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## Safety and immunogenicity of an intranasal vaccine against pneumonic mannheimiosis of sheep and goats following field vaccination

Zaid Ahmad Mohd Zamri, Norazrina Pakiman, Radin Mohammad Arif Abu-Bakar, Mohd Jamil Aizat Jamaluddin and Mohd Zamri-Saad\* 

BioAngle Vacs Sdn Bhd, UPM-MTDC Technology Center III, Universiti Putra Malaysia, Serdang, Malaysia

### ABSTRACT

**Background:** Pneumonic mannheimiosis is an important respiratory disease that inhibits production in sheep and goats. It is associated with stress, resulting in 10% to 40% mortality. The disease should be effectively controlled.

**Aim:** This field vaccination trial evaluates the safety and immunity of a newly manufactured vaccine (STVac7™) against pneumonic mannheimiosis in sheep and goats.

**Methods:** A sheep and a goat breeder farm were identified, before 2 groups of breeder sheep with 80 animals per group, and 2 groups of breeder goats with 50 animals per group were selected. Group 1 sheep and goats were vaccinated intranasally, while Group 2 remained unvaccinated. Vaccinations were conducted on weeks 0 and 2. Throughout the 24-week (sheep) and 12-week (goats) study periods, animal management in both farms remained unchanged. Serum samples were collected from both groups before and at weekly intervals after vaccination, while nasal swabs were collected at monthly intervals. Adverse effects, including sneezing, coughing, nasal discharge, and mortality, were noted weekly throughout the study period.

**Results:** Intranasal vaccination did not cause significant side effects in sheep and goats, although mild nasal discharge and coughing were occasionally observed. Only one non-vaccinated ewe (1.3%;  $p > 0.05$ ) died despite the identification of *M. haemolytica* in the nasal cavity of the majority of the sheep and goats. Following vaccination, antibody levels showed significant increase and remained significantly high ( $p < 0.05$ ) throughout the study period.

**Conclusion:** Intranasal vaccination of sheep and goats with STVac7™ is safe and well tolerated, resulting in high serum antibody levels and maintaining the health status of the animals during the stressful monsoon season.

**Keywords:** Field trial, Pneumonic mannheimiosis, STVac7™, Vaccination.

### Introduction

Pneumonic mannheimiosis (previously pneumonic pasteurellosis) is an infectious disease caused by *Mannheimia haemolytica* (previously *Pasteurella haemolytica*), leading to widespread financial losses through death, reduced live weight, delayed marketing, treatment costs, and un-thriftiness among survivors (Rahman *et al.*, 2023). *Mannheimia haemolytica* is a gram-negative rod or coccobacillus that is part of the normal commensal of the upper respiratory tract of animals (Cozens *et al.*, 2019). Stresses, such as transportation stress, concurrent infection, and climate change, are among the important risk factors that influence disease development (Jilo *et al.*, 2020). With a prevalence rate of 10% to 40%, and mortality exceeds 20% in young ruminants (Zamri *et al.*, 2025), pneumonic mannheimiosis continues to inhibit the progression of small ruminant production (Abdullah *et al.*, 2015; Abbas *et al.*, 2019).

STVac7™, a newly developed and manufactured vaccine, is an innovative intranasal spray vaccine against pneumonic mannheimiosis of sheep and goats. The vaccine comprises inactivated whole *M. haemolytica* cells that exhibit cross-protection against common *M. haemolytica* serotypes. Under controlled experimental conditions, the vaccine was shown to stimulate respiratory tract mucosal immunity (IgA) (Effendy *et al.*, 1998; Emikpe and Ajisegiri, 2011) and systemic immunity (IgG) that protects animals from infection (Zamri-Saad *et al.*, 1999). Subsequent field trials using the vaccine prepared under laboratory conditions revealed similar efficacy (Sabri *et al.*, 2013). Set to be commercialized soon, the product is easy to apply, requires less training for vaccine administration, and reduces the vaccination period. It is administered intranasally prior to stress, such as monsoon seasons, to stimulate both local and systemic immunity, thus preventing the establishment of *M. haemolytica* in

\*Corresponding Author: Mohd Zamri-Saad. BioAngle Vacs Sdn Bhd, UPM-MTDC Technology Center III, Universiti Putra Malaysia, Serdang, Malaysia. Email: [mzamri@upm.edu.my](mailto:mzamri@upm.edu.my)

the respiratory tract and subsequent development of pneumonic mannheimiosis in goats and sheep (Sabri *et al.*, 2013). Nevertheless, a newly developed vaccine must be tested in the field to ensure safety and efficacy, as those reported in experimental trials before commercialization.

This study evaluates the adverse effects and health status of sheep and goats following intranasal vaccination using STVac7<sup>TM</sup> and determines the duration and pattern of serum antibody in sheep (24 weeks) and goats (12 weeks) in a field vaccination trial.

## Materials and Methods

### Farm information

A sheep breeding farm located in Johor, in the southern part of peninsular Malaysia, was identified for the study. It is located in an oil palm plantation and houses 1,200 Santa Ines and Blackhead Dorper sheep. The farm used a semi-intensive farming system in which animals were fed soybean hulls and pellets in the morning before being released for grazing in the evening. Water was available *ad libitum* in the farm through an automatic drinking water system. The feces were regularly collected, at least once a week, to be used as fertilizer for plants and crops. As this is a breeder farm, breeding was conducted as scheduled using the natural breeding system. Groups of breeder females and males at a female/male ratio of 20:1 were kept in separate pens, whereas new-born kids and mothers were kept separately in separate pens.

For goats, a breeder farm located in Selangor, in the central part of peninsular Malaysia, was selected. The farm consisted of approximately 120 goats of various ages, mainly of the Boer breed, with a few local Katjang goats. The farm also used a semi-intensive farming system, where goats were mostly confined in pens and were occasionally released for grazing between 11 am and 3 pm. Goats were fed pellets, cut Napier grass, alfalfa, and soybean hulls when they were in the pens. Napier grass was usually freshly cut and fed either in the morning or evening, while alfalfa was offered to the sick and inactive animals to increase appetite. Drinking water was available *ad libitum* and replenished with fresh water every morning. A mineral salt block was provided for the goat as a supplement. Feces were collected once a week for use as fertilizer.

### Experimental design

The field trials involving sheep and goats lasted 24 and 12 weeks, respectively, by agreement with the respective farm owners. The 24-week sheep vaccination trial was conducted during the monsoon season, when more cases of pneumonic mannheimiosis are usually reported. The trial started in September 2024 and ended in March 2025, when the monsoon season usually occurs between October and January (between weeks 3 and 15 of the trial). At the same time, the 12-week goat vaccination trial was also conducted at the start of

the monsoon season in October and ended in December 2024 (between weeks 1 and 12 of the trial).

A total of 160 adult, healthy breeder sheep with an average weight of  $31.9 \pm 2.5$  kg were included in the study. They consisted of 150 ewes and 10 rams and were divided into 2 groups, vaccinated (Group 1) and non-vaccinated (Group 2), with 80 animals per group. Animals were kept in 8 pens with 20 animals per pen, ensuring that vaccinated and non-vaccinated pens were physically separated. A total of 100 Boer breeder goats with an average of  $37.7 \pm 2.6$  kg were selected for the trial. They consisted of 10 males and 90 females and were divided into 2 major groups, vaccinated (Group 1) and non-vaccinated (Group 2), with 50 animals per group. They were kept in smaller groups of 15 females per pen within 6 pens, while a group of 10 males was kept in 2 separate pens with 5 males per pen. The vaccinated and non-vaccinated groups were physically separated.

Before the start of the study, serum samples were randomly collected from both groups and subjected to an enzyme-linked immunosorbent assay (ELISA) to ensure that they had low antibody levels against pneumonic mannheimiosis. There was no history of vaccination against this disease. At the start of the trial, sheep and goats of Group 1 were vaccinated intranasally with 1 ml of the newly manufactured STVac7<sup>TM</sup> vaccine, while sheep and goats of Group 2 remained un-vaccinated. A booster dose was similarly administered 2 weeks later. BioAngle Vac Sdn Bhd supplied the STVac7<sup>TM</sup> vaccine. Except for vaccination, neither farm's management practices were changed nor modified.

### Monitoring of adverse events and physical conditions

Following intranasal administrations of the first dose at Week 0 and the booster dose at Week 2, all sheep and goats were closely monitored for coughing, sneezing, lethargy, loss of appetite, nasal discharge, and mortality. Monitoring was conducted once a week after vaccination, and the number of animals with any adverse event in both vaccinated and non-vaccinated groups was recorded.

The body condition score (BCS) of all animals was assessed weekly, based on the scoring criteria of Ghosh *et al.* (2019) and Thomas and Bailey (2021). Body scores of 3 and 4 were considered good and acceptable under this scoring system. The weekly body scores of each animal were recorded before the data were analyzed at the end of this field vaccination trial.

### Blood sampling and nasal swab collection

Before vaccination and at weekly intervals after vaccination, blood samples were collected for 12 weeks in goats and 24 weeks in sheep. Approximately 30% of the animals in each group were randomly selected before 6 to 8 ml of blood were collected from the jugular vein of each animal into plain tubes. Blood samples were allowed to clot at room temperature, centrifuged at  $2,000 \times g$  for 10 minutes before the sera

were collected. The sera were stored at  $-20^{\circ}\text{C}$  and later subjected to ELISA to determine the IgG antibody levels. Nasal swabs were collected from approximately 30% of the animals in each group before vaccination and every 4 weeks after vaccination to identify *M. haemolytica* using PCR (Hawari *et al.*, 2008). The swabs were submerged in transport medium and transferred to the laboratory on the same day, stored at  $4^{\circ}\text{C}$ , and processed for bacterial identification the next day.

#### **Monitoring of serum antibody levels using ELISA**

Serum antibody levels were measured using an in-house indirect ELISA method based on Sabri *et al.* (2000) and Intan-Shameha and Zamri-Saad (2005). A 96-well microplate was coated with 50  $\mu\text{l}$  coating antigen containing  $10^9$  cfu/ml of *M. haemolytica* A7 and incubated at  $37^{\circ}\text{C}$  for 1 hour. Then, the microplate was washed three times with buffer before adding blocking buffer to each well and incubating at  $37^{\circ}\text{C}$  for 1 hour. The microplate was then washed three times with PBST before adding diluted serum samples (diluted to 1:200 in PBS) in triplicate. The plate was again incubated at  $37^{\circ}\text{C}$  for 1 h and washed three times with PBST. Diluted anti-goat IgG horseradish peroxidase or anti-sheep IgG horseradish peroxidase was then added into the wells, incubated for 1 h at  $37^{\circ}\text{C}$ , and washed three times with PBST. Then, substrate (1X TMB Substrate Solution) was added into each well and incubated for 30 minutes. Subsequently, the reaction was stopped with 0.2 M sulfuric acid, and the results were read in a microplate reader at 450 nm.

#### **Bacterial identification**

DNA was extracted directly from swab samples using a standard DNA extraction kit (QIAGEN, Malaysia). The obtained DNA samples were then subjected to polymerase chain reaction (PCR) analysis with a general 16S primer for the detection of *M. haemolytica* (Tabatabaei and Abdullahi, 2018). Gel electrophoresis was used to amplify the PCR products. After completion, the gel was removed and observed under an ultraviolet light transilluminator, where bands could be observed. The samples that showed the same band as the positive control were considered to have *M. haemolytica*.

#### **Statistical analysis**

All data were methodically arranged into tabular formats before comparative analysis among the different groups was performed using one-way analysis of variance (ANOVA). The pattern was compared between different weeks within the same group, whereas the effect of different treatments was compared between different groups within the same week.

#### **Ethical Approval**

All animal use and experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Universiti Putra Malaysia, under the reference number UPM/IACUC/

AUP-R004/2023. Ethical guidelines were followed for blood and nasal swab collection.

## **Results**

### **Health status**

Following the first vaccination, the health status of all sheep and goats in both the vaccinated and non-vaccinated groups remained good with no adverse events ( $p > 0.05$ ). The majority of the animals (90% vaccinated and 88% non-vaccinated sheep, 98% vaccinated and 94% non-vaccinated goats) had a BCS of 4, showing no significant difference ( $p > 0.05$ ) between the two groups ( $p > 0.05$ ).

Following booster vaccination in week 2, nasal discharge was observed in 3.8% vaccinated and 2.5% non-vaccinated ( $p > 0.05$ ) sheep. The symptoms appeared mild and did not progress to severe conditions. None of the goats in both groups showed any adverse effects. The majority of the sheep and goats (90% vaccinated and 81% non-vaccinated sheep, 100% vaccinated and non-vaccinated goats) had a BCS of 4 ( $p > 0.05$ ).

Table 1 summarizes the health status of both vaccinated and non-vaccinated animals throughout the study period. Nasal discharge and coughing were detected among some of the vaccinated and non-vaccinated sheep, but no obvious pattern was observed. Nasal discharge was recorded at the highest ( $>10\%$  of the vaccinated animals) in weeks 3, 4, 8, 11, and 14, which were significantly higher ( $p < 0.05$ ) than in the non-vaccinated animals. Difficulty in breathing was only observed among  $<7.5\%$  of vaccinated animals, and a non-vaccinated ewe with a BCS of 2 died due to malnutrition.

Coughing was detected among the non-vaccinated sheep at weeks 5 and 24, and nasal discharge was detected at weeks 1 to 20, except at weeks 4, 6, and 7. High numbers of sheep with nasal discharge ( $>10\%$  of the animals) were detected in weeks 8, 10, and 11. While the vaccinated group showed high numbers with nasal discharge between weeks 3 and 16 and decreased after week 16, the non-vaccinated group showed initially low number before increasing and consistently higher than the vaccinated group after week 12. Both groups showed coughing symptoms in week 6 and at the end of the field trial in weeks 20 and 22 for the vaccinated group and week 24 for the non-vaccinated group.

Table 2 summarizes the clinical signs observed in vaccinated and non-vaccinated goats throughout the study period. Coughing and nasal discharge were occasionally observed, whereas sneezing was present only at weeks 1 and 12 in both groups ( $p > 0.05$ ). Nasal discharge was present in the vaccinated group from week 0 ( $p < 0.05$ ) to week 6, decreasing following vaccination until the end of the field trial. Coughing was detected mostly in the middle of the trial among vaccinated goats. Similarly, a higher percentage of non-vaccinated goats showed nasal discharge at the

**Table 1.** Percentage of vaccinated and non-vaccinated sheep showing clinical signs during the study period.

| Week | Vaccinated (n = 80) |              |                      |                         |               | Non-vaccinated (n = 80) |              |                      |                         |               |
|------|---------------------|--------------|----------------------|-------------------------|---------------|-------------------------|--------------|----------------------|-------------------------|---------------|
|      | Sneezing (%)        | Coughing (%) | Nasal discharge (%)  | Difficult breathing (%) | Mortality (%) | Sneezing (%)            | Coughing (%) | Nasal discharge (%)  | Difficult breathing (%) | Mortality (%) |
| 0    | 0.00                | 0.00         | 0.00                 | 0.00                    | 0.00          | 0.00                    | 0.00         | 0.00                 | 0.00                    | 0.00          |
| 1    | 0.00                | 0.00         | 0.00                 | 0.00                    | 0.00          | 0.00                    | 0.00         | 1.25                 | 0.00                    | 0.00          |
| 2    | 0.00                | 0.00         | 0.00                 | 0.00                    | 0.00          | 0.00                    | 0.00         | 2.50                 | 0.00                    | 0.00          |
| 3    | 0.00                | 0.00         | 13.80 <sup>a,y</sup> | 0.00                    | 0.00          | 0.00                    | 0.00         | 2.50 <sup>z</sup>    | 0.00                    | 0.00          |
| 4    | 0.00                | 0.00         | 12.50 <sup>a,y</sup> | 0.00                    | 0.00          | 0.00                    | 0.00         | 0.00 <sup>z</sup>    | 0.00                    | 0.00          |
| 5    | 0.00                | 1.25         | 5.00                 | 7.50                    | 0.00          | 0.00                    | 1.25         | 1.25                 | 0.00                    | 0.00          |
| 6    | 0.00                | 0.00         | 0.00                 | 0.00                    | 0.00          | 0.00                    | 0.00         | 0.00                 | 0.00                    | 0.00          |
| 7    | 0.00                | 0.00         | 0.00                 | 2.50                    | 0.00          | 0.00                    | 0.00         | 0.00                 | 0.00                    | 0.00          |
| 8    | 0.00                | 0.00         | 12.50 <sup>a,y</sup> | 0.00                    | 0.00          | 0.00                    | 0.00         | 11.25 <sup>a,y</sup> | 0.00                    | 0.00          |
| 9    | 0.00                | 0.00         | 6.25                 | 0.00                    | 0.00          | 0.00                    | 0.00         | 3.75                 | 0.00                    | 0.00          |
| 10   | 0.00                | 0.00         | 3.75 <sup>y</sup>    | 0.00                    | 0.00          | 0.00                    | 0.00         | 12.50 <sup>a,z</sup> | 0.00                    | 0.00          |
| 11   | 0.00                | 0.00         | 12.50 <sup>a</sup>   | 0.00                    | 0.00          | 0.00                    | 0.00         | 10.00                | 0.00                    | 0.00          |
| 12   | 0.00                | 0.00         | 8.75                 | 0.00                    | 0.00          | 0.00                    | 0.00         | 8.75                 | 0.00                    | 0.00          |
| 14   | 0.00                | 0.00         | 11.25 <sup>a,y</sup> | 0.00                    | 0.00          | 0.00                    | 0.00         | 7.50 <sup>z</sup>    | 0.00                    | 0.00          |
| 16   | 0.00                | 0.00         | 5.00                 | 0.00                    | 0.00          | 0.00                    | 0.00         | 7.50                 | 0.00                    | 0.00          |
| 18   | 0.00                | 0.00         | 5.00                 | 0.00                    | 0.00          | 0.00                    | 0.00         | 7.50                 | 0.00                    | 0.00          |
| 20   | 0.00                | 1.25         | 1.25 <sup>y</sup>    | 0.00                    | 0.00          | 0.00                    | 0.00         | 7.50 <sup>z</sup>    | 0.00                    | 0.00          |
| 22   | 0.00                | 1.25         | 2.50                 | 0.00                    | 1.25          | 0.00                    | 0.00         | 0.00                 | 0.00                    | 0.00          |
| 24   | 0.00                | 0.00         | 1.25                 | 0.00                    | 0.00          | 0.00                    | 2.50         | 0.00                 | 0.00                    | 0.00          |

<sup>a,b</sup>Indicate significant difference ( $p < 0.05$ ) compared with other weeks within the same group

<sup>y,z</sup>Indicate significant difference ( $p < 0.05$ ) between groups within the same week

**Table 2.** Percentage of vaccinated and non-vaccinated goats showing clinical signs during the study period

| Week | Vaccinated (n = 50) |              |                      |                         |               | Non-vaccinated (n = 50) |              |                     |                         |               |
|------|---------------------|--------------|----------------------|-------------------------|---------------|-------------------------|--------------|---------------------|-------------------------|---------------|
|      | Sneezing (%)        | Coughing (%) | Nasal discharge (%)  | Difficult breathing (%) | Mortality (%) | Sneezing (%)            | Coughing (%) | Nasal discharge (%) | Difficult breathing (%) | Mortality (%) |
| 0    | 0.00                | 0.00         | 10.00 <sup>a,y</sup> | 0.00                    | 0.00          | 0.00                    | 0.00         | 0.00 <sup>z</sup>   | 0.00                    | 0.00          |
| 1    | 2.00                | 2.00         | 2.00                 | 0.00                    | 0.00          | 2.00                    | 2.00         | 2.00                | 0.00                    | 0.00          |
| 2    | 0.00                | 0.00         | 4.00                 | 0.00                    | 0.00          | 0.00                    | 0.00         | 4.00                | 0.00                    | 0.00          |
| 3    | 0.00                | 4.00         | 4.00                 | 0.00                    | 0.00          | 0.00                    | 3.00         | 0.00                | 0.00                    | 0.00          |
| 4    | 0.00                | 4.00         | 6.00 <sup>a,y</sup>  | 0.00                    | 0.00          | 0.00                    | 4.00         | 2.00 <sup>z</sup>   | 0.00                    | 0.00          |
| 5    | 0.00                | 0.00         | 3.00                 | 0.00                    | 0.00          | 0.00                    | 0.00         | 0.00                | 0.00                    | 0.00          |
| 6    | 0.00                | 2.00         | 2.00                 | 0.00                    | 0.00          | 0.00                    | 0.00         | 0.00                | 0.00                    | 0.00          |
| 7    | 0.00                | 2.00         | 0.00                 | 0.00                    | 0.00          | 0.00                    | 2.00         | 0.00                | 0.00                    | 0.00          |
| 8    | 0.00                | 0.00         | 0.00                 | 0.00                    | 0.00          | 0.00                    | 2.00         | 0.00                | 0.00                    | 0.00          |
| 9    | 0.00                | 0.00         | 4.00 <sup>a</sup>    | 0.00                    | 0.00          | 0.00                    | 0.00         | 2.00                | 0.00                    | 0.00          |
| 10   | 0.00                | 2.00         | 0.00                 | 0.00                    | 0.00          | 0.00                    | 0.00         | 0.00                | 0.00                    | 0.00          |
| 11   | 0.00                | 0.00         | 2.00                 | 0.00                    | 0.00          | 0.00                    | 0.00         | 2.00                | 0.00                    | 0.00          |
| 12   | 0.00                | 0.00         | 0.00                 | 0.00                    | 0.00          | 2.00                    | 10.00        | 4.00                | 0.00                    | 0.00          |

<sup>a,b</sup>Indicate significant difference ( $p < 0.05$ ) compared with other weeks within the same group

<sup>y,z</sup>Indicate significant difference ( $p < 0.05$ ) between groups within the same week



beginning and end of the trial, particularly at weeks 1 and 2 and at weeks 11 and 12; however, the difference remained insignificant ( $p > 0.05$ ). The non-vaccinated goats had coughing in the middle of the field trial, but it was significantly ( $p < 0.05$ ) highest at the end of the trial (week 12).

Generally, no significant differences ( $p > 0.05$ ) were observed in the number of vaccinated and non-vaccinated goats showing clinical signs throughout the study period. Nevertheless, all observed clinical signs were mild and did not progress to become severe.

Table 3 summarizes the BCS of vaccinated and non-vaccinated sheep throughout the 24-week study period. Most vaccinated and non-vaccinated sheep had a BCS score of 4 from weeks 1 to 12, indicating good health and feeding management, whereas almost half of the sheep were pregnant. The number of BCS 4 in both groups started to decrease after week 12 because some pregnant sheep had lambbed.

At the end of the study period at week 24, the number of vaccinated sheep with BCS 4 was significantly higher ( $p < 0.05$ ) than that of non-vaccinated sheep, while the number of sheep with BCS 2 was significantly ( $p < 0.05$ ) lower than that of the non-vaccinated sheep.

Table 4 shows the BCS of vaccinated and non-vaccinated goats throughout the 12-week field trial.

Most goats (92% vaccinated and 100% non-vaccinated) had a BCS of 4 starting from week 2 and remained so until week 10 ( $p > 0.05$ ).

#### Identification of *Mannheimia haemolytica*

*Mannheimia haemolytica* was detected (Fig. 1) in most animals (>80%) throughout the study duration (Table 5), indicating that *M. haemolytica* had already existed in the sheep flock, with the risk of triggering the infection when animals are exposed to stressful environments, such as transportation, weaning, change of diets, bullying, and monsoon season. Vaccination was able to reduce, although insignificantly ( $p > 0.05$ ), the number of animals carrying *M. haemolytica* during the monsoon season between November and December. All vaccinated goats carried *M. haemolytica* in their nasal cavity in the first 2 months of the study, but the percentage reduced to 60% from 80% in November and December. However, all non-vaccinated goats carried *M. haemolytica* throughout the 12-week trial. Nevertheless, the differences were insignificant ( $p > 0.05$ ).

#### Serum antibody levels

Figure 2 shows the antibody pattern throughout the 24-week trial period. Following vaccination, the antibody levels of vaccinated sheep had increased significantly ( $p < 0.05$ ), higher than week 0, as compared to the non-

**Table 3.** Weekly body condition score (BCS) of vaccinated and non-vaccinated sheep throughout the study period.

| Week | Vaccinated (n = 80) |                    |                     | Non-vaccinated (n = 80) |                    |                    |
|------|---------------------|--------------------|---------------------|-------------------------|--------------------|--------------------|
|      | Score 2 (%)         | Score 3 (%)        | Score 4 (%)         | Score 2 (%)             | Score 3 (%)        | Score 4 (%)        |
| 0    | 0.00 <sup>a</sup>   | 13.75 <sup>b</sup> | 86.25 <sup>a</sup>  | 2.50 <sup>a</sup>       | 0.00 <sup>a</sup>  | 97.50 <sup>a</sup> |
| 1    | 5.00 <sup>a</sup>   | 6.25 <sup>a</sup>  | 88.75 <sup>a</sup>  | 16.25 <sup>b</sup>      | 8.75 <sup>a</sup>  | 75.00 <sup>a</sup> |
| 2    | 1.25 <sup>a</sup>   | 3.75 <sup>a</sup>  | 95.00 <sup>a</sup>  | 23.75 <sup>b</sup>      | 3.75 <sup>a</sup>  | 72.50 <sup>b</sup> |
| 3    | 0.00                | 10.00 <sup>a</sup> | 90.00 <sup>a</sup>  | 10.00 <sup>a</sup>      | 10.00 <sup>a</sup> | 80.00 <sup>a</sup> |
| 4    | 1.25 <sup>a</sup>   | 12.50 <sup>b</sup> | 86.25 <sup>a</sup>  | 10.00 <sup>b</sup>      | 7.50 <sup>a</sup>  | 82.50 <sup>a</sup> |
| 5    | 2.50 <sup>a</sup>   | 5.00 <sup>b</sup>  | 92.50 <sup>b</sup>  | 11.25 <sup>a</sup>      | 8.75 <sup>a</sup>  | 80.00 <sup>a</sup> |
| 6    | 0.00 <sup>a</sup>   | 0.00 <sup>a</sup>  | 100.00 <sup>a</sup> | 7.50 <sup>a</sup>       | 3.75 <sup>a</sup>  | 88.75 <sup>a</sup> |
| 7    | 0.00 <sup>a</sup>   | 0.00 <sup>a</sup>  | 100.00 <sup>a</sup> | 3.75 <sup>a</sup>       | 3.75 <sup>a</sup>  | 92.50 <sup>a</sup> |
| 8    | 0.00 <sup>a</sup>   | 0.00 <sup>a</sup>  | 100.00 <sup>b</sup> | 3.75 <sup>a</sup>       | 10.00 <sup>b</sup> | 86.25 <sup>b</sup> |
| 9    | 1.25 <sup>a</sup>   | 2.50 <sup>a</sup>  | 96.25 <sup>a</sup>  | 1.25 <sup>a</sup>       | 2.50 <sup>a</sup>  | 96.25 <sup>a</sup> |
| 10   | 1.25 <sup>a</sup>   | 2.50 <sup>a</sup>  | 96.25 <sup>a</sup>  | 3.75 <sup>b</sup>       | 10.00 <sup>b</sup> | 86.25 <sup>b</sup> |
| 11   | 0.00 <sup>a</sup>   | 2.50 <sup>a</sup>  | 97.50 <sup>a</sup>  | 0.00 <sup>a</sup>       | 2.50 <sup>a</sup>  | 97.50 <sup>a</sup> |
| 12   | 10.00 <sup>a</sup>  | 11.25 <sup>a</sup> | 78.75 <sup>a</sup>  | 8.75 <sup>a</sup>       | 8.75 <sup>a</sup>  | 82.50 <sup>a</sup> |
| 14   | 13.75 <sup>a</sup>  | 16.25 <sup>a</sup> | 70.00 <sup>a</sup>  | 28.75 <sup>a</sup>      | 48.75 <sup>b</sup> | 22.50 <sup>b</sup> |
| 16   | 10.00 <sup>a</sup>  | 40.00 <sup>a</sup> | 50.00 <sup>a</sup>  | 31.25 <sup>a</sup>      | 58.75 <sup>a</sup> | 10.00 <sup>b</sup> |
| 18   | 13.75 <sup>a</sup>  | 51.2 <sup>a</sup>  | 35.00 <sup>a</sup>  | 23.75 <sup>a</sup>      | 65.00 <sup>a</sup> | 11.25 <sup>a</sup> |
| 20   | 18.75 <sup>a</sup>  | 46.25 <sup>a</sup> | 35.00 <sup>a</sup>  | 33.75 <sup>b</sup>      | 53.75 <sup>a</sup> | 12.50 <sup>b</sup> |
| 22   | 16.25 <sup>a</sup>  | 46.25 <sup>a</sup> | 37.50 <sup>a</sup>  | 35.00 <sup>b</sup>      | 55.00 <sup>a</sup> | 10.00 <sup>b</sup> |
| 24   | 27.50 <sup>a</sup>  | 37.50 <sup>a</sup> | 35.00 <sup>a</sup>  | 41.25 <sup>b</sup>      | 56.25 <sup>a</sup> | 2.50 <sup>b</sup>  |

<sup>a,b</sup>Different superscripts in each row indicate significant difference ( $p < 0.05$ ) between the vaccinated and non-vaccinated sheep within the same week.

**Table 4.** Weekly body condition score (BCS) of vaccinated and non-vaccinated goats throughout the study period.

| Week | Vaccinated (n = 50) |                   |                     | Non-vaccinated (n = 50) |                   |                     |
|------|---------------------|-------------------|---------------------|-------------------------|-------------------|---------------------|
|      | Score 2 (%)         | Score 3 (%)       | Score 4 (%)         | Score 2 (%)             | Score 3 (%)       | Score 4 (%)         |
| 0    | 0.00 <sup>a</sup>   | 6.00 <sup>a</sup> | 94.00 <sup>a</sup>  | 0.00 <sup>a</sup>       | 0.00 <sup>b</sup> | 100.00 <sup>a</sup> |
| 1    | 2.00 <sup>a</sup>   | 6.00 <sup>a</sup> | 92.00 <sup>a</sup>  | 0.00 <sup>a</sup>       | 2.00 <sup>b</sup> | 98.00 <sup>a</sup>  |
| 2    | 0.00 <sup>a</sup>   | 4.00 <sup>a</sup> | 96.00 <sup>a</sup>  | 0.00 <sup>a</sup>       | 2.00 <sup>a</sup> | 98.00 <sup>a</sup>  |
| 3    | 0.00 <sup>a</sup>   | 2.00 <sup>a</sup> | 98.00 <sup>a</sup>  | 0.00 <sup>a</sup>       | 0.00 <sup>a</sup> | 100.00 <sup>a</sup> |
| 4    | 0.00 <sup>a</sup>   | 0.00 <sup>a</sup> | 100.00 <sup>a</sup> | 0.00 <sup>a</sup>       | 0.00 <sup>a</sup> | 100.00 <sup>a</sup> |
| 5    | 0.00 <sup>a</sup>   | 2.00 <sup>a</sup> | 98.00 <sup>a</sup>  | 0.00 <sup>a</sup>       | 0.00 <sup>a</sup> | 100.00 <sup>a</sup> |
| 6    | 0.00 <sup>a</sup>   | 0.00 <sup>a</sup> | 100.00 <sup>a</sup> | 0.00 <sup>a</sup>       | 0.00 <sup>a</sup> | 100.00 <sup>a</sup> |
| 7    | 0.00 <sup>a</sup>   | 0.00 <sup>a</sup> | 100.00 <sup>a</sup> | 0.00 <sup>a</sup>       | 0.00 <sup>a</sup> | 100.00 <sup>a</sup> |
| 8    | 0.00 <sup>a</sup>   | 2.00 <sup>a</sup> | 98.00 <sup>a</sup>  | 2.00 <sup>a</sup>       | 6.00 <sup>b</sup> | 92.00 <sup>a</sup>  |
| 9    | 0.00 <sup>a</sup>   | 0.00 <sup>a</sup> | 100.00 <sup>a</sup> | 0.00 <sup>a</sup>       | 0.00 <sup>a</sup> | 100.00 <sup>a</sup> |
| 10   | 0.00 <sup>a</sup>   | 0.00 <sup>a</sup> | 100.00 <sup>a</sup> | 0.00 <sup>a</sup>       | 0.00 <sup>a</sup> | 100.00 <sup>a</sup> |
| 11   | 0.00 <sup>a</sup>   | 2.0 <sup>a</sup>  | 98.00 <sup>a</sup>  | 0.00 <sup>a</sup>       | 2.00 <sup>a</sup> | 98.00 <sup>a</sup>  |
| 12   | 0.00 <sup>a</sup>   | 6.00 <sup>a</sup> | 94.00 <sup>a</sup>  | 0.00 <sup>a</sup>       | 2.00 <sup>a</sup> | 98.00 <sup>a</sup>  |

<sup>a,b</sup>Different superscripts in each row indicate significant difference ( $p < 0.05$ ) between the vaccinated and non-vaccinated sheep within the same week.

**Table 5.** Isolation rate (%) of *Mannheimia haemolytica* before and monthly following vaccination.

| Month             | Sheep (n = 48) |                | Goats (n = 30) |                |
|-------------------|----------------|----------------|----------------|----------------|
|                   | Vaccinated     | Non-vaccinated | Vaccinated     | Non-vaccinated |
| Pre-vaccination:  |                |                |                |                |
| September         | 48 (100%)      | 48 (100%)      | 30 (100%)      | 30 (100%)      |
| Post-vaccination: |                |                |                |                |
| October           | 48 (100%)      | 48 (100%)      | 30 (100%)      | 30 (100%)      |
| November          | 43 (90%)       | 34 (80%)       | 18 (60%)       | 30 (100%)      |
| December          | 34 (80%)       | 43 (90%)       | 24 (80%)       | 30 (100%)      |
| January           | 48 (100%)      | 48 (100%)      | ND             | ND             |
| February          | 43 (90%)       | 34 (80%)       | ND             | ND             |
| March             | 48 (100%)      | 48 (100%)      | ND             | ND             |

ND = not done.

vaccinated group. Figure 2 shows that the antibody levels of the vaccinated group revealed a significant ( $p < 0.05$ ) increasing pattern from weeks 1 to 2, and remained significantly higher ( $p < 0.05$ ) than the non-vaccinated animals, reaching the highest levels between weeks 12 and 18.

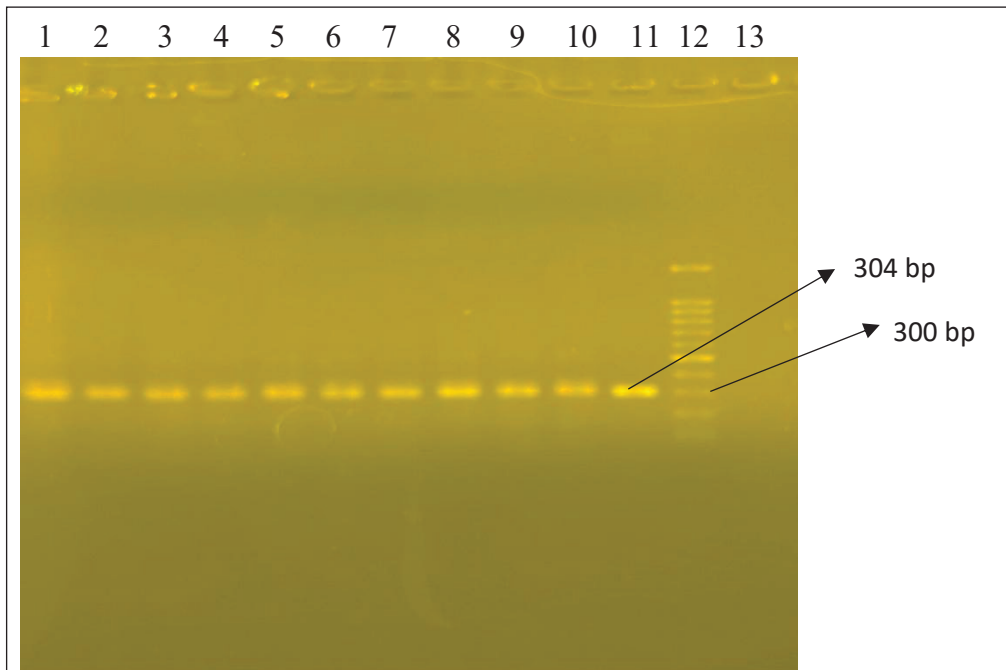
The antibody levels of the non-vaccinated group remained significantly ( $p < 0.05$ ) lower than those of the vaccinated group throughout the 24-week field trial, except at week 10. Although the antibody levels of the non-vaccinated group were slightly increased from week 2 to week 18, they remained significantly low throughout the study period.

Figure 3 shows the pattern of goat antibodies throughout the trial period. The antibody levels of

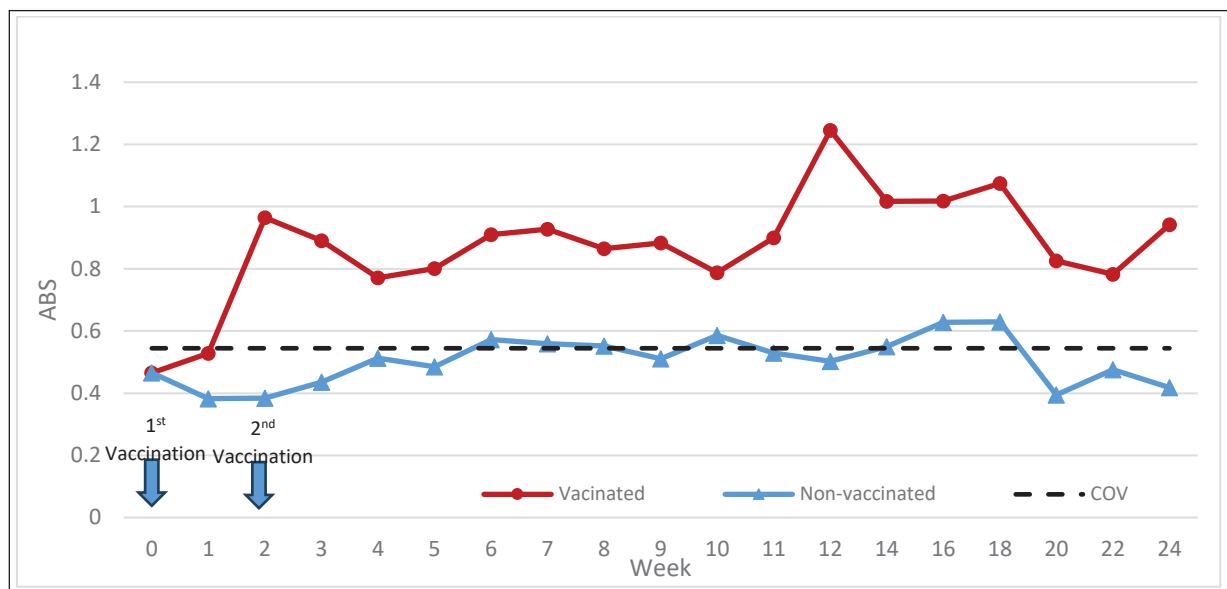
vaccinated goats increased significantly ( $p < 0.05$ ) and remained high throughout the 12-week trial period. On the other hand, the antibody levels of the non-vaccinated goats remained significantly low ( $p < 0.05$ ) throughout the study period, except weeks 3 and 11, but remained significantly lower ( $p < 0.05$ ) than the vaccinated goats.

## Discussion

This field trial was conducted to evaluate the safety and immunogenicity of a newly manufactured mannheimiosis vaccine, the STVac7<sup>TM</sup> on sheep and goats for a period of between 12 (3 months) and 24 weeks (6 months). The field vaccinations did not cause significant side effects within the first 24 and



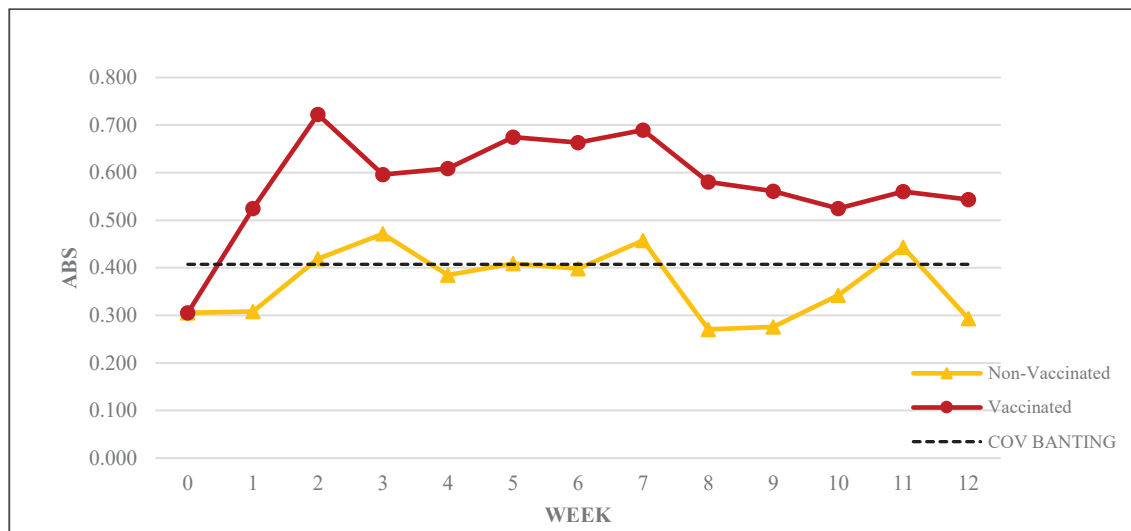
**Fig. 1.** Results of polymerase chain reaction for swab samples of sheep collected from Johor. Positive results reveal the 304-bp band. Lane 1–10 = swab samples, lane 11 = *Mannheimia haemolytica*-positive sample, lane 12 = DNA ladder, lane 13 = negative sample.



**Fig. 2.** Antibody levels of vaccinated and non-vaccinated sheep following vaccination with STVac7. Antibodies of vaccinated sheep remained high throughout the 24-week study period.

48 hours after the first and booster doses, when mild and brief nasal discharge was occasionally observed, involving 3.1% of the animals, which is considerably low. Similarly, nasal discharge was occasionally observed throughout the 12- and 24-week trial periods, followed by brief coughing, which are among the

common clinical signs of pneumonic mannheimiosis (Laishevtsev, 2020). Since these signs involved both vaccinated and non-vaccinated groups, the possibility of dusty concentrate supplementation contributing to these conditions could not be ruled out (Anderson *et al.*, 2010).



**Fig. 3.** Antibody levels of vaccinated and non-vaccinated goats following vaccination with STVac7. Antibodies of vaccinated goats remained high throughout the 12-week study period.

*Mannheimia haemolytica* was already present in the sheep and goats before the start of the experiment. The most common serotype worldwide is *M. haemolytica* A2 (Zamri et al., 2025). This condition is common in most sheep and goat farms worldwide (Abbas et al., 2019), indicating that the animals have been exposed to the bacterium and may be susceptible to infection following stress. However, this field trial that involved vaccinating sheep and goats prior to the monsoon season (September) seems to be able to maintain the livestock's health during the monsoon months of October to January, as observed in previous controlled experiments. A similar concept of vaccinating livestock against pneumonic manheimiosis before stressful conditions was also recommended by Sabri et al. (2013) and Abate and Kassa (2023). Intranasal vaccination has been shown to stimulate mucosal immunity of the respiratory tract via IgA production (Hill et al., 2019; Uddin et al., 2024), which prevents bacterial adherence to the respiratory tract (Joseph, 2022). Simultaneously, systemic immunity is stimulated via IgG (Kehagia et al., 2023) that protects hosts from systemic infection throughout the stressful period. However, vaccinating animals against pneumonic manheimiosis during stress may lead to vaccination failure (Intan-Shameha and Zamri-Saad, 2005; Roth, 2007). Stress causes immunosuppression, which increases susceptibility to infections (Alotiby, 2024).

Despite the occasional nasal discharge and coughing, the number of vaccinated sheep and goats with BCS 4 was similar to that of non-vaccinated goats and sheep. The fact that vaccination against pneumonic manheimiosis does not enhance the live weight of livestock has been previously reported by Goodwin-Ray et al. (2008); Phythian (2020) and Gardner et al. (2024). Similarly, this field vaccination study

concluded that vaccination of sheep and goats against pneumonic manheimiosis has no negative effect on the overall body condition of sheep and goats.

Antibody level is an important indicator of pneumonic manheimiosis vaccination (Abera and Mossie, 2023). Following vaccination, antibody levels significantly increased and remained significantly high throughout the study period, reaching the highest levels between weeks 12 and 18. It should be noted that under an uncontrolled field trial, such as this study, the immune responses of vaccinated animals were believed to be due to the effect of vaccination, especially when the non-vaccinated groups did not show obvious stimulation. In fact, an earlier experiment concluded that vaccination stimulates antibody production and prevents pneumonic manheimiosis (Sabri et al., 2000), whereas manheimiosis vaccines prepared at the laboratory scale resulted in a similar antibody pattern (Sabri et al., 2013) that could protect vaccinated animals from challenge infection (Bkiri et al., 2023). The serum antibody levels in this field trial were found to be high and stable throughout the 24-week study period. Thus, 6-monthly boosters recommended for STVac7<sup>TM</sup> should be able to maintain the animals' immune status during the anticipated stressful conditions, particularly the stressful seasonal change, such as monsoon, winter, and summer seasons, and other stressful conditions, including transportation and weaning stresses (Sabri et al., 2013; Jesse et al., 2018). Most injectable pneumonic pasteurellosis vaccines recommend yearly boosters (Tizard, 2021; Abera and Mossie, 2023). It is important to note that the vaccination regimen is an extremely important aspect of vaccination against pneumonic manheimiosis, and it depends on the type of vaccine and the immunization trials of the particular vaccine (Ahmad et al., 2014).



Nevertheless, the current field study involves a sheep and a goat farm. Therefore, these results provide a preliminary indication of the safety and efficacy of the STVac7<sup>TM</sup> vaccine. Perhaps more farms should be included in future studies to ensure vaccine efficacy.

### Conclusion

Intranasal vaccination with STVac7<sup>TM</sup> is generally safe and well tolerated by sheep and goats in the field. Intranasal vaccination resulted in high serum antibody levels while retaining the animals' health status and body condition throughout the 6-month study period, particularly during the monsoon season.

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### Authors' contributions

MZS and NP conceptualized the study and reviewed the manuscript. ZAMZ and NP contributed to the initial drafting of the manuscript, whereas RMAAB and MJAJ were responsible for data collection and compilation.

### Conflict of interest

The authors have no conflicts of interest to declare.

### Data availability

Data are available upon request from the corresponding author.

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