

Available on <http://www.pjbt.org>
 Pakistan Journal of Biotechnology
 (PJB)
 (P-ISSN: 1812-1837 and E-ISSN: 2312-7791)



IMPROVING MCCP VACCINE IN SHEEP: NOVEL STRATEGIES FOR DEVELOPMENT, EFFICACY AND CROSS-PROTECTION

Haider Ali Yousafzai¹, Shahid Hussain Abro¹, Abdul Sajid², Hanif ur Rahman², Rani Abro³, Baitullah², Farhan Anwar khan⁴, Dildar Hussain Kalhoro¹

¹Department of Veterinary Microbiology, Sindh Agriculture University Tandojam

²Veterinary Research Institution Peshawar Khyber Pakhtunkhwa

³College of Veterinary Sciences, Agriculture University Peshawar, Pakistan

⁴Faculty of Animal Husbandry & Veterinary Sciences, Sindh Agriculture University Tandojam

*Corresponding author: shahidabro9@yahoo.com

Article Received 20-01-2024, Article Revised 11-02-2024, Article Accepted 27-04-2024.

ABSTRACT

Vaccination serves as preventing infectious contagious caprine pleuropneumonia (CCPP), a severe respiratory system affecting small ruminants. This research aimed to create a vaccine utilizing *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) isolated from KPK, Pakistan. The local Mccp strain was rendered inactive alongside saponin, to produce the vaccine. The viable count was determined to be 1×10^8 CFU/mL from the stock culture, with an estimated protein content of 0.2g/ml. The developed vaccine underwent testing and comparison with a standard CCPP vaccine in sheep to assess safety and efficacy. Twelve experimental sheep were divided into three groups: A, B, and C. Group A received the new vaccine, group B received the standard vaccine, and group C acted as the control. Groups A and B were further split into two subsets: one remained healthy while the other fell ill. The sheep were closely monitored for any changes over a 75-day post-vaccination period. Blood samples were analyzed for antibodies, showing average antibody GMT titers of 24.3, 84.4, and 128 on days 21, 28, and 35 post-immunization. By day 35, sheep vaccinated with the new vaccine displayed the highest antibody levels. The quadratic relationship (R^2) indicated that both saponized and lyophilized vaccines were influenced by the number of days by 57.4% and 55%, respectively. The study successfully deactivated microorganism with saponin, ensuring a sterile vaccine. In vivo testing in rabbits confirmed the vaccine's safety, while serum analysis in sheep revealed protective antibody responses, with the saponized vaccine eliciting higher titers than the lyophilized version.

Keywords: CCPP, Mccp, Vaccine, Sheep, Saponin, Khyber Pakhtunkhwa

INTRODUCTION

Caprine pleuro-pneumonia (CCPP) is a highly contagious and fatal disease of small ruminates (sheep and goat) flocks. The disease is caused by microbial agent called *Mycoplasma capricolum capripneumoniae* (Mccp). The diseases have diagnosed in several countries of the globe (OIE, 2019). The six *Mycoplasma* strains are involved for the infections in dairy flocks across the various territories of the world. The effective immunization and control of infection is based on the prevalence of the type of strain infecting flocks in the region. Various bacterial pathogens of *Mycoplasma* species such as *Mycoplasma LC mycoides* (*Mycoides Mycoides*) *Mycoplasma putrefaciens*, *Mycoplasma agalactiae*, *Mccp*, and *Mycoplasma mucoiedes mucoiedes* were found production of ovine and caprine mycoplasmosis (Sadique *et al.*, 2012; Hira *et al.*, 2015; Banaras *et al.*, 2016).

Traditional immunization against the disease using killed, attenuated and live vaccines, sonicated

bacteria, saponin based products and mass spectrometry have limitations in quality, efficacy and practical application of immunization (Thiaucourt *et al.*, 2018; Tesgera *et al.*, 2017; Ayelet *et al.*, 2007). The immunizing agents consisting of attenuated cultures of *Mycoplasma capricolum capripneumoniae* vaccine had been fail to control of the disease in vaccinated herds (Rurangirwa and McGuire, 2016; MacOwan and Minette, 1978). Afterwards, several immunization with vaccines produced short term immunity/efficacy against the caprine mycoplasmosis. This type of vaccines were consisted of saponin based lyophilized *Mccp*, sonicated antigens conjugated Freund adjuvant (Tesgera *et al.*, 2017; Gelagay *et al.*, 2007; Rurangirwa *et al.*, 1987). However, immunization using vaccine (saponin based *Mycoplasma*) produced promising results for controlling of mycoplasma infections in dairy flocks in Kenya (OIE, 2014).

However, little focus were on development of vaccines consisting of various adjuvants used for

immunization against MCCP infection in sheep and goat population (Rahman *et al.*, 2003). Despite mass vaccination strategies applied for control of *Mycoplasma* infection in herds in several regions of Pakistan, These vaccine programs fail to confirm protection and/or control MCCP outbreaks in the country (Shahzad *et al.*, 2012).

Vaccines contain various types adjuvant, among these sponin is well known for its activity and ability of inactivation. Sponin derived substance derived from *Guillaia saponaria* plant in South America. Sponin have been applied for inactivation of *Mycoplasma* species in feed of livestock (Thiaucourt *et al.*, 2018; Mulira *et al.*, 1988). Generally, the vaccine confirms immunity approximately 14 months to immunized animals for CCPP infections. However, booster dose is recommended after one year of immunization (OIE, 2014). Development of MCCP vaccines based on whole-cell culture encounters to different challenges and/or limitations including inhibitory effects and purification of immunoglobulin IgG (March *et al.* 2000; March and Jones, 1998). Considering limited efficiency of available commercial vaccines for protection to CCPP infections were observed in environmental conditions. The current research aims to develop a based vaccine from local MCCP strains to evaluate the cross-protectivity of improved MCCP vaccine in sheep.

MATERIALS AND METHODS

The purpose of this research was to develop a saponized vaccine from of *Mycoplasma* strains identified locally. Developed vaccine was tested and comparison performed with commercially available lyophilized CCPP vaccine for immunogenicity.

Development of *Mycoplasma* sponin based vaccine: *Mccp* strains isolated from local outbreaks were utilized for development of vaccine. The confirmation of *Mccp* strains was performed by cultural, biochemical tests and molecular detection targeting housekeeping genes.

Culture preparation: The cultures confirmed using biochemical and molecular methods culture of *Mccp* exhibited mass turbidity was sub-culture for 72 hours at 37 °C in modified Hayflick medium containing 5% CO₂ (OIE, 2004). The pure culture 20.0 ml was used to inoculate 200 ml of production medium. For five days, it was incubated at 37 °C with 5% CO₂ to achieve the desired turbidity and growth rate (OIE, 2014).

Inactivation of *Mccp* antigen: The pure culture was centrifuge at 12000 rpm (4°C) for 15 minutes. The pellet washed with PBS three times followed by supernatant discard and re-suspension. The pellet was resuspended in 0.1M phosphate buffer saline (pH 7.2) and washed three times. 3.0 mg/mL saponin (S4521; Sigma, Aldrich®, Germany) was added in cultured *Mycoplasma* species and incubated at 37°C for 8 hours. Risk of microbial contamination was

determined by culture of vaccine on various media (Thiaucourt *et al.*, 2018; Nicholas *et al.*, 2004)..

Protein estimation of vaccine culture: *Mycoplasma* organisms titer was enhanced to get maximum concentration. Protein content (0.2 mg/ml) was processed and mixed with bicinchoninic acid (BCA) (Sigma-Aldrich, Germany). The procedure for protein estimation of vaccine culture was determined, as recommended by (Albers and Fletcher, 1982). The standard prepared by combining BCA solution and cultured *Mycoplasma* species followed by incubation for 1 hour at 37°C. The optical density (OD) was measured at 562 nm using ELISA plate reader. Protein concentration was determined using a standard curve created by comparing the *Mccp* culture result to a known BSA concentration.

Quality control of saponized vaccine

Determination of developed vaccine sterility: To determine sterility of whole cell saponized *Mccp* vaccine was cultured on fluid thioglycollate broth with and without Tween 80, Sabourad dextrose agar and tryptic soya broth.

Safety of vaccine: To assess the safety of the newly produced whole cell saponified *Mccp* vaccine, 12 rabbits were housed at animal house Veterinary Research Institute, Peshawar. They were divided into four groups of three rabbits each: (G1-G4). Groups 1 to 3 received subcutaneous injections of various concentrations of whole cell saponized trial vaccine at 1.0, 2.0, and 3.0mL, respectively. G4 was used as a negative control, receiving 1.0mL of normal saline. For 14 days after immunization, the experimental rabbits examined for any clinical / physiological problems twice daily.

Vaccinal trial in experimental animals: Experimental animals (Bulkhi sheep) were used to evaluate effectiveness of an indigenous saponin based *Mccp* vaccination and a commercially available lyophilized *Mccp* vaccine.

Sheep grouping and inoculation: To assess the vaccine's immunogenic effectiveness, One year old 12 male Bulkhi sheep were kept field animal farm in Nowshera. Three categories of animals were created: A, B, and C. Each group was kept separately and provided green feed, concentrate in diet, and clean water for 15-days. De-worming of experimental animals was performed using 10.5mg/kg body weight of Nilzan Plus (ICI, Pakistan). The animals were tested for *Mycoplasma* infection in the past. Nasal swabs from all sheep cultured on Hayflick broth were obtained to confirm the presence of contamination. Experimental Group A and Group B were injected subcutaneously 1.0 ml saponin based *Mccp* vaccine and commercial lyophilized *Mccp* vaccine. The booster dose of 1.0 ml vaccine was administered on the fourteenth day after immunization. Sterilized Hayflick medium 1.0 ml was injected to group C, as a negative control was established.

Evaluation of blood profile and immunized experimental animals: To assess the vaccine's

immunogenic effectiveness in 12 male Bulkhi sheep after immunization, the animals were closely observed for clinical symptoms and the development of lesions. The detailed physical and clinical examinations of the animals were performed in the morning and evening. The data of the examinations was recorded daily. On days 0, 7, 14, 21, 28, 35, 42, 49, 56, 60, and 75 after immunization, a 5ml blood sample was taken in a vacutainer from each experimental animal. The blood samples were taken from experimental animals at various intervals and left to remain at room temperature for two hours before being centrifuged for five minutes at 2500 rpm for serum extraction (Tuck *et al.*, 2009). The extracted serum stored at -20 °C until further analysis. It was used to detect antibodies against *Mccp* using IHA as previously reported by (De Dieu *et al* 2019).

Mycoplasma antigen development: The inoculum comprising pure *Mycoplasma* colonies cultured on Hayflick agar medium followed by transferring it to a new broth and growth was maintained at 1×10^4 CFU/ml.

Erythrocytes (RBC) sensitization: Sheep erythrocytes RBC were mixed to 0.2 % glutaraldehyde solution before being incubation for 20 minutes at 37 °C. Three times washings of cells were using 0.1% sodium azide (SigmaAldrich) containing normal saline. RBCs were mixed with 0.01M PBS to maintain a 20% working solution.

Indirect Hemagglutination (IHA) test: Separate hyperimmune sera were produced in sheep using a conventional procedure as reported before by Rahman *et al* (2003). The test was conducted using the technique with minor modifications. Serum samples from sheep were inactivated in a water bath for 30 minutes at 56°C. 1% sera of sheep blood as well as a control group mixture, were diluted serially in normal saline (25 ul). Separately, on a 96-well microtitration plate, 2 percent sensitized sheep RBC were added to each blood sample. After one hour of incubation at

37°C, the plate was assessed for hemagglutinating activity. Antibodies titer against *Mccp* considered as the reciprocal of the maximum dilution that demonstrated antigen-sensitized agglutination of sheep RBCs.

Data analysis: The data processed into a Microsoft Excel spreadsheet and the geometric mean (GMT) value computed for antibody titers. The relationship between the antibody titer and the days was determined using a polynomial two-degree quadratic equation. The t-test was performed for the independent samples to evaluate the statistical significance of the difference between the average GMTs of two vaccinations.

RESULTS

Protein concentration and viable counts

assessment: Stock culture of the *Mycoplasma mycoides* subsp. *capripneumoniae* (*Mccp*) exhibited viable count 1.0×10^8 CFU/mL (Figure 1). Concentration of 0.2g/ml protein content was obtained from the stock culture. *Mycoplasma* species were effectively deactivated by autoclaved saponin at a concentration of 3mg/mL for eight hours at 37°C, following three washes in 1% PBS. To check for contamination and *Mycoplasma* growth, the deactivated cells were culture on modified Hayflick agar and blood agar, respectively. After 96 hours, no growth was observed on the agar plates, indicating successful deactivation of the *Mycoplasma*.

The investigation of sterility of *Mccp* saponin based vaccine carried out for any contamination. The *Mycoplasma* species were cultured on tryptose soya broth (TSB), SBCDM agar, fluid thioglycollate medium (FTM) and Sabourad dextrose agar, There was no any microbial (bacteria and fungi) contamination / growth detected on the used media. These findings suggest that the vaccine meets standard sterility requirements of vaccine and may be considered safe for use in dairy herds.

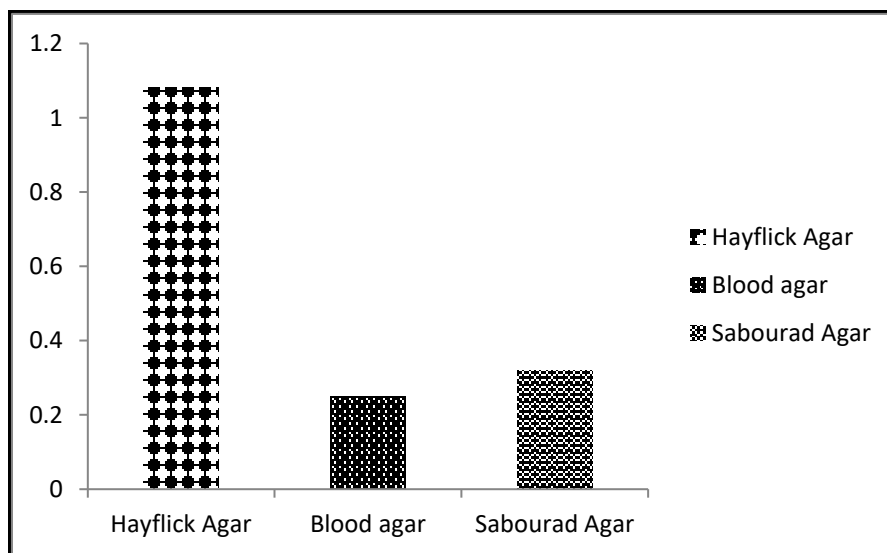


Figure 1 Showing locally isolated *Mccp* strains growth on various media used

Assessment of the safety of the cell saponized vaccine in rabbits: All rabbits examined and monitored for clinical and physiological issues, such as fever, behavioral changes, restlessness, coughing, salivation, palpable edema at the vaccine injection site, gastrointestinal abnormalities and nasal discharge. In addition no detrimental clinical and physiological complications were observed in either the vaccinated or control group animals after 14 days of monitoring. Consequently, the vaccine was deemed safe for in vivo use.

Quantification antibodies titer: Quantification analyses was determined from vaccinated and control groups samples of sheep.. The samples derived at specific intervals: days 0, 7, 14, 21, 28, 35, 42, 49, 56, 60, and 75 days after vaccination. The obtained data subjected calculation of IHA antibody titers generated in experimental animals by immunizing commercial lyophilize and cell saponin based Mccp vaccine (Figure 2). In group A, which received the Saponized vaccinated animals (Group A) , showed the average

antibody titer 27.86 after 21 days vaccination. The increased titer GMT values were recorded following vaccination at 28, 35, 42 128 and 147 days respectively. The GMT titer remained at 147 on days 35 and 42, then decreased to 48 on day 49. By day 75 post-vaccination, the lowest GMT titer observed was 4.59. Group B, which received the VRI Lahore Mccp vaccine, exhibited average antibody GMT titers of 24.3, 84.4, and 128 at days 21, 28, and 35 post-immunization. By days 42 and 49, the titer reduced to GMT 111.4 and 32 respectively. The lowest GMT value observed at day 75 was 3.5.

According to the results, both vaccinations elicited protective antibodies in the bloodstream of experimental sheep (Figure 3). However, group A, which received the whole-cell culture saponized vaccine, exhibited a higher GMT value of 147 on days 35 and 42 post-immunization. The quadratic relationship (R²) indicated that both saponized and lyophilized vaccines were dependent on the number of days by 57.4 % and 55%, respectively.

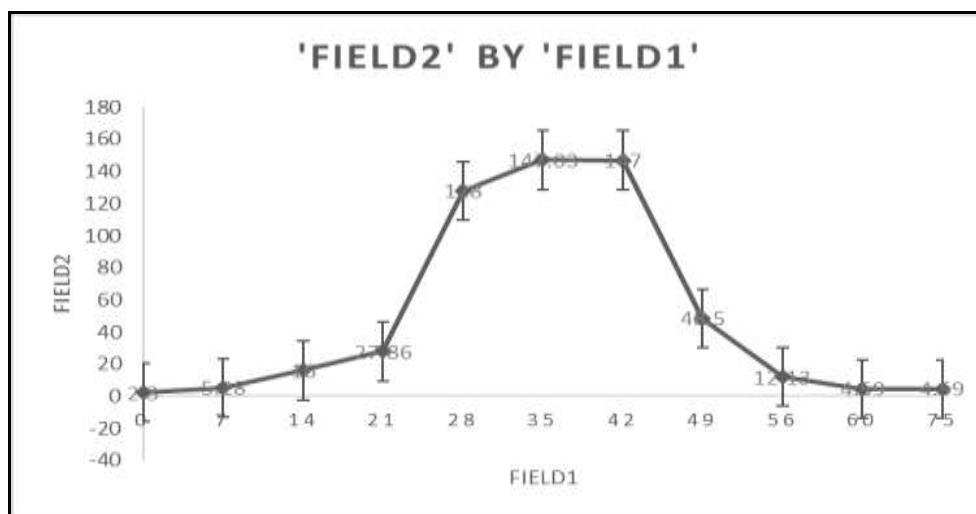


Figure 2. Vaccinations elicited antibodies response in the bloodstream of experimental sheep

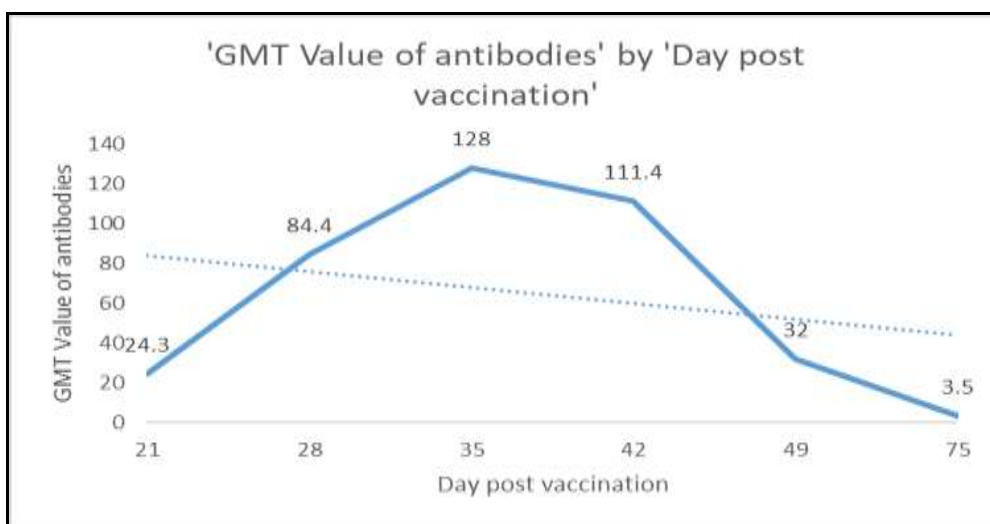


Figure 3 GMT value of antibodies after Mccp vaccination in the experimental animals

DISCUSSION

To manage and control CCPP infection in small ruminants in Pakistan, only one species-specific vaccination is utilized. Despite widespread immunization, the disease persists in spreading across the country. Local isolates of CCPP were effectively inactivated using saponin as an adjuvant (Nicholas *et al.*, 2002). The immunogenic effectiveness of saponin-inactivated Mycoplasma vaccine varies depending on the location (Nicholas and Churchward, 2012).

The pathogenic Mycoplasma species identified from spontaneously infected small ruminants was Mycoplasma capricolum subsp. capripneumoniae (Mccp). Therefore, this pathogen was evaluated for vaccine development against CCPP in the research region. A saponin-inactivated Mccp vaccine was developed and tested in sheep. The dosage of 3.0 mg/ml saponin proved to be efficient in inactivating Mycoplasma whole cells. The results demonstrate that saponin functions as both a Mycoplasma cell inactivator and a vaccination adjuvant, supporting previous research (Kensil *et al.*, 1991; Tesgera *et al.*, 2017; Gelagay *et al.* 2007).

Vaccines against infectious Mycoplasma agalactiae (saponized) provided superior protection compared to formalized or heat-destroyed vaccines. Saponin was certified as primary antigenic substance for untreated microbial agent (Mycoplasma species). A previous study demonstrated that saponin at 2.0 mg/mL is enough for successful inactivation of *Mycoplasma bovis* cultured organisms (Thiaucourt *et al.*, 2018; Tola *et al.*, 1999).

Efficacy of vaccines was tested on 12 sheep comprising of groups: A, B and C. On first day after vaccination, all group animals produced adequate antibody titers, with a geometric mean titer (GMT) of 1.7 in serum. It has had been documented that after first day of immunization to experimental animals, there was no evidence of antibodies presence (Rahman *et al.*, 2003; Manimaran *et al.*, 2006). Low antibody titer may be in relation to antibodies need time to develop and activate.

On days 35 and 42 post-vaccination, a group inoculated with whole-cell saponized Mccp vaccine had the highest antibody titer of 47.1. In contrast, the lyophilized Mccp vaccine generated a maximum GMT of 128 on day 35. An isolated field strain vaccine produced the highest antibody titer in a group. The current study's findings are supported by the fact that vaccines produced from any local pathogen strain provide optimal outcomes and disease prevention (OIE, 2014). The peak antibody titer was observed 6-7 weeks after immunization. In goats, maximal antibody titer was attained 6-8 weeks after immunization using a lyophilized Mccp vaccine (Manimaran *et al.*, 2006).

Based on the quadratic relation (R²), saponized and lyophilized vaccines showed 57.4% and 55%

dependency on day immunized. Influences on antibody development were not ruled out. Both vaccines demonstrated immunogenic potential, but the saponized vaccine elicited higher antibody titers. Throughout the 75-day study, no physiological and/or clinical signs were detected in the vaccinated animals, confirming vaccine's safety. Following immunization, the vaccinated sheep experienced a slight rise in body temperature (104-104.8 °C) for 12-36 hours. This modest increase in body temperature supports the immunogenic reaction of the whole-cell saponized vaccine. The results indicate that vaccines act as antigens, activating the host immune system and inducing various cellular and metabolic mediators, resulting in elevated body temperature.

Goats are highly susceptible to CCPP infection with high mortality and morbidity rates, and prominent severe clinical signs. The study aimed to evaluate the immunogenicity of saponized and lyophilized Mccp vaccines. On day 28 post-immunization, animals in the unchallenged group A (A1, A2, A3) exhibited the highest antibody GMT of 176. By days 35 and 42, the GMT had decreased to 160 and 112, respectively.

The current research anticipated that maximal antibody titers would be achieved in sheep. On day 75 after immunization, the GMT was 8. To determine the effectiveness and protective efficiency of indigenous vaccine, animals were challenged with isolated Mccp antigen from the locally isolated strain. GMT value (224) was the highest on 28 and 35 days after vaccination. A decrease to GMT 176 and 128 was recorded on days 42 and 49 post-vaccination. In animals, saponin based vaccine generated high amount of antibodies after 42 days post immunization (Thiaucourt *et al.*, 2018; Manimaran *et al.*, 2006). The antibody titer continuously decreased until 75th day, with GMT value of 16.8 to a low level.

The data indicated that the saponin based vaccine challenged animals produced the highest level of antibodies in comparison to the non-vaccinated animals. These findings indicated swift response by immune system of animal followed by receiving of Mccp antigen, resulting in enhanced antibody levels in the blood. To calculate the relationship between vaccine immunogenicity and number days, a quadratic equation was applied.

The results shown high antibody levels were come by a booster dose, followed by challenge with antigen, and subsequently declined progressively until 75 days. These results assert the preventive efficiency and efficacy of the saponin based vaccine against the Mycoplasma infection in experimental animals. As challenged microbial organisms are susceptible to the host immunity, high amounts of antibody are generated in the blood of challenged animals. The experimental animals were closely monitored for physiological and/or clinical symptoms/symptoms issues twice daily for 75 days. No clinical symptom /

signs, abnormal physiological issues were noticed in any of the vaccinated, challenged, or control group animals. The results are contrast to reports with minor clinical symptoms recorded in calves after immunization (Thiaucourt *et al.*, 2018; Tesgera *et al.*, 2017; Nicholas *et al.*, 2004). It has been reported model for assessing immunization efficacy in small number experimental animals with controlled conditions (Tesgera *et al.*, 2017; Gelagay *et al.*, 2007; Roth and Flaming, 1990).

In this study, group B received lyophilized Mccp vaccination, was assessed for its efficiency comparison to Saponin based vaccine. Post-vaccination on day 21, the GMT value of antibodies in non vaccinated groups (B1, B2, B3) was 96. The highest GMT value 160 was recorded at day 28 that was maintained till 35th day post-vaccination, continuous reduced to 96 and 64 on 42nd and 49th day, respectively. After immunization on 75th day, the low level of titer observed 6.3. For vaccinated challenged animals (B4, B5, B6), on 21st day after vaccination, antibodies were calculated with a high GMT value of 80.63. By days 28 and 35 after immunization, the challenged goats exhibited the highest antibody titer of 192. The GMT then partially reduced to 160 and 96 on 42nd and 59th after vaccination, respectively. The lowest GMT titer recorded was 16 on day 75 post-vaccination. The quadratic relation (R²) revealed that antibodies production by saponized and lyophilized vaccines depended on days by 61.2% and 54.8%, respectively, in challenged animals.

Variables influencing antibody formation in test group animal ca not be ruled out. Since the Mccp antigen had been previously exposed through vaccination, it activated the host immune system. First-exposure memory cells responded rapidly, and activated B-cells quickly generated high levels of antibodies against the identified antigen. The vaccinated and challenged goats were examined twice daily for respiratory distress, gastrointestinal disturbances, and fever.

The results indicated that no abnormal signs were observed, although the experimental animals experienced a slight increase in body temperature ranging from 104.6 to 105.2 °C during the 24-48 hours following immunization. The specificity of antibodies in their activity and function in combating the antigen justifies the protection observed in the challenged animals. The profound immunogenic response was detected using saponin derived vaccine. This immunogenic response needed to activate various pathways immune complex mechanisms. Also, several research studies shown that increase in body temperature followed immunization (Stipkovits *et al.*, 2001; Tesgera *et al.*, 2017; Gelagay *et al.*, 2007).

It was observed that saponin base Mccp vaccine efficiently illicit protective immune response in the animals. The findings of this study correlate to research reported by (Tesgera *et al.*, 2017; Gelagay *et al.*,

2007). The small ruminant herd vaccinated *Mm LC* and *M. agalactiae* vaccine (containing saponin showed promising results safety against the *Contagious agalactiae* (De Fe *et al.*, 2007), pneumonia in calves (Nicholas, 2002).

In summary, *Mycoplasma mycoides capri* was deactivated by using saponin for development of vaccine. The developed vaccine safety index and protective antibody response was confirmed.

REFERENCES

- Ayelet, G., Laekemariam, Y., Aschalew, Z., Esayas, G., & Kassahun, A. (2007). Validation of immunity induced by inactivated CCPP vaccine with different adjuvants. *Small Ruminant Research*, 73, 200–205.
- Banaras, F., Abbas, F., Awan, M. A., Riaz, M., & Khan, I. A. Molecular survey on the prevalence of caprine *Mycoplasma* in the goats of Quetta City of Pakistan. *International Journal of Veterinary Science*, 5, 158-163. (2016)
- De la Fe, C., Martín, A. G., Amores, J., Corrales, J. C., Sánchez, A., Poveda, J. B., & Contreras, A. (2010). Latent infection of male goats with *Mycoplasma agalactiae* and *Mycoplasma mycoides* subspecies *capri* at an artificial insemination centre. *The Veterinary Journal*, 186(1), 113-115.
- De Dieu, J., Baziki, Charles, Bodjo S. Nwankpa, Nick, Chitsungo, Ethel, Moustapha Boukary, Cisse Rahamatou Maina, Naomi, Tefera, Takele A., Nwankpa, Rume Veronica, Mwangi, Nduta Mathurin Koffi, Yao. (2019). Development and Evaluation of Epitope-Blocking ELISA for Detection of Antibodies against Contagious Caprine Pleuropneumonia in Goat Sera. *Veterinary Sciences*, 6(4), 82-88.
- Gagea, M.I., Bateman, K.G., Dreumel, T.V., McEwen, B.J., Carman, S., Shanahan, R.A., & Caswell, J.L. Diseases and pathogens associated with mortality in Ontario beef feedlots. *Journal of Veterinary Diagnostic Investigation*, 18, 18-28. (2006)
- Gelagay, A., Teshale, S., Amsalu, W., & Esayas, G. Prevalence of contagious caprine pleuropneumonia in the Borana pastoral areas of Ethiopia. *Small Ruminant Research*, 70(2), 131-135. (2007).
- Hira, E., Hira, H., Awan, M.A., Kakar, M.A., & Tariq, M. Molecular study on the prevalence of respiratory *Mycoplasma* species in small ruminants of Kuchlak, District Quetta and Khanazai, District Pishin, Balochistan. *Pakistan Journal of Zoology*, 47, 473-478. (2015)
- Jaffri, K.T., Gill, Z.J., Bhatti, A.R., & Raza, A. Immune Response of Buffalo Calves to Haemorrhagic Septicemia Oil Adjuvant and Alum Precipitated Vaccine. *International Journal of Agriculture and Biology*, 8(5), 645-647. (2006)
- Kensil C.R., Patel U., Lennick M., Marciani D. Separation and characterization of saponins with adjuvant activity from *Quillaja saponaria* Molina cortex. *J. Immunol.* 146:431–437. (1991).
- Lakew, M., Sisay, T., Ayelet, G., Eshetu, E., Dawit, G., & Tadele, T. Sero-prevalence of contagious caprine pleuropneumonia and field performance of

- inactivated vaccine in Borana pastoral area, southern Ethiopia. *African Journal of Microbiology Research*, 8(24), 2344-2351. (2014)
- MacOwan, K.J., & Minnette, J.E. The effect of high passage Mycoplasma strain F38 on the course of contagious Caprine pleuropneumonia (CCPP). *Tropical Animal Health and Production*, 10, 31-35. (1978).
- Manimaran, K., Singh, V.P., Ltu, K., Das, S., Kumar, A.A., & Srivastava, S.K. Immune responses against different inactivated vaccines of Mycoplasma mycoides subsp. capri in goats. *Journal of Applied Animal Research*, 29, 117-120. (2006).
- McMartin, D.A., MacOwan, K.J., & Swift, L.L. A century of classical contagious pleuropneumonia: from original description to aetiology. *British Veterinary Journal*, 136, 507-515. (1980).
- Mulira, G.L., Masiga, W.N., & NandoKha, E. Efficacy of different adjuvants to potentiate the immune response to Mycoplasma strain F-38. *Tropical Animal Health and Production*, 20, 30-34. (1988).
- March J.B., Gammack C., Nicholas R. Rapid detection of contagious caprine pleuropneumonia using a Mycoplasma capricolum subsp. capripneumoniae capsular polysaccharide-specific antigen detection latex agglutination test. *Journal of Clinical Microbiology*. 38(11):4152-4159. (2000).
- March J.B., Jones G.E., Inhibitory effects of vaccines containing subunit fractions of Mycoplasma capricolum subsp. capripneumoniae. In: Leori G, Santini F, Scanziani E, Frey J, editors. *Mycoplasmas of ruminants: pathogenicity, diagnostics, epidemiology and molecular genetics*. Vol. 2. Luxembourg: European Union; p. 44-49. (1998).
- Nicholas, R., & Churchward, C. Contagious caprine pleuropneumonia: a new aspects of an old disease. *Transboundary and Emerging Diseases*, 59, 189-196. (2012).
- Nicholas, R.A.J., Tjipura-Zaire, G., Scacchia, M., Frey, J., & bschle, O.J.B. An inactivated vaccine against CBPP exacerbates disease in cattle. In: *Proceedings of FAO/OIE/AU Consultative meeting, Rome*. pp. 91-97. (2004).
- OIE. Contagious caprine pleuropneumonia. OIE Terrestrial Manual. Chapter 2.7.6. Office International Des Epizootics, Paris, France. 704-719. (2014).
- OIE (World Organization for Animal Health). Contagious Caprine Pleuropneumonia. Retrieved from http://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/contagious_caprine_pleuro.pdf. (2019).
- Rahman, S.U., Siddique, M., Iftekhar, H., Muhammad, K., & Rasool, M.H. Standardization of indirect haemagglutination test for monitoring Mycoplasma mycoides subspecies capri antibodies raised in rabbits and goats. *International Journal of Agriculture and Biology*, 5(3), 295-297. (2003)
- Roth, J.A., & Flaming, K.P. Model systems to study immunomodulation in domestic food animals. *Advances in Veterinary Science and Comparative Medicine*, 35, 21-41. (1990).
- Rurangirwa, F.R., & McGuire, T.C. Contagious caprine pleuropneumonia: Diagnosis and control. Retrieved from <http://www.fao.org/wairdocs/ilri/x5473b/x5473b11>. (2016).
- Rurangirwa, F.R., Kouyate, B., Niang, M., & McGuire, T.C. CCPP: Antibodies to F38 polysaccharide in Mali goats. *Veterinary Record*, 127, 353. (1990).
- Rurangirwa, F.R., McGuire, T.C., Kibor, A., & Chema, S. (1987). An inactivated vaccine for contagious caprine pleuropneumonia. *Veterinary Record*, 121, 397-402.
- Rurangirwa, F.R., Masiga, W.N., & Muthomi, E.K. (1984). Immunization of goats against contagious caprine pleuropneumonia using sonicated antigens of F-38 strain of mycoplasma. *Research in Veterinary Science*, 36, 174-176.
- Sadique, U., Zafer, R., Younas, Z.U., Hassan, M., Idrees, M., Mushtaq, A., Sajid, A., & Sabtain, M. (2012). Molecular characterization of contagious caprine pleuropneumonia (CCPP) in small ruminants of Khyber Pakhtunkhwa. *Pakistan Journal of Animal and Plant Sciences*, 22, 33-37.
- Samiullah, S. (2013) Contagious caprine pleuropneumonia and its current picture in Pakistan: a review. *Veterinaria Medicina*, 58(8), 389-398.
- Shahzad, A., Rashid, M., Muhammad, S.K., Mansur, D.A., & Rashid, A. (2012). Characterization, molecular diagnosis and prevalence of caprine mycoplasmosis in different areas of Pakistan. *Pakistan Journal of Zoology*, 44(2), 559-568.
- Tesgera, T., Sori, H., Yami, M., & Mamo, B. (2017). Evaluation of safety and immunogenicity of inactivated whole culture contagious caprine pleuropneumonia trial vaccine in National Veterinary Institute, Ethiopia. *African Journal of Microbiology Research*, 11(11), 466-473.
- Thiaucourt, F., Pible, O., Miotello, G., Nwankpa, N., & Armengaud, J. (2018). Improving quality control of Contagious Caprine Pleuropneumonia vaccine with tandem mass spectrometry. *Proteomics*, 1800088.
- Tuck, M.K., Chan, D.W., Chia, D., Grizzle, W.E., Krueger, K.E., & Rom, W. (2009). Standard Operating Procedures for Serum and Plasma Collection: Early Detection Research Network Consensus Statement Standard Operating Procedure Integration Working Group. *Journal of Proteome Research*, 8(1), 113-117.

Publisher's note: PJBT remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. To

view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>