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Development and Evaluation of Inactivated Whole-Cell Contagious Caprine Pleuropneumonia Vaccine Against *Mycoplasma capricolum* Subsp. *capripneumoniae* Pakistan Strain

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HK, FAK, US, and IA designed and conceived the study. HK, FA and MS carried out the research. HK, FAK, and HR analyzed the data. HK and FAK wrote the manuscript. FAK, MS, and HR critically reviewed and revised the manuscript.

Key words
Whole-cell vaccine, Saponin, CCPP, Mccp, Saponized vaccine, Contagious caprine pleuropneumonia, *Mycoplasma capricolum* Subsp. *Capripneumoniae*, Pulmovac vaccine

ABSTRACT

To control and prevent various types of infectious diseases that affect the animal population, vaccination is a simple and efficient solution. The goat population suffers greatly from the serious respiratory disease contagious caprine pleuropneumonia (CCPP). Being a highly contagious disease, CCPP control is of major concern in the country. In this study, an inactivated whole-cell (WC) CCPP vaccine was developed and evaluated from the Mccp local strain isolate. The isolates were confirmed by PCR to have 0.15 mg/mL of proteins and were inactivated with saponin at a dose rate of 3.0 mg/mL. The inactivated saponized WC-CCPP vaccine and the commercially available Pulmovac CCPP vaccine (Türkiye) were inoculated in the experimental goats for evaluation and comparison. A total of 30 goats were used in this trial, in which 24 goats were divided randomly into three groups inoculated with inactivated WC CCPP vaccine, pulmovac CCPP vaccine, and sterile PBS as a negative control. In a safety trial, the goats remained healthy with no pyrexia and no pathological changes post-vaccination. On day 49, the mean percent inhibition was higher (84.768%) in goats vaccinated with the pulmovac CCPP live vaccine than in goats vaccinated with the inactivated WC CCPP vaccine (79.604). The antibody titers were measured using cELISA for 90 days post-vaccination. Goats vaccinated with saponized Mccp vaccine and pulmovac CCPP vaccine showed an increase in the antibody titer and reached the maximum level in the 7th week with a geometric mean titer (GMT) value of 169.24 and 177.3, respectively. Goats challenged after three months of vaccination were resistant to infection, while two non-vaccinated goats died of CCPP. After six months of challenge, one goat in group A and two goats in group B developed signs of CCPP, while one goat died of CCPP in the control group. These findings revealed that the goats require two doses of the inactivated WC CCPP vaccine annually, as it provides immunity against CCPP for six months.

INTRODUCTION

The goat population in Pakistan is 84.6 million and is the highest among the country's animal populations (225 million). Goats produce 1046,000 metric tons of milk and 799,000 tons of mutton annually, making up 14.51% of the world's meat supply despite their small size (Economic Survey of Pakistan, 2022–23). The goat population in Pakistan faces several challenges such as inadequate management practices and fodder, extreme weather conditions, and various infectious diseases (Shahzad *et al*., 2012). Among all infectious diseases, CCPP is one of the most severe threats to goats with high morbidity and mortality (WOAH, 2021). *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) is the causative agent of contagious caprine pleuropneumonia (CCPP), a highly contagious and deadly respiratory disease that affects goats. The typical signs of the disease

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include coughing, respiratory distress, and extremely high morsbidity and mortality (Thiaucourt and Bolske, 1996). Close contact between infected and susceptible animals is required for Mccp transmission since *Mycoplasma* species are highly susceptible (Thiaucourt *et al*., 1996). Stress factors like overcrowding, malnutrition, and long-distance travel increase the risk of disease transmission and morbidity (Lefevre *et al*., 1987; Mekuria and Asmare, 2010). The Mccp was successfully isolated from CCPP-affected goats in Pakistan (Ahmad *et al*., 2021). According to Akhtar *et al*. (2022), the Mccp strains in Asia exhibit significant genetic variation when analyzed using multi-locus sequence analysis (MLSA).

Prophylaxis is an efficient strategy in good husbandry methods for controlling and preventing a variety of infectious diseases in animals. Worldwide, vaccination campaigns against bacterial and viral diseases are conducted with varying degrees of success. More than a century ago, Hutcheon subcutaneously injected goats with lung extract from CCPP-affected animals, demonstrating that protection against the disease was achievable (McMartin *et al*., 1980). Goats immunized with a broth culture of attenuated Mccp prevented the CCPP from spreading (MacOwan and Minette, 1978). This showed explicitly that control over *Mycoplasma* is achievable. Since then, other preparations have been developed that promise to provide strong immunity for up to a year. Some of these vaccines include one that uses lyophilized Mccp that is inactivated with saponin right before immunization and another that uses sonicated antigens emulsified with inadequate Freund's adjuvant (Rurangirwa *et al*., 1987). Vaccines are prepared using a variety of adjuvants, each with varying degrees of success. Saponin has been effectively used as an adjuvant for preparing an inactivated vaccine from local isolates (Nicholas *et al*., 2002). In many parts of Kenya, the Mccp vaccine inactivated with saponin has been used with success in recent years (WOAH, 2021). A local field isolate of *Mycoplasma bovis* was used for the preparation of an inactivated adjuvanated vaccine and its efficacy was evaluated in calves (Ahmad *et al*., 2013). Compared to formalized or heat-killed vaccines, saponized vaccines for contagious agalactiae provided good protection (Tola *et al*., 1999). Different commercially available vaccines are produced including live CCPP vaccine Pulmovac and Capridol (Türkiye) and killed vaccine Caprivac (Kenya), no such vaccines against CCPP are produced in Pakistan.

The main objective of this study was to develop and evaluate the safety and immunogenicity of saponized inactivated WC CCPP vaccine in comparison to the Pulmovac live CCPP vaccine in goats.

MATERIALS AND METHODS

Mccp culturing and molecular confirmation

The samples were collected from goats from across the Khyber Pakhtunkhwa (KP) and Gilgit Baltistan regions. The Mccp from the freeze-dried master seed samples were used for vaccine production. The isolate was cultured in pleuropneumonia-like organism (PPLO) broth for 10–14 days in a carbon dioxide (CO_2) incubator at 37 °C with the provision of 5% CO_2. The change in color of the broth from pink to yellow with the whirling movement was the evidence of positive growth. The culture was confirmed through Mccp species-specific primers (F:5′-ATCATTTTTAATCCCTTCAAG-3′, R:5′-TACTATGAGTAATTATAATATATGCAA-3′) described previously by Woubit *et al*. (2004) using polymerase chain reaction (PCR).

Vaccine preparation

A concentrated and purified Mccp culture was obtained for vaccine preparation. The culture was centrifuged at 12,000 x g for 20 min and the supernatant was discarded. The pellet was centrifuged a second time after a sufficient volume of sterile PBS was added for washing. The whole-cell proteins were extracted from the pellet. As an adjuvant for the antigen's inactivation, 3 mg of saponin per dosage was used.

The protein concentration was measured using a Bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific).

Experimental goats and antibody screening

Clinically healthy goats (n= 30; male: 07 and female: 23), 6-12 months of age with no previous history of exposure of goats to CCPP and tested negative for Mccp-specific antibodies using cELISA used in the experiment were housed at the VTH, College of Veterinary Sciences, The University of Agriculture Peshawar. Three goats were used for the safety evaluation of the vaccine, three were used for experimental infection with live Mccp isolate, and the remaining twenty-four goats were randomly allocated into three groups A, B, and C for a vaccine trial.

Adjuvant preparation

A stock solution of saponin 10% was prepared using PBS and autoclaved for 15 min at 121 °C. The sterility of the saponin solution was checked by culturing it on sabouraud dextrose agar, thioglycollate broth, tryptose soya broth, and soybean casein digest medium (SBCDM) agar.

Quality control tests

Inactivation, sterility and safety tests were performed

for determining the quality of the formulated in activated WC CCPP vaccine for in activation test, 10 test tubes (15 ml) were taken each containing 10 ml PPLO broth. The inactivated vaccine (1 ml) was inoculated into four tubes, another four tubes were inoculated with live Mccp culture and 2 tubes were kept as negative control. All the tubes were incubated for 10 days at 37 □ and were observed for the absence and presence of Mccp growth (Takele *et al*., 2017). The broth culture was then grown on PPLO agar for the Mccp colony. For sterility test the vaccine samples were cultured on thioglycollate broth, tryptone soya broth, SBCDM agar, and sabouraud dextrose agar for 24 – 48 h at 37 □. For safety test six seronegative goats were injected subcutaneously with 1ml of inactivated WC CCPP vaccine and three goats were kept as negative control by injecting sterile PBS. The goats were observed daily for systemic and local reaction and rectal temperature was recorded twice daily for 14 days (Takele *et al*., 2017; WOAH, 2021).

In-vivo trial of vaccine

The goats of group A (*n*= 08) were vaccinated twice, 21 days apart between the first and booster immunization with inactivated WC CCPP vaccine 1 ml subcutaneously. Group B (*n*= 08) was inoculated with commercially available Pulmovac live CCPP vaccine twice (Türkiye), and Group C (*n*= 08) was kept as a negative control using sterile PBS 1ml/goat. The animals were thoroughly monitored for any clinical signs after vaccination. The site of inoculation was examined daily by palpation for any swelling. The rectal temperatures were recorded daily twice (morning and afternoon) for two weeks. The blood (3ml) was collected for serum samples from goats before vaccination (day 0), day 14, 21, 35, 49, 63, 77 and day 90.

For antibody response induced by vaccination competitive ELISA was used and the manufacturer protocol was followed. The serum samples from goats at days 0, 14, 21, 35, 49, 63, 77 and 90 were analyzed. The serum antibody titers were calculated by geometric mean titer (GMT). The cELISA values for percent inhibition (PI) were interpreted as Positive SPI > 55%; Negative SPI<55%.

Challenge of immunized goats

Three susceptible naive goats were experimentally infected intratracheally with local Mccp isolate culture dose of $1.7x10^8$ CFU/ml. After 90 days of immunization, four goats from each group were challenged by housing them together with the experimentally infected goats. The goats were observed daily and the rectal temperature was recorded until the typical signs of CCPP appeared in the control group. The remaining four goats from each group after six months of vaccination were similarly challenged.

Statistical analysis

The data collected were compiled into Microsoft Excel and analyzed through a t-test on Statistical Package for Social Sciences (SPSS) version 19.0 for comparison between the GMT and mean PI of the two vaccines. The geometric mean value was calculated for antibody titer quantification. The confidence level was taken to 95% and the p-value <0.05 for significance in all analyses.

RESULTS

Mccp viable count

The viable count of Mccp local isolate was obtained from a stock culture with an estimated $1x10^7$ CFU/ml. The Mccp WC proteins were washed three times with 1% PBS, inactivated with 3 mg/ml saponin, and incubated at 37 °C for 8 hours. The final quantity of protein concentration in the reconstituted vial was adjusted to 0.15 mg per dose.

Quality of vaccine

The inactivated vaccine was cultured in PPLO broth and agar for 14 days. There was no growth observed for the vaccine both in broth and on agar, indicating that Mccp had been successfully inactivated. The vaccine was found sterile after sterility test. Besides that, the rectal temperature recorded for the safety-tested goats was 101.6 °F - 102.9 °F which is within the normal range as for healthy goats. There was no significant difference between the temperatures of the two groups (Table I).

Table I. Morning and afternoon body temperature of goats vaccinated with inactivated WC CCPP vaccine and control group for safety test.

Group	Days	Mean±SD (Range)	SE	p value
Morning observation (n=3)				
Vaccinated	14	101.9±0.850 (100.1-103.2)	0.131	0.105
Non vaccinated	14	101.5±1.016 (99.4-103.0)	0.156	
Afternoon observation (n=3)				
Vaccinated	14	101.8±0.955 (100.0-103.5)	0.147	0.103
Non vaccinated	14	101.6±1.086 (99.9-103.7)	0.167	

Clinical examination of vaccinated goats

The goats were thoroughly monitored but no clinical signs were observed in the vaccinated goats and non-vaccinated goats. The temperature for all the goats

of group A was in the range of 100.8 – 103.8 °F in the morning and 101.3 – 103.4 °F in the afternoon. Similarly, the mean temperature of goats vaccinated with pulmovac vaccine in the morning and afternoon was 102.4 °F and 103.1 °F, respectively (Table II).

Table II. Morning and afternoon body temperature of goats vaccinated with inactivated WC CCPP vaccine (A), Pulmovac CCPP vaccine (B) and negative control group (C).

Group	Days	Mean±SD (Range)	SE	p value
Morning observation (n=8)				
A	14	102.7±1.103 (100.8-103.8)	0.139	0.081
B	14	102.4±0.986 (101.1-103.5)	0.143	
C	14	101.8±0.914 (100.6-102.9)	0.156	
Afternoon observation (n=8)				
A	14	102.9±1.217 (101.3-103.4)	1.217	0.095
B	14	103.1±1.052 (100.9-103.7)	1.052	
C	14	102.2±0.975 (99.8-103.1)	0.975	

Table III shows the seroconversion/ percent inhibition (PI) test results of the vaccinated and non-vaccinated goats for the period of up to 90 days. The PI >55% was considered as seropositive for Mccp. The mean PI for group C in all observed days was below 43% which indicates no seroconversion. The mean PI for goats of group A was 40.847 on day 0 and reached 56.013 on day 14 after being vaccinated with inactivated WC CCPP vaccine. The highest PI for groups A and B was 79.604 and 84.768, respectively on day 49 which then gradually decreased and reached the PI value of 59.860 and 62.195, respectively. Figure 1 shows the mean PI of vaccinated and non-vaccinated goats.

Antibody response induced by vaccination

The first dose of vaccines induced a low antibody response, as detected by cELISA. The goats immunized with the first dose of Mccp saponized vaccine showed a rise in average antibody titers, with a GMT value of 16.3 on day 14 and 28.14 on day 21st. After the booster dose, the antibody titer reached a maximum level of 169.24 on day 49 and then decreased to the antibody titer of 154.8 on day 90 (Fig. 2).

Table III. Mean percent inhibition and summary statistic of seroconversion for different vaccinated and control groups up to 90 days.

Blood collection days	Group	PI Mean±SD (n=8)	SE	95% confidence interval for mean	
				Lower	Upper
0	A	40.85±5.91 (28.81-49.41)	2.09	36.753	44.941
	B	38.17±4.51 (33.09-47.78)	1.59	35.047	41.293
	C	42.27±5.62 (34.93-49.41)	1.99	38.381	46.169
14	A	56.01±1.18 (55.59-58.14)	0.42	55.198	56.829
	B	56.87±1.08 (55.98-58.50)	0.38	56.138	57.634
	C	35.67±5.86 (27.99-45.13)	2.07	31.607	39.735
21	A	58.97±1.71 (59.41-63.48)	0.61	57.785	60.157
	B	61.46±1.78 (58.10-62.04)	0.63	60.225	62.695
	C	37.12±7.91 (27.79-48.19)	2.79	31.642	42.607
35	A	68.73±2.82 (68.48-71.25)	0.99	66.771	70.684
	B	71.16±1.49 (67.97-71.28)	0.53	70.126	72.196
	C	39.99±4.21 (32.07-45.46)	1.49	37.076	42.918
49	A	79.60±1.57 (79.28-90.23)	0.55	78.517	80.690
	B	84.77±3.52 (80.21-88.13)	1.24	82.329	87.207
	C	39.61±5.72 (31.01-47.51)	2.02	35.647	43.582
63	A	70.85±2.69 (72.91-78.42)	0.95	68.984	72.709
	B	76.27±1.85 (73.42-77.01)	0.65	74.990	77.553
	C	38.85±4.18 (33.42-44.66)	1.48	35.951	41.749
77	A	65.81±3.42 (66.47-71.73)	1.21	63.439	68.178
	B	69.19±1.34 (67.35-71.46)	0.48	68.259	70.123
	C	40.86±4.09 (33.47-46.87)	1.45	38.021	43.695
90	A	59.86±1.78 (56.71-62.02)	0.63	58.623	61.097
	B	62.19±2.06 (59.00-64.91)	0.73	60.769	63.620
	C	40.19±3.71 (34.19-45.55)	1.31	37.623	42.774

Group A: Goats vaccinated with inactivated WC CCPP vaccine, Group B: Goats vaccinated with pulmovac CCPP vaccine, Group C: Inoculated with sterile PBS (Negative control). PI: Percent inhibition.

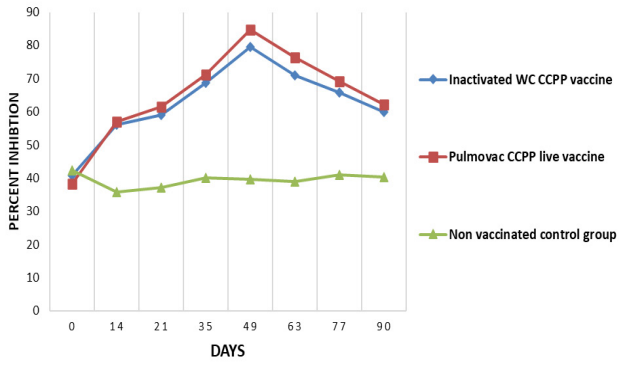

Fig. 1. Mean percent inhibition of seroconversion for vaccinated goats.

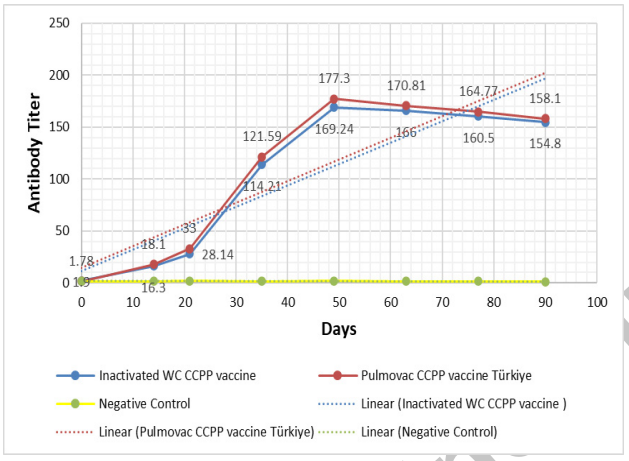

Fig. 2. Comparative antibody titer of inactivated WC CCPP vaccine, pulmovac CCPP live vaccine, and control group in goats. T-test was applied to compare mean GM value of both vaccine (t value= 0.63, df=27, p=0.391), non-significant difference (p>0.05) was found between the two vaccines.

The goats of group B administered with a single dose of pulmovac vaccine showed antibody titers ranging from 18.1 on day 14 to 33 on day 21st. The antibody titer rose to a GMT value of 177.3 on day 49 after a booster dose and declined to the average antibody titer of 158.1 on the 90th day (Fig. 2). These findings showed that the local Mccp saponized vaccine elicits an immunological response. Comparative antibody titer of inactivated WC CCPP vaccine, pulmovac CCPP live vaccine, and control group in goats is shown in Figure 2.

Immunity exhibited by goats challenged after three and six months of vaccination

There was no pyrexia and any signs of disease 3 months after vaccinations in goats vaccinated with inactivated WC CCPP vaccine and pulmovac CCPP vaccine. All the goats of the negative control group developed pyrexia and respiratory signs 15.3±2 days after exposure to experimentally infected goats and one goat died 3 days later. Thus, 90 days after immunization, all the vaccinated goats show resistance to infection and remain healthy.

Six months after vaccination all goats in the control group, one of the goats immunized with inactivated WC CCPP vaccine, two goats immunized with pulmovac CCPP vaccine developed pyrexia and respiratory signs 12.7, 14.9, and 14.0 days after in-contact exposure with experimentally infected goats, respectively. One goat in the control group died 21.0±1 days later of typical CCPP (Table IV).

Postmortem examination of goats

The postmortem of goats was performed after mortality occurred due to CCPP. The gross lesions were present in the pericardium, pleura, and lungs. Fibrinous exudate was present on the parietal pleura with straw color fluid in the thoracic cavity. The lungs were friable and firmly adhered to the thoracic wall.

Table IV. Immunity exhibited by goats challenged with live Mccp local isolate after three and six months of vaccination.

Months after vaccination	Group	Days to pyrexia and respiratory signs	Days to death	Number of goats survived/ number of goats in group
3	WC CCPP vaccine	0	0	4/4
	Pulmovac vaccine	0	0	4/4
	Negative control	15.3±2	18.0±1	3/4
6	WC CCPP vaccine	14.9	0	4/4
	Pulmovac vaccine	14.0	0	4/4
	Negative control	12.7	21.0±1	3/4

DISCUSSION

Vaccines against CCPP have been developed, including inactivated or live vaccines, each type has its advantages and disadvantages. Antigen-based live vaccines can provide immunity for a longer duration, reducing the overall cost and dose requirements, however, there is a risk of infection (Salt *et al*., 2019; Yatoo *et al*., 2019a, b). The inactivated CCPP vaccine requires a higher dose (Yatoo *et al*., 2019a). The use of sonicated antigens instead of live attenuated antigens inoculated intratracheally for vaccination against CCPP has several advantages. Protection by sonicated antigen is up to 100% and is for a longer duration with no chances of disease (MacOwan and Minette, 1978; Rurangirwa *et al*., 1984).

The vaccine was prepared following the standard operational procedure and subjected to all the quality control tests including sterility test, inactivation test, safety test, and immunogenicity test (WOAH, 2021). The safety test was performed on three seronegative goats before the immunogenicity test. During a 14-day observation period, there were no adverse reactions such as swelling on the site of injection, pyrexia, and respiratory distress in the goats. The rectal temperature taken twice daily for 14 days was in the normal range and there was no significant difference ($p>0.05$) in the vaccinated and non-vaccinated goats of the safety trial. This study is in accordance with the results by Takele *et al*. (2017), who performed vaccine safety evaluation and immunogenicity in Ethiopia. According to the recommendations by WOAH (2021), animals should not exhibit any major pathological changes except slight swelling at the site of injection.

To evaluate the seroconversion during the experimental period the mean percent inhibition (PI) of sera of the goats of the three groups were analyzed using cELISA. On day 0 the mean PI for groups A, B, and C was <55% which indicates no anti-Mccp antibodies. The mean PI increases after a booster dose of vaccination. The results showed that the mean PI of the goats vaccinated with Pulmovac CCPP vaccine was the highest (84.678%) and the mean PI of goats vaccinated with inactivated WC CCPP vaccine was 79.604 on day 49. The mean PI declined to 59.860 and 62.195 on day 90 for groups A and B, respectively. Litamoi *et al*. (1989) investigated the efficacy of inactivated Mccp strain F-38 vaccine in natural infection with contagious caprine pleuropneumonia by vaccinating 10,000 goats, out of which 400 were observed regularly for 6 months post-vaccination. Throughout the monitoring period, the immunized goats remained free from infection. The immunogenicity test was performed using slide agglutination and complement fixation tests. Young beef cattle were successfully immunized against *M. bovis* using a saponin-inactivated vaccine, which prevented the disease's spread to other organs and reduced the symptoms of pneumonia, fever, and lung lesions in the protected animals (Nicholas *et al*., 2002). The seroconversion of live Mccp vaccination was found to be higher (84.2%) than that of inactivated vaccine (68.4%) (Tarekegn *et al*., 2012). After receiving the killed CCPP vaccine, 253 out of 414 goats (61.1%) were found to be seroconverted, and the rate of seroconversion was higher in young goats than in adult goats (Lakew *et al*., 2014). Takele *et al*. (2017) reported mean seropositivity and percent inhibition of 60.71% and 61.52%, respectively, in the experimental goats.

The goats of the vaccinated and control groups were challenged after three and six months of vaccination. A live Mccp local isolate was administered intratracheally into three goats and the immunized goats were housed together with these goats. The goats were examined daily until the typical signs of CCPP appeared in the control group. The goats challenged after three months of vaccination showed resistance to infection with no pyrexia and respiratory signs. In the current study, one of the four goats died in the control group challenge after three months and one out of four goats died after six months of challenged trial. Only one of the four goats in group A and two goats in group B after six months of immunization developed pyrexia and showed signs of CCPP but no mortality was observed in the challenged goats vaccinated with inactivated WC CCPP vaccine and pulmovac vaccine. The signs of CCPP in vaccinated goats developed after six months of vaccination may be due to individual variation or some unknown cause. Secondly, the antibody titer decreases gradually after 90 days, which may also be a factor in disease development. This study is in line with the study by Rurangirwa *et al*. (1984) who performed a vaccine trial in experimental goats using a sonicated antigen of Mccp F-38. In this study, three types of adjuvant were used including IFA, aluminum hydroxide, and phosphate buffer saline (PBS). The goats were challenged after 3 and 6 months of vaccination in which all the goats in the control group died of CCPP.

The antibody titer for vaccinated goats was quantified and compared with the control group. The GMT values for goats vaccinated with inactivated WC CCPP vaccine and pulmovac vaccine before vaccination were 1.9 and 1.78, respectively. The goats vaccinated with inactivated WC CCPP vaccine have a GMT value of 28.14 and 114.21 on day 21 (before a booster dose) and 35, respectively. A maximum GMT value of 169.24 was obtained on day 49 post-vaccination. In contrast, goats immunized with the pulmovac CCPP vaccine had the highest GMT value of 177.3 on day 49. The increase in antibody titer in group B

as compared to group A may be due to the live nature of the pulmovac vaccine. These results are in agreement with the work of Tarekegn *et al*. (2012), who reported that live vaccines are more effective in seroconversion than killed vaccines. During the 90-day trial, there was no increase in GMT values in the control group. The maximum antibody titer was found during the sixth and eighth week after vaccination, the antibody titer then gradually decreases. Manimaran *et al*. (2006), reported similar results of the highest antibody titer reached in 6-8 weeks after vaccination with Mmc lyophilized vaccine in goats. Naturally, as part of the immune response, antibodies decrease with time. After vaccination, antibody levels steadily decline after a peak, as the vaccine antigen no longer stimulates the immune system constantly. These findings revealed that the vaccines provide immunity for six months, therefore; two doses of inactivated WC vaccine are required annually. This statement is supported by the findings that revaccination of goats using inactivated vaccines against CCPP is recommended after every six months (Yatoo *et al*., 2019; Jores *et al*., 2020).

CONCLUSION

Based on the present study we concluded that no significant difference was observed in the humoral response of goats immunized with inactivated WC CCPP vaccine and pulmovac CCPP live vaccine. The mean PI and antibody titer for both vaccines reached to higher level at the seventh week post-vaccination. The vaccinated goats challenged after six months of vaccination developed pyrexia and respiratory signs. These results showed that, since the inactivated WC CCPP vaccine confers immunity for six months, goats need two doses each year.

DECLARATIONS

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IRB approval

The Advanced Studies and Research Board (ASRB) in its 51st meeting held on September 25, 2020, approved this study vide notification No.1549/*ASRB*-51/UAP dated October 5, 2020.

Ethical statement

The study was carried out according to the standard animal rights and duly approved by the Animal Ethics Committee of the Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture Peshawar vide letter No.6681/LM, B&G/AUP dated October 10, 2020.

Statement of conflict of interest

The authors have declared no conflict of interest.

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