gidim, Kewet, Menz Gera, and Menz Mama Districts) and on-station at the Ataye Boer Nucleus Site of the Debre Birhan Agricultural Research Center (DBARC). Menz Mama and Menz Gera are found in the central highlands of the country at distances of 254-360 kilometres from Addis Ababa, respectively. Geographically, the areas lie between 09°35'45" and 10°18'0" N

latitude and 39°29'40" to 39°40'0" E longitude, with an average elevation range of 2800 to 3200 meters above sea level and a mean annual temperature range of 12.2 to 19.9°C. The average annual rainfall of the areas ranges from 897.8 to 1149mm, and it is characterized by a bimodal pattern with a cold, harsh climate that occasionally has frost, particularly between November and January (Menz Gera and Menz Mama Livestock Offices, 2014).

Kewet and Efratana-gidim are located 200 to 250 km from Addis Ababa. Geographically, the areas are located at 10°21'0" N and 39°55'60" E with average elevation ranges of 1280-1,468 meters above sea level. The temperature ranges from 25.4-27.0 °C; June is the hottest month of the year. December has the lowest average temperature of the year. The climate is characterized by bimodal rainfall consisting of the long rainy season (June-September), short rainy season (February-May), and dry season (October-January). In a year, the average rainfall is 1085 mm (Fekadu, 2015).

Study animals and their management

The animals used for this study were sheep and goats. Under smallholder farm conditions, animals were managed under an extensive system; they spent all day on grazing pasture on fallow lands, were fed crop residues, usually with no extra-supplement, and were sheltered during the night. They were vaccinated against sheep and goat pox, contagious caprine pleuropneumonia (CCPP), PPR, and ovine pasteurellosis diseases. Moreover, these animals were treated using anthelmintic drugs that included albendazole, oxclozanide, tetramisole, ivermectin, and triclobandazole. The drugs were applied based on the manufacturers' recommendations.

On-station, animals were managed in a semi-intensive production system; they were supplemented with extra feed sources in addition to grazing. The supplement includes *adlibitum* grass hay, chopped pasture (Napier grass, *Desmodium species*, and vetch) and commercial concentrate. They were vaccinated against sheep and goat pox, CCPP, PPR, and ovine pasteurellosis diseases. Moreover, these animals were treated using anthelmintic drugs that included albendazole, oxclozanide, tetramisole, ivermectin, and triclobandazole. Also, animals were sprayed against ectoparasites using diazinon (60%) and amitrazine (12.5%). Regular vigilance was performed by animal health and production experts to ensure feeding, herd health care, proper breeding, and cleanliness of the farm.

Study designs

The strategy for implementation of vaccination against ovine Pasteurellosis Biotype A and PPR to evaluate their impact on animals' morbidity and mortality was divided into two components:

Vaccination of animals: The specific targets were to improve the handling, administration, timing, and coverage compared to the usual practice and to follow a holistic approach. The types of vaccines used were ovine Pasteurellosis (*P. multocida* biotype A; two times per year) and PPR (once per year), which were produced by the National Veterinary Institute, Ethiopia. The vaccines were administered through a sub-cutaneous (SC) route around the lateral cervical vertebrae.

Longitudinal investigation of cases: a clinical study was conducted in two purposively selected model sheep breed improvement villages, *Sinambanaboda* from Menz Gera and *Keyafer* from Menz Mama District. There were intensive surveillance and longitudinal monitoring systems for the occurrence of diseases in the small ruminant flock to investigate the impact of preventive measures in providing protection from the diseases and to clarify the causes of diseases. Clinical diagnosis was made on the basis of clinical manifestations and their probable signs. This was done to find which system was affected and causing health disturbances. As respiratory diseases, clinical cases of pneumonia, cough, severe to mucopurulent nasal discharge, pasteurollosis, and any respiratory embarrassment were classified.

Sampling and serological examination

A total of 553 (359 for ovine Pasteurellosis and 194 for PPR) serum samples were collected from sheep and goats to evaluate the flock immune response of small ruminants to ovine Pasteurellosis Bio type A and Peste des petits ruminants (PPR) vaccines from four districts of north Shewa, Ethiopia.

Serological examinations

Sampling had two consecutive phases, i.e., for ovine Pasteurellosis, the samples were collected after 28 days and six months of vaccination. For PPR, the samples were collected after 28 days and 12 months of vaccination. The samples were collected from the same flocks of vaccinated animals.

Blood samples were collected from the jugular vein of randomly selected (with systematic random sampling technique) small ruminants above six months of age. The collected blood samples were allowed to clot for 1-2 hrs at room temperature, then stored horizontally overnight at 4°C and finally, the serum was separated from the clot. The separated serum was transferred to new cryo-vials, labelled, transported to the laboratory using a cold chain and stored at -20°C until processed and upon arrival. The serum samples were centrifuged to remove the remaining red blood cells before being analysed.

Ovine Pasteurellosis

The samples were examined using an indirect haemagglutination inhibition (IHA) test for ovine Pasteurellosis. The IHA test was conducted according to the manufacturer's instructions. Based on the manufacturer's recommendation, a titer greater than or equal to 1:10 was taken as positive. The laboratory tests were conducted at the National Veterinary Institute (NVI), Ethiopia.

Peste des petits ruminants (PPR)

Peste des petits ruminants (PPR) laboratory tests were conducted at the National Animal Health Diagnostic and Investigation Centre (NAHDIC) in Sebeta, Ethiopia. A monoclonal antibody (MAb) based competitive enzyme linked immunosorbent assay (cELISA) (OIE, 2013) was used for the detection of antibodies directed against the nucleoprotein of the PPR virus using an approved competitive ELISA kit. The optical density (OD) was recorded at 450 nm using a microplate reader. The sensitivity and specificity of the competitive ELISA test kit as provided by the manufacturer was 95.4% and 98.4%, respectively. The cut-off points were calculated as percentage inhibition (S/N) from the optical densities (OD), as described by Libeau et al. (1995):

$$S/N (\%) = (OD_{\text{test sample}}/OD_{\text{blank}}) \times 100$$

Where OD_{blank} is the OD of the negative control,

Test samples that showed S/N values of less than or equal to 50% were considered positive, those with S/N values of > 50% but less than or equal to 60% were considered doubtful, while test samples that showed S/N values above 60% were considered negative.

The same procedure was used in this study to convert the OD values to percentage inhibition for PPR detection by using the following formula, as described by Libeau et al. (1995):

$$PI = [100 - (OD_{\text{sample}} / OD_{\text{NC}})] \times 100$$

All sera with a percentage inhibition (PI) of greater than 50% were considered positive. The percent of color inhibition indicated using a c-ELISA provided an indirect measure of antibody levels in the serum samples (Singh et al., 2004).

Data management and analysis

Data collected during sampling and laboratory results were entered into an MS-Excel spreadsheet and analyzed by using SPSS software (version 20). Descriptive statistics were used to determine the antibody titer of ovine Pasteurellosis and the percent color inhibition of PPR after 28 days, six months and 12 months of vaccinations, in small ruminants, respectively. A Chi-square (χ^2) test was used to measure the level of association between the protective antibody developments after 28 days and six months of vaccinations for ovine Pasteurellosis and also after 28 days and 12 months of vaccinations for PPR. A significance level ($p < 0.05$) was set to determine the presence or absence of a statistically significant difference between the given parameters.

Results

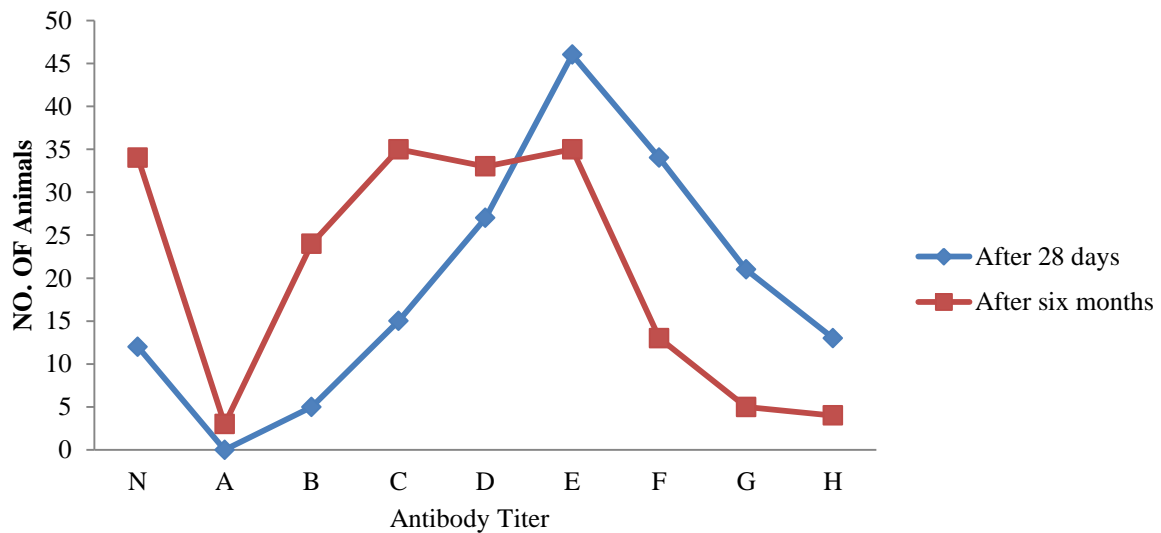
The level of protective antibody titer against ovine pasteurellosis after 28 days of vaccinations, was 161 (93.1%), while after six months of vaccination the antibody titer in response to *P. multocida* Bio-type A vaccine was 152 (81.7%). There was a significant difference ($p = 0.001$) between these two antibody titers. Small ruminant flock immunity response against ovine pasteurellosis after 28 days and six months of vaccination was above the threshold level of 70% (Table 1).

The level of antibody against PPRV after 28 days of vaccination was 82 (86.3%), while after 12 months of vaccination, the antibody level in response to the PPR vaccine was 69 (69.7%). There was a significant difference ($p = 0.001$) between the antibody response after-28 days and 12 months of vaccination antibody response against the PPR vaccine. The immune response against the PPR vaccine after 28 days and 12 months of vaccination was above the threshold level of 80% (Table 1).

Table 1. A comparison of antibody titers ($\geq 1:10$) in response to *P. multocida* Bio-type A and PI > 50% of PPR Vaccination in the North Shewa Zones of Ethiopia

Variables	No. Examined	No. animals $\geq 1:10$ (%)	X^2 -value	<i>p</i> -value
Ovine Pasteurellosis Biotype A vaccine				
After 28 days of vaccination	173	161 (93.1)	10.323	0.001*
After 6 months of vaccination	186	152 (81.7)		
Total	359	313 (87.2)		
Peste des petits ruminants (PPR) vaccine				
After 28 days of vaccination	95	82 (86.3)	7.761	0.005*
After 12 months of vaccination	99	69 (69.7)		
Total	194	151 (77.8)		

Figure (1) shows the frequencies of antibody titer responses of small ruminants to the *P. multocida* Bio-type A vaccine. After 28 days of vaccination, 81% of the animals were above 1:80 titers, while after six months of vaccination, most of the animals (55.3%) antibody response to vaccine was within the range of 1:40 - 1:160.



Note: N = Negative, A = 1:10, B = 1:20, C = 1:40, D = 1:80, E = 1:160, F = 1:320, G = 1:640, and H = 1:1280

Figure 1. Antibody titer response of small ruminants to *P. multocida* Bio-type A vaccine. During three years of intervention, 500 (21.3%) cases from a study population of 2350 showed respiratory problems, with 45 (1.9%) animals dying. Relatively, the highest and

lowest respiratory problems were recorded from July to September and January, respectively (Figure 2).

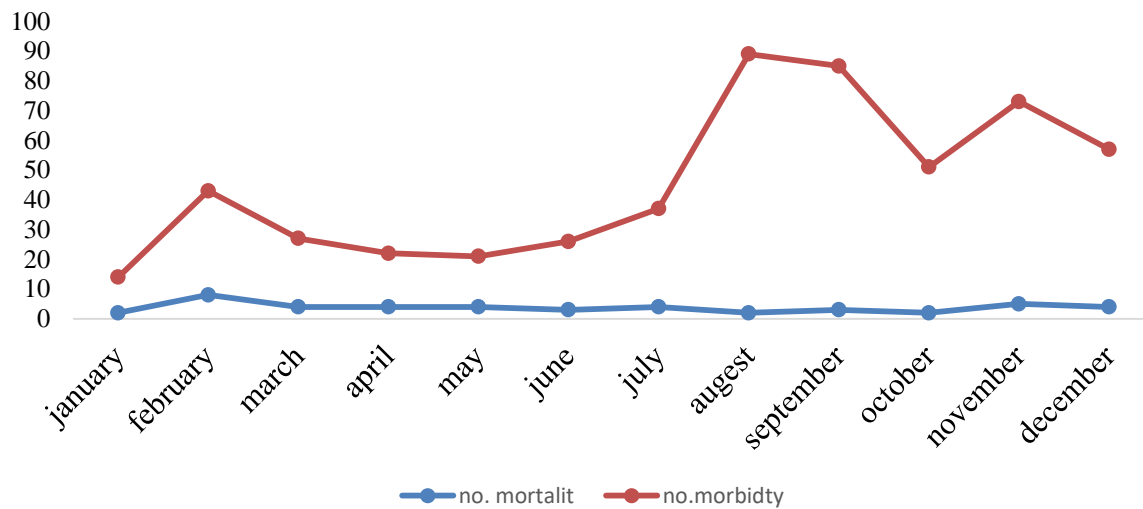


Figure 2. The pattern of respiratory problems in sheep across months in the average of the last three years

Discussion

The protective level of *Pasteurella* vaccine effectiveness was evaluated by measuring specific serum antibody titers produced against *P. multocida* Bio-type A in small ruminants. In the study, a higher protective antibody titer was recorded in the vaccinated population, which could be due to the result of the *P. multocida* Bio-type A vaccine that induced a higher level of *in vivo* antibody production. This finding is consistent with the epidemic theory, which suggests that if 70% of the population is immune, an outbreak is unlikely to occur because there are not enough susceptible populations to propagate an epidemic (Thrusfield, 1995).

The 93.1% post vaccination (after 28 days) antibody response against *P. multocida* Bio-type A in this study was comparable with the reports of Fisseha et al. (2016) and Yeshwas et al. (2013) that they reported 87.5% and 98.1% of antibody titers after vaccination in north-western Ethiopia and SNNPRS, respectively. However, the post vaccination antibody response result of the study was higher than that of the previous finding of Yeshimebet and Musa (2016) that reported 16.7% of protective antibody titers for post vaccination in north Shewa, Ethiopia. Such inconsistency in the post-vaccination antibody response might be due to the variation in the storage and handling condition of the vaccine and the underlying health status of the vaccinated groups. Even though the antibody titer of pre-vaccination was above the herd immune threshold level, it was relatively lower than the post-vaccination titer. The

relative decrease in herd immunity after six months of vaccination might be associated with population turnover and/or the antibody titration levels being reduced gradually and reaching below the protection level after six to eight months of vaccination.

The level of positive antibody against PPRV (percent of colour inhibition, PI > 50%) after 28 days of vaccination was 86.3%, while after 12 months of vaccination the antibody PI in response to PPR vaccine was 69.7%. The herd immunity to block the effective transmission of the virus has been expected to be greater than 80% after mass vaccination (Kumar et al., 2014). The high level of herd immunity achieved at post-vaccination may indicate good vaccine quality, cold chain maintenance, effective vaccine delivery, and good vaccine coverage in the district's vaccination campaigns.

The 86.3% post-vaccination (after 28 days) antibody response against PPR in this study was comparable with the reports of Yirga et al. (2019), who indicated 93.9% in vaccinated small ruminants in northwest Ethiopia. However, the study's post-vaccination antibody response against PPR was higher than previous reports by Biruk (2014), who reported 64.5% of vaccinated small ruminants in the eastern Amhara region, and Faris (2011), who reported 61.3% of vaccinated small ruminants in the Awash Fentale. Inconsistency in post-vaccination antibody response may be due to population turnover, vaccine handling practices, cold chain, host factor, vaccine delivery, and vaccine coverage.

The finding of morbidity was less than 64%, while the mortality was comparable to 0.1% of deaths associated with respiratory problems in the central highland of Ethiopia (Ayelet et al., 2004). The mortality rates due to respiratory problems in intervention villages in a follow-up period were lower as compared to the previous national reports, where annual mortality ranged from 12 to 14% for sheep and 11 to 13% for goats (MoA and ILRI, 2013). Even though low levels of morbidity and mortality were recorded, such cases might happen due to multiple causes such as *M. haemolytica* serotypes which could not be cross-protected by the *P. multocida* bio-type A vaccine and/or the presence of other causes of respiratory complexes, including lung worm. This is supported by Yeshimebet and Musa (2016), who reported *M. haemolytica* serotypes A1, A2, and A7 were present in community-based breeding programs and health monitoring villages of north Shewa, Ethiopia. Similarly, this finding is supported by Ayelet et al. (2004), who reported that the incompleteness of the available vaccine for pasteurellosis, which does not include all species and serotypes of *P. haemolytica*, could not completely protect small ruminants from ovine pasteurellosis.

In this finding, relatively higher respiratory problems were recorded from July to September, which might be associated with a change in the factors. Due to long rainy days, the animals

become confined to their pens and predispose them to starvation, which might aggravate factors for respiratory problems that determine the animals' resistance to respiratory infection. The findings agreed with the findings of Zegeye et al. (2014) and Ayelet et al. (2004) that reported pneumonia peaked in the months of July. Marked changes in weather and other factors that impair innate or adaptive resistance increase susceptibility to pneumonia (Radostits, 2007). In such cases, most of the bacteria that are normally resident in the upper respiratory tract have the ability to establish themselves in the lower respiratory tract (lung) and cause disease when the defence mechanism of the host is compromised (Carlton and McGavin, 1995).

Even though farmers and animal health experts had complained about the effectiveness of biannual vaccination against ovine Pasteurellosis with a monovalent vaccine (inactivated *P. multocida* biotype A) and annual vaccinations against PPRV, the finding showed that the antibody response against *P. multocida* bio-type A and PPR was above the threshold level of 70% and 80% in the study population, respectively. Moreover, during the study periods, there was no outbreak of any respiratory diseases in the study villages. So, the finding revealed that the vaccines employed were effective against targeted respiratory diseases of small ruminants.

Conclusions

The results showed that PPR and *P. multocida* Bio-type A serotype vaccines applied in the field were effective in developing antibodies above the threshold level of 80% and 70% in the vaccinated population, respectively. The morbidity and mortality of animals were low in the study flocks of small ruminants. Regular vaccination against respiratory diseases (PPR and ovine Pasteurellosis) in small ruminants according to the epidemiological dynamics of these diseases can result in a significant reduction in morbidity and mortality of animals. Therefore, the current ongoing efforts to control ovine Pasteurellosis and PPR of small ruminants through vaccination should be encouraged and strict sero-surveillance and monitoring of these diseases needs to be done side by side.

Conflict of interests

The authors have not declared any conflict of interests.

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