

Preliminary Study on the Safety and Efficacy of a Newly Manufactured STVac7 Vaccine against Pneumonic Mannheimiosis of Goats

Annas Salleh^{1*}, Norazrina Pakiman², Syafiqah Adilah Sharidon², Nuralia Raihana Rohzaili², and Mohd Zamri-Saad^{1,2}

¹Department of Veterinary Laboratory Diagnosis, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia

²BioAngle Vacc Sdn. Bhd., Unit DG2, UPM-MTDC Technology Centre 3, 43400 UPM Serdang, Malaysia

ABSTRACT

Pneumonic manheimiosis is a respiratory disease of small ruminants caused by *Mannheimia haemolytica*. It leads to deaths and great economic loss, and the most effective control measure is through vaccination. This study evaluates the safety and efficacy of a newly manufactured STVac7 vaccine in goats against *M. haemolytica*. Five groups of goats with 3 goats per group received either freshly prepared vaccines (Group 1), 21-month-old formulations (Group 2), high dose vaccination (Group 3), vaccine containing a stabiliser and preservative (Group 4) and control, no vaccination (Group 5). Key clinical signs, including respiratory rates, body temperatures and adverse events, particularly sneezing and coughing, were monitored throughout the 12-week study period. All goats were challenged with 10⁹ CFU/mL of live *M. haemolytica* in week 10. Following vaccination, mild sneezing and coughing were occasionally observed. Post-challenge, clinical signs were most severe in the control Group 5, while *M. haemolytica* was isolated from all groups, with a higher frequency in the control Group 5. This was consistent with the more severe gross and histopathological lung lesions in control Group 5, while bronchus-associated lymphoid tissue (BALT) was mostly observed in Group 1 and least observed in control Group 5. Subsequently, high levels of IgG were detected in

vaccinated goats, particularly Group 4. Fibrinous bronchopneumonia, a key lesion of pneumonic manheimiosis was observed only in the control Group 5. In summary, the STVac7 vaccine is safe and effective in protecting goats from severe pneumonic manheimiosis.

ARTICLE INFO

Article history:

Received: 04 May 2025

Accepted: 24 February 2026

Published: 27 February 2026

DOI: <https://doi.org/10.47836/pjtas.49.1.23>

E-mail addresses:

annas@upm.edu.my (Annas Salleh)

norazrina@bioanglevac.com (Norazrina Pakiman)

syafiqah@bioanglevac.com (Syafiqah Adilah Sharidon)

nuraliaraihana@bioanglevac.com (Nuralia Raihana Rohzaili)

mzamri@upm.edu.my (Mohd Zamri Saad)

*Corresponding author

Keywords: Efficacy, goats, manheimiosis, safety, STVac7

INTRODUCTION

Mannheimiosis, a significant respiratory disease affecting goats and sheep, is caused by the bacterium *Mannheimia haemolytica* (Shiferaw et al., 2006), a Gram-negative bacterium belonging to the Pasteurellaceae family. This pathogen is renowned for its role in causing acute and chronic pneumonia, particularly in ruminants (Taunde et al., 2019). *Mannheimia haemolytica* thrives in the respiratory tract of goats, causing damage to the lung tissues that impair the respiratory function. The bacterium's potent toxins and virulence factors contribute to the severity of the disease (Zecchinon et al., 2005).

Mannheimiosis is globally distributed and can affect small ruminant populations in various countries. It is more prevalent in regions with intensive livestock production and can lead to considerable economic losses due to reduced productivity, increased mortality rates, and treatment costs (Wang et al., 2018; Girma et al., 2023). Goats exposed to stressors such as inclement weather, transportation, parasitism and malnourishment are highly susceptible to the disease (Abera & Mossie, 2022; Taunde et al., 2019). The widespread occurrence of the disease underscores its importance and highlights the need for effective control and prevention strategies.

Pneumonic mannheimiosis typically presents with dyspnoea, coughing, nasal discharge, and increased respiratory rate (Rawat et al., 2019). Affected goats may exhibit decreased feed intake and lethargy. Severe cases can lead to high morbidity and mortality rates, particularly in young and immune-compromised animals. Gross pathological findings often include lung consolidation, pleuritis and fibrinous adhesions, particularly in the cranioventral aspect of the lungs (Emikpe & Akpavie, 2012; Rawat et al., 2019). On histopathological examination, lung lesions are characterised by bronchopneumonia, interstitial pneumonia, emphysema and haemorrhage. These lesions collectively contribute to impaired lung function and compromised respiratory efficiency.

Efforts to manage pneumonic mannheimiosis primarily involve disease prevention through vaccination and biosecurity measures (Shahudin et al., 2018). Intranasal vaccines, such as those containing live attenuated *M. haemolytica* antigens, have proven effective in inducing local immunity in the respiratory tract (Tenuche & Emikpe, 2015). These vaccines can help reduce the severity of clinical signs and lessen the economic impact of the disease. Intranasal vaccines offer the advantage of stimulating a rapid mucosal immune response at the site of entry, enhancing the animal's ability to fend off the bacterium upon exposure, while also ensuring ease of delivery and better vaccine coverage (Annas & Zamri-Saad, 2021). Proper vaccination protocols, including timing and booster doses, are critical for optimal protection.

STVac7 is an intranasal spray vaccine that has been developed by Universiti Putra Malaysia. It contains inactivated whole cells of *M. haemolytica* and was found to cross-protect against serotypes A2, A7, and A9 (Sabri et al., 2000). While studies on the efficacy

of laboratory-scaled STVac7 spray vaccine have been carried out, the safety and shelf-life efficacy of this vaccine following manufacturing under a GMP facility have yet to be determined. This paper reports the impact of administering a newly manufactured STVac7 vaccine and a 21-month-old STVac7 vaccine in goats. At the same time, the study also assesses the toxicity or adverse effects of administering a high dose of a freshly prepared STVac7 and the STVac7 containing polysorbate 80 and benzyl alcohol as a stabiliser.

MATERIALS AND METHODS

Ethical Approval

All use of animals and experimental protocols was granted approval by the Institutional Animal Care and Use Committee (IACUC) of Universiti Putra Malaysia, under the reference number UPM/IACUC/AUP-R004/2023.

Animal Selection and Management

A group of 15 adult Boer breed goats between 25 and 30 kg bodyweight, comprising both male and female specimens, was carefully chosen for the study. These selected goats were unvaccinated and exhibited apparent good health. Before the final selection, serum samples were collected from each goat and subjected to ELISA to determine the antibody levels against *M. haemolytica*. Those with low antibody levels against *M. haemolytica* were subsequently chosen. These selected goats underwent a 7-day acclimatisation period and were housed at the Animal Experimental House within the Faculty of Veterinary Medicine at Universiti Putra Malaysia (UPM) for the duration of the study.

During acclimatisation, all goats received a subcutaneous administration of ivermectin at 1 ml per 50 kg bodyweight, as a component of a routine deworming protocol. Throughout the course of the study, the goats' diet consisted of freshly harvested Napier grass and a commercial goat pellet, both supplied at a rate equivalent to 1.0% of their body weight. Additionally, they were provided unrestricted access to drinking water.

Experimental Design

At the start of the experiment, selected goats were randomly divided into five groups with 3 goats in each group. Groupings were done by a member of the research team based on animal ID. Each group was kept in a separate enclosure. Subsequently, different types of STVac7 vaccine were administered intranasally into each group, respectively. The vaccines were manufactured in a GMP facility at BioAngle Vacs Sdn. Bhd., based on the protocol described by Sabri et al. (2013) and were administered to the respective group as outlined in Table 1.

Vaccinations were performed on two separate occasions, on days 1 and 14 of the study. Subsequently, on day 70, all goats across the groups were challenged with an inoculum containing 10^9 CFU/ml of live *M. haemolytica* A7 through an intratracheal administration method to ensure disease development (Smith et al., 2020). On day 91 of the study, or 21 days after challenge, all goats were euthanised by means of slaughtering and were immediately followed by necropsy procedures.

Table 1

Summary of grouping and the various types of vaccine preparation administered

Group	Treatment
Group 1	Freshly manufactured STVac7 vaccine (< 1 month; 10^6 cells/mL)
Group 2	STVac7 that has been kept for 21 months. The vaccine was stored at room temperature and was checked for stability and presence of intact antigen at 3-month interval
Group 3	High-dose freshly prepared STVac7 vaccine (< 1 month; 10^8 cells/mL)
Group 4	Freshly manufactured STVac7 vaccine containing 0.01% Polysorbate 80 and 2% Benzyl Alcohol as vaccine stabilisers (Corcoran & Ray, 2014; Ieven et al., 2021)
Group 5	PBS placebo vaccine

Clinical Observations and Samplings

Following administrations of the vaccine, all goats were closely monitored for adverse effects of vaccination, which included coughing, sneezing, lethargy, loss of appetite, oronasal discharge and mortality. The monitoring was conducted at 24- and 48-hours post-vaccination.

After the challenge with the live *M. haemolytica* A7, daily observations were carried out for three consecutive weeks to monitor for clinical signs of pneumonic manheimiosis. These signs included oronasal discharge, pyrexia (rectal temperature $\geq 40^\circ\text{C}$), coughing, sneezing, dullness or lethargy, abnormal respiration, loss of appetite, depression, and death.

Throughout the study, all animals were consistently observed for health status. Any abnormal clinical signs or adverse effects were meticulously recorded by a veterinarian. The severity of these events was assessed, with particular attention to the frequency and duration of coughing and sneezing, which were classified as mild or severe. Mild reactions were defined by occasional or intermittent symptoms, while severe reactions were characterised by continuous or frequent occurrence.

Prior to and at weekly intervals following vaccination, serum samples were collected from all goats throughout the 12-week study period. The sera were subjected to ELISA according to Poonsuk et al. (2023) to determine the antibody levels. The serum was diluted to 1:1,200 while the secondary antibody was diluted to 1:10,000.

For dead or euthanised animals, a thorough examination of all organs was carried out by a veterinary pathologist, with particular emphasis on the lungs, while liver and kidney

lesions were observed for goats in Groups 3 and 4. Gross lesions that were present within the lungs were documented and described. Subsequently, pneumonic lesions were scored based on the percentage of areas affected (Table 2) according to Bkiri et al. (2023). Samples of lung, liver, and kidney were collected into sterile plastic bags for the isolation of *M. haemolytica* (Abate & Fentie Kassa, 2023). Similarly, samples were collected for histopathology analysis, where lungs were examined for pneumonic lesions, while liver and kidneys were examined for toxicity lesions, particularly for Group 3 and 4. The lungs encompassed the right apical lobe and the cranial part of the left apical lobe, preserved by immersion in a solution of 10% neutral buffered formalin, allowing for fixation to occur over a period of no less than 28 h.

The formalin-fixed lung samples were subjected to routine processing for histopathology examination involving paraffin embedding, sectioning at 4 μ m and stained with haematoxylin and eosin. Each slide was blindly examined under light microscopy by a veterinary pathologist, involving five microscopic fields at magnification 200 \times , before the severity of the histopathology lesions was scored. Score 0 represented a normal tissue, signifying unaffected tissue; score 1 represented mild severity, involving less than 25% of the tissue; score 2 denoted moderate severity, affecting between 25% and 50% of the tissue; and score 3 represented severe lesions, involving more than 50% of the tissue. At the same time, all bronchi and bronchioles were examined at 100 \times and 200 \times magnifications and their numbers, along with the number of bronchus-associated lymphoid tissue (BALT), were counted and noted before the numbers of BALT were then normalised by dividing the number of BALT by the number of bronchi.

Statistical Analysis

All data were methodically arranged into tabular formats before comparative analysis among the different groups was performed using the One-way Analysis of Variance (ANOVA) statistical method. This was followed by the implementation of Tukey's post hoc test to identify specific group differences. Additionally, cumulative severity scores for each organ and respective group were calculated, facilitating a comprehensive assessment of the overall impact. Similar comparative evaluations were conducted to analyse the cumulative severity scores across the groups. All statistical analyses were done using SPSS version 20.

Table 2
Summary of gross lung lesion scoring

Extent of the Lesion	Score
0% (normal)	0
1–24%	2
25–49%	4
\geq 50%	6

RESULTS

Adverse Effects

Between 24 and 48h post-initial vaccination, one goat from each of Groups 1, 2, 4, and 5 showed mild, transient sneezing ($p>0.05$). Similarly, a goat from Group 1 showed mild sneezing, while a goat from each of Group 2 and Group 3 showed mild, transient coughing following a booster dose on week 2 ($p>0.05$). Table 3 summarises the adverse events among goats following challenge with live *M. haemolytica*. Analyses revealed no significant ($p>0.05$) differences between all treated groups.

Respiratory Rate and Body Temperature

Table 4 summarises post-challenge respiratory rate and body temperature for the five groups of goats. Both respiratory rate and body temperature were within the normal range, although there were significant ($p<0.05$) differences in the respiratory rates of treated goats compared to control but not the body temperatures ($p>0.05$). Groups 1 and 2 showed significantly ($p<0.05$) higher, while Group 3 showed significantly ($p<0.05$) lower respiratory rates than control Group 5. Groups 1, 3, and 4 showed significantly ($p<0.05$) lower body temperature than that of control Group 5.

Table 3

Adverse events among goats following challenge with live Mannheimia haemolytica

Adverse Events	Group 1 (n=3)	Group 2 (n=3)	Group 3 (n=3)	Group 4 (n=3)	Group 5 (n=3)
Mild sneezing	2	2	1	2	2
Severe sneezing	0	1	0	0	0
Mild coughing	2	1	1	0	2
Severe coughing	0	0	0	1	0
Dullness/lethargy	1	2	1	0	0
Oronasal discharge	1	1	2	2	1
Loss of appetite	0	0	0	1	0
Depression	0	0	0	0	0
Death	0	0	0	0	0

Note. All data showed no significant differences between groups

Table 4

Average respiratory rate and body temperature following challenge of goats subjected to different treatments

Parameter	Group 1 (n=3)	Group 2 (n=3)	Group 3 (n=3)	Group 4 (n=3)	Group 5 (n=3)
Respiratory rate (breaths/min)	36.8 ± 8.4 ^c	36.2 ± 13.6 ^c	31.1 ± 5.3 ^b	34.8 ± 9.5 ^a	33.9 ± 9.3 ^a
Body temperature (°C)	38.6 ± 0.3 ^a	38.9 ± 0.3 ^b	38.6 ± 0.4 ^a	38.6 ± 0.2 ^a	38.8 ± 0.4 ^b

Note. ^{a,b} Different superscripts in the same row indicate significant differences ($p<0.05$)

Gross Pathology

The lungs of all animals in Group 1 showed consolidated and pneumonic lesions in the right apical and accessory lobes, with areas of petechiations. Cut surface revealed the presence of frothy oedema fluid. One goat showed pneumonia and mild petechiations with meaty lung consistency involving the left side of the lung. Group 2 showed acute pneumonia with a reduction in sponginess, involving the entire lungs or some parts of the lungs. A low amount of oedema fluid was observed on the cut surface. One goat of this group showed mild congestion and haemorrhage, involving the three left lung lobes. Similarly, Group 3 showed acute pneumonia with a reduction of sponginess involving the entire lungs or some parts of the lungs. The lung parenchyma of all goats showed congestion in the left cranial and caudal parts of the apical lobes, and the right apical lobe. No consolidation was noted. A low amount of oedema fluid was observed from the cut surface of all lungs, particularly in the pneumonic and congested areas.

In Group 4, only one goat showed mild oedema and acute pneumonia involving the right apical lobe. No other lesion was seen. On the other hand, all goats of Group 5 showed lesions consistent with fibrinous pneumonia. In two goats, fibrin tags were seen on the visceral pleural surface of the left cranial and caudal parts of the apical lobes, as well as the right apical lobe. The parenchyma of the left cranial and caudal parts of the apical lobes, the right apical lobe, the accessory lobe, and some parts of both caudal lobes were consolidated. The other goat showed fibrinous deposition leads to adhesion of the left cranial part of the apical lobe to the pericardium, and the right goat showed moderate consolidation and pneumonia in the middle lung lobe. These lobes were consolidated, along with the left and right caudal lobes. Congestion and oedema were observed in the lungs of all goats, involving most of the lung lobes.

Total lung lesion scoring of all treated groups was mild (range between 0.22-0.15 and 1.56 ± 0.47) with no significant difference ($p > 0.05$) between the treated groups but were significantly ($P < 0.05$) lower than the control Group 5 that showed moderate lung lesions of 3.67 ± 0.46 . Similarly, consolidation and presence of fibrin, which are among the major gross lung lesions of pneumonic mannheimiosis were significantly ($p < 0.05$) more severe in control Group 5 and were insignificant ($p > 0.05$) among the four treated groups. Acute pneumonia is another important lung lesion, which was significantly ($p < 0.05$) less severe in Groups 1, 2 and 4 than the control Group 5 (Table 5). Pulmonary congestions were extremely low in treated groups but was moderately high in control Group 5 ($p < 0.05$). Pulmonary haemorrhages (petechiation) are not a consistent lesion in pneumonic mannheimiosis, and this study revealed insignificant ($p > 0.05$) differences between all groups.

Table 5

Gross lesion scoring in the lungs of goats experimentally infected by Mannheimia haemolytica

Lesions	Group 1	Group 2	Group 3	Group 4	Group 5
Pneumonia	2.00 ± 0.00 ^b	4.00 ± 1.15 ^{a,b}	5.33 ± 0.67 ^a	0.67 ± 0.67 ^b	5.33 ± 0.67 ^a
Consolidation	1.33 ± 0.67 ^b	1.33 ± 0.67 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	4.67 ± 0.67 ^a
Congestion	0.00 ± 0.00 ^b	1.33 ± 1.76 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	4.00 ± 1.15 ^a
Fibrin	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	2.67 ± 0.67 ^a
Oedema	1.33 ± 0.67 ^b	2.00 ± 0.00 ^b	2.00 ± 0.00 ^b	0.67 ± 0.67 ^b	4.67 ± 0.67 ^a
Haemorrhage	1.33 ± 0.67 ^a	0.67 ± 0.67 ^a	0.67 ± 0.67 ^a	0.00 ± 0.00 ^a	0.67 ± 0.67 ^a
Total score	1.00 ± 0.24 ^b	1.56 ± 0.41 ^b	1.56 ± 0.47 ^b	0.22 ± 0.15 ^b	3.67 ± 0.46 ^a

Note. ^{a,b} Different superscripts in the same row indicate significant differences (p<0.05)

Histopathology Description

In this study, the histological examinations of the lungs of goats infected with *M. haemolytica* revealed several distinct lesions. Many of the bronchi of goats in Group 1 showed large-sized and activated BALT (Figure 1a). Mild to moderate bronchopneumonia was noted, characterised by a low to moderate number of lymphocytes surrounding several bronchi and bronchioles (Figure 1b), but rarely involving the neighbouring lung parenchyma. However, inflammatory reactions in the lungs were characterised by interstitial pneumonia involving alveolar macrophages, which were seen in all goats of Group 1 (Figure 1c) but affected only certain areas of the lung, causing mild to moderate thickening of the alveolar septa. Most areas of the lung parenchyma appeared normal with occasional mild atelectasis, while mild to moderate emphysema was occasionally seen in some areas of the lung sections. Pulmonary haemorrhage was mild and was seen only in one lung lobe of one goat. On the other hand, the number of lymphocytes was especially high in the wall of bronchi and bronchioles that showed the presence of BALT.

Lungs of Group 2 revealed a very low number of respiratory airways with the presence of BALT. When BALT was present, they appeared small to moderate in size (Figure 2a). Nevertheless, moderate to very severe bronchopneumonia was noted (Figure 2b), characterised by moderate to high numbers of lymphocytes found surrounding many bronchi and bronchioles, which sometimes involved the neighbouring lung parenchyma. Inflammations of the lungs were characterised only by interstitial pneumonia, causing moderate to severe thickening of the alveolar septa. Therefore, areas of normal lung parenchyma were rarely identified, with the exception of one lung lobe of one animal that showed extensive normal lung parenchyma. Atelectasis was mild, while emphysema was mild to moderate, seen in many areas of the sections. Severe pulmonary haemorrhage was seen only in one lung lobe of one goat. Pulmonary oedema (Figure 2c) and interlobular oedema were none to moderate.

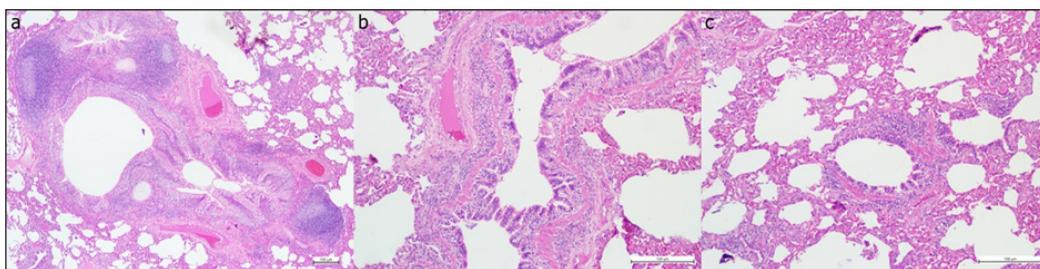


Figure 1. Examples of histopathological findings in the lungs of Group 1. a: Large BALT surrounding the respiratory airways (bar=100 μ m); b: Mild bronchopneumonia characterised by a low infiltration of lymphocytes (bar=100 μ m); c: Moderate bronchopneumonia and interstitial pneumonia (bar=100 μ m)

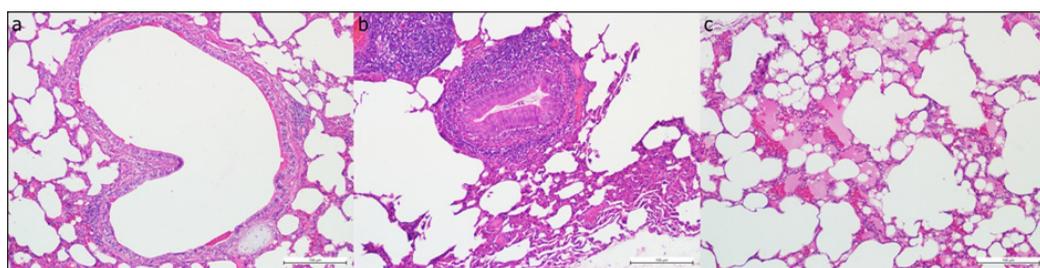


Figure 2. Examples of histopathological findings in the lungs of Group 2. a: Rare observation of an extremely small BALT (bar=100 μ m); b: Severe bronchopneumonia with emphysema in the surrounding parenchyma (bar=100 μ m); c: Mild pulmonary oedema and haemorrhage (bar=100 μ m)

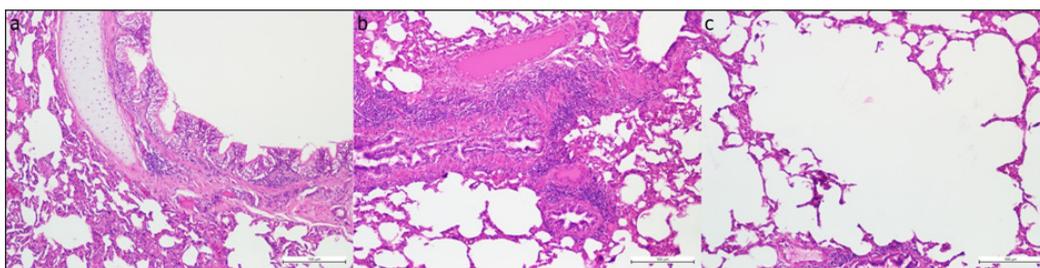


Figure 3. Examples of histopathological findings in the lungs of Group 3. a: Rare observation of a small BALT (bar=100 μ m); b: Severe bronchopneumonia (bar=100 μ m); c: An area of pulmonary emphysema (bar=100 μ m)

Lungs of Group 3 showed a low number of respiratory airways with the presence of BALT. When BALT was present, they appeared small in size (Figure 3a). Moderate to severe bronchopneumonia was apparent (Figure 3b), featuring a moderate to high influx of lymphocytes surrounding many bronchi and bronchioles, and their neighbouring lung parenchyma. However, inflammations of the lungs were characterised only by interstitial pneumonia, which was extensive, leading to moderate to severe thickening of the alveolar septa. Areas of normal lung parenchyma were occasionally identified, and emphysema was

mild to moderate (Figure 3c), seen at many areas of the sections, while atelectasis was mild. Perivascular oedema and pulmonary oedema were not observed.

The lungs of Groups 4 showed some bronchi with medium- to large-sized, activated BALT (Figure 4a). Mild to severe bronchopneumonia was noted, characterised by a low to high number of lymphocytes surrounding several bronchi and bronchioles that occasionally involved the neighbouring lung parenchyma. As Group 1, the number of lymphocytes tends to be high, especially in the wall of bronchi and bronchioles that show the presence of BALT. Inflammations of the lungs were characterised by interstitial pneumonia, which was seen in all goats of Group 4, but affected only certain areas of the lung, causing mild to moderate thickening of the alveolar septa (Figure 4b). Areas of normal lung parenchyma were frequently identified (Figure 4c), occasionally accompanied by limited regions of mild atelectasis and mild to moderate emphysema.

The lungs of Group 5 showed an extremely low number of respiratory airways, which showed the presence of BALT. When present, they were extremely small. However, broncho-pneumonia was generally severe to very severe, characterised by a high to very high number of lymphocytes surrounding several bronchi and bronchioles, and tends to involve the surrounding lung parenchyma (Figure 5a). Inflammations of the lungs were

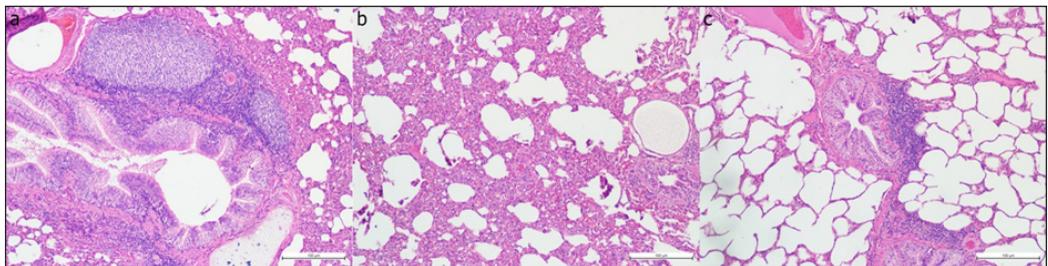


Figure 4. Examples of histopathological findings in the lungs of Group 4. a: Large-sized BALT surrounding a respiratory airway (bar=100 µm); b: Moderately severe interstitial pneumonia (bar=100 µm); c: Moderate bronchopneumonia, note that the surrounding parenchyma is not affected (bar=100 µm)

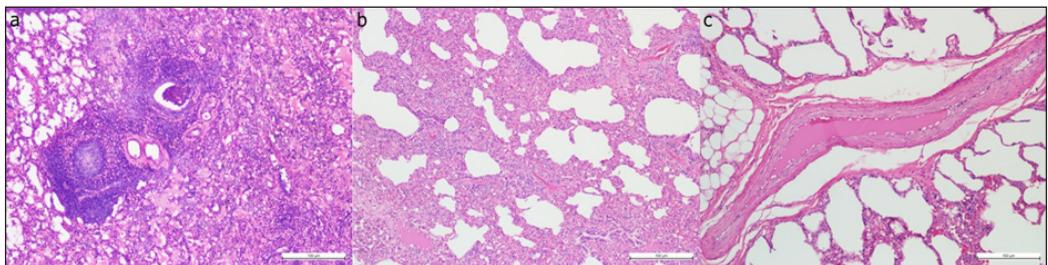


Figure 5. Examples of histopathological findings in the lungs of Group 5. a: Severe bronchopneumonia and pneumonia, accompanied by pulmonary oedema (bar=100 µm); b: Severe interstitial pneumonia (bar=100 µm); c: Perivascular oedema as evidenced by separation and lifting of the connective tissue surrounding the blood vessel (bar=100 µm)

characterised by interstitial pneumonia, which was seen in all goats and affected extensive areas of the lung sections, leading to severe to very severe thickening of the alveolar septa (Figure 5b). Areas of normal lung parenchyma were only occasionally identified; emphysema was moderate to severe, while atelectasis was mild. Mild to severe pulmonary haemorrhage was observed in two goats, and mild presence of fibrin in the alveolar space and pleural surface was seen in all goats of this group. Mild pulmonary oedema was observed in one goat; pleural oedema was moderate to severe (Figure 5c), while interlobular oedema was severe. None of the groups showed significant gross and histopathology lesions in the livers and kidneys.

Histopathology Evaluation

Table 6 summarises the mean scores of lung lesions along with the standard errors of mean (SEM) for each lesion in each group. Among the lesions, bronchopneumonia, emphysema, presence of fibrin, interlobular oedema, perivascular oedema, and total lesion score displayed significant ($p < 0.05$) variations across groups. Scores for bronchopneumonia were notably high in Group 5 compared to the other groups ($p < 0.05$). Pneumonia was primarily observed as interstitial pneumonia with thickening of the alveolar septa. Despite the severity of pneumonia in Group 5, no significant difference ($p > 0.05$) was observed among all groups.

Emphysema was observed in all groups, with the most severe in Group 4 and the least severe in Group 1. In fact, emphysema was significantly less severe ($p < 0.05$) in Group 1 compared to Groups 4 and 5, but not significant ($p > 0.05$) compared to Groups 2 and 3. On the other hand, the presence of fibrin was only noted in Group 5, a significant ($p < 0.05$)

Table 6

Histopathological lung lesion scores in goats following an experimental infection by Mannheimia haemolytica

Lesions	Group 1	Group 2	Group 3	Group 4	Group 5
Bronchopneumonia	1.17 ± 0.20 ^{a,b}	1.33 ± 0.15 ^{a,b}	1.10 ± 0.16 ^{a,b}	0.97 ± 0.18 ^a	1.67 ± 0.18 ^b
Pneumonia	1.50 ± 0.22 ^a	1.53 ± 0.21 ^a	1.23 ± 0.21 ^a	1.37 ± 0.21 ^a	2.00 ± 0.21 ^a
Emphysema	0.37 ± 0.10 ^a	0.77 ± 0.14 ^{a,b}	0.70 ± 0.13 ^{a,b}	0.90 ± 0.11 ^b	0.87 ± 0.13 ^b
Atelectasis	0.20 ± 0.07 ^a	0.17 ± 0.07 ^a	0.37 ± 0.13 ^a	0.17 ± 0.10 ^a	0.57 ± 0.15 ^a
Haemorrhage	0.53 ± 0.09 ^a	0.67 ± 0.15 ^a	0.30 ± 0.10 ^a	0.33 ± 0.10 ^a	0.63 ± 0.14 ^a
Fibrin	0.00 ± 0.00 ^a	0.33 ± 0.10 ^b			
Pleural oedema	1.07 ± 0.14 ^a	1.70 ± 0.20 ^a	1.43 ± 0.19 ^a	1.50 ± 0.18 ^a	1.70 ± 0.16 ^a
Interlobular oedema	0.37 ± 0.12 ^a	0.53 ± 0.13 ^a	1.63 ± 0.19 ^b	0.63 ± 0.17 ^a	0.93 ± 0.20 ^a
Perivascular oedema	0.00 ± 0.00 ^a	0.33 ± 0.13 ^b	0.33 ± 0.12 ^b	0.20 ± 0.09 ^a	0.77 ± 0.19 ^b
Pulmonary oedema	0.00 ± 0.00 ^a	0.07 ± 0.05 ^a	0.27 ± 0.14 ^a	0.00 ± 0.00 ^a	0.30 ± 0.13 ^a
Total score	0.52 ± 0.05 ^a	0.71 ± 0.05 ^{a,b}	0.74 ± 0.06 ^b	0.61 ± 0.05 ^{a,b}	0.98 ± 0.06 ^c

Note. ^{a,b} Different superscripts in the same row indicate significant differences ($p < 0.05$)

observation compared to other groups. However, interlobular oedema was significantly more severe ($p < 0.05$) in Group 3 compared to the remaining groups, while perivascular oedema was most severe in Group 5, and was significant ($p < 0.05$) compared to Groups 1 and 4.

Ultimately, the mean total lung lesion score indicated that Group 5 exhibited the highest lesion severity score (0.98 ± 0.06), which was significant ($p < 0.05$) compared to the other groups. In contrast, Group 1 showed the lowest total lung lesion score of 0.52 ± 0.05 , significantly ($p < 0.05$) lower compared to Groups 3 and 5, but not significantly ($p > 0.05$) compared to Groups 2 and 4.

BALT Evaluation

Among the five groups, Group 1 exhibited the highest number of BALT (17.67 ± 5.55), followed by Group 4 (12.17 ± 3.74), while Group 2 showed the lowest number of BALT (5.50 ± 1.57). However, no significant difference ($p > 0.05$) was observed between all five groups. For the number of bronchi and bronchioles observed, Group 5 recorded the highest count of these respiratory airways at 50.83 ± 3.74 , while the lowest count was observed in Group 1 (34.00 ± 3.62). Similarly, no significant difference ($p > 0.05$) was noted for the number of respiratory airways evaluated. The BALT count was normalised and compared between the groups, revealing that Group 1 displayed the highest normalised BALT count (52.86 ± 13.92), followed by Group 4 (25.51 ± 8.34). No significant difference ($p < 0.05$) was observed between Groups 1 and 4. The remaining Groups 2, 4, and 5 generally exhibited low normalised BALT counts ranging between 13.92 ± 3.87 and 16.96 ± 4.46 . The normalised BALT count of Group 1 was significantly higher ($p < 0.05$) compared to Groups 2, 4, and 5 (Table 7).

Table 7

Number of BALT in the lungs of goats following an experimental infection by Mannheimia haemolytica

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
No. of BALT	17.67 ± 5.55	5.50 ± 1.57	7.33 ± 2.80	12.17 ± 3.74	8.00 ± 1.41
No. of bronchi and bronchioles	34.00 ± 3.62	38.00 ± 3.93	35.33 ± 6.60	48.50 ± 4.28	50.83 ± 3.74
Normalised BALT count	52.86 ± 13.92^b	13.92 ± 3.87^a	16.96 ± 4.46^a	$25.51 \pm 8.34^{a,b}$	16.61 ± 4.01^a

Note. ^{a,b} Different superscripts in the same row indicate significant differences ($p < 0.05$)

No superscripts in the same row indicate no significant difference ($p < 0.05$) between all groups

Bacterial Isolation

Table 8 summarises the rate of bacterial isolation from the lung, liver, and kidney of goats from different groups following vaccination and subsequent challenge with live *M.*

haemolytica. The bacterium was successfully isolated from all five groups, with Group 5 exhibiting the highest percentage of isolation at 89.0%, which was significantly ($p < 0.05$) higher than Group 3 at 44.0%. Groups 1, 2 and 4 displayed similar bacterial isolation rates of 67.0%, which was not significant ($p > 0.05$) compared to Group 5.

Table 8
Rate of isolation of *Mannheimia haemolytica* from lungs, liver, and kidneys of goats of different groups

Organ	Group 1 (n = 3)	Group 2 (n = 3)	Group 3 (n = 3)	Group 4 (n = 3)	Group 5 (n = 3)
Lungs	2 (66.7%)	2 (66.7%)	2 (66.7%)	3 (100%)	2 (66.7%)
Liver	2 (66.7%)	2 (66.7%)	0 (0.0%)	1 (33.3%)	3 (100%)
Kidneys	2 (66.7%)	2 (66.7%)	1 (33.3%)	2 (66.7%)	3 (100%)
Average %	67% ^{a,b}	67% ^{a,b}	44% ^b	67% ^{a,b}	89% ^a

Note. ^{a,b} Different superscripts in the same row indicate a significant difference ($p < 0.05$)

Serum Antibody Response

Figure 6 summarises the level and pattern of antibody response by goats of different groups. Following administration of STVac7 at Week 0, there was a significant ($p < 0.05$) elevation of antibody against *M. haemolytica* in Groups 1, 3, and 4 compared to control Group 5 at Week 1. Group 2 displayed lower antibody levels and remained similar level ($p > 0.05$) as the control Group 5. At Week 2 post-vaccination, all vaccinated groups showed

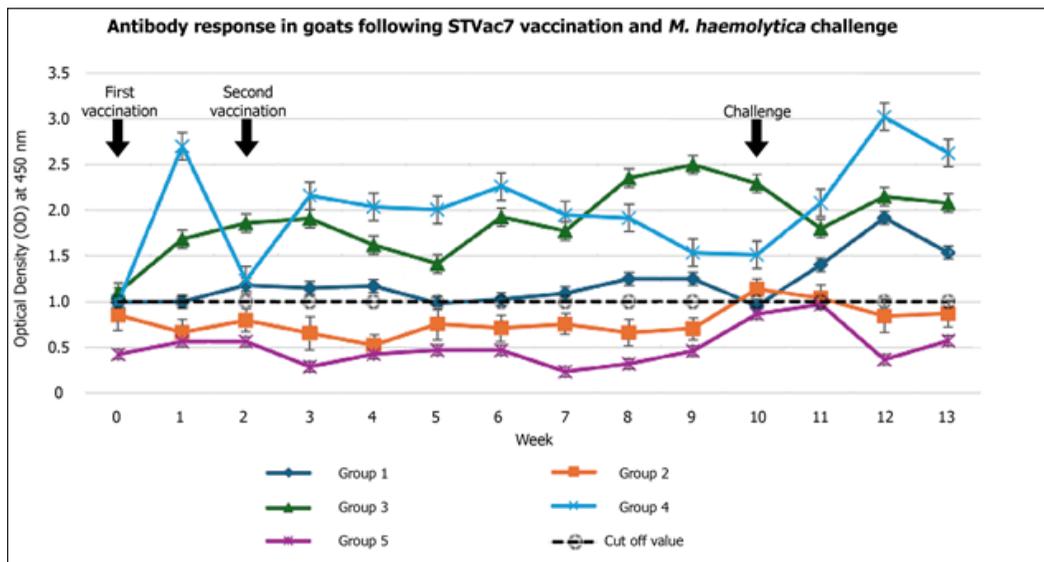


Figure 6. Antibody levels and pattern of different groups of goats following vaccination with STVac7 at weeks 0 and 2 followed by challenge with live *M. haemolytica* at week 10

significantly ($p < 0.05$) higher antibody levels than the control group. Following the booster dose at Week 2, antibody levels of all vaccinated groups remained significantly ($p < 0.05$) higher than the control Group 5 for 5 weeks post-second vaccination.

At the challenge in Week 10, all vaccinated groups showed higher antibody levels than the control Group 5. Following the challenge, antibody levels increased further in Group 3 and 4, with Group 4 showing ($p < 0.05$) higher antibody levels than all other groups for 2 weeks post-challenge. Group 3 experienced a decline in IgG levels at Week 11 post-challenge but exhibited a gradual increase in the subsequent week.

In general, all vaccinated groups showed higher antibody levels than the control group throughout the 12-week study period. Upon challenge at week 10, the antibody levels of all vaccinated groups were high, indicating protection against infection except Group 5 (Figure 6). Nevertheless, it seemed that vaccination with STVac7 containing stabilisers and preservatives was most effective in inducing a robust humoral immune response against *M. haemolytica*.

DISCUSSION

Findings from this study suggest that GMP-manufactured STVac7 is safe and effective. All groups did not show significant clinical signs following vaccination, either with freshly prepared vaccine, with 21-month-old vaccine, with a higher dose or with vaccine that contains a stabiliser and preservative. The few animals that showed mild sneezing and coughing were regarded as normal (Stockler et al., 2020), possibly due to inhaling the powder-formed concentrate supplementation given to them each morning. Furthermore, all vaccinated groups showed high antibody levels, except those vaccinated with the 21-month-old vaccine, but remained higher than the control unvaccinated throughout the study period. Therefore, at the time of challenge, the antibody levels of all four vaccinated groups were high, indicating the efficacy of these vaccine preparations. In fact, pathology findings revealed that all vaccinated groups developed various types of microscopic and macroscopic lesions, but were extremely mild compared to the control unvaccinated goats of Group 5, which showed fibrinous pneumonia involving the antero-ventral aspect of the lungs. Fibrinous pneumonia is recognised as a classical pulmonary lesion in cases of pneumonic manheimiosis (Sharma et al., 2011). Although pneumonia was noted in all vaccinated groups, it was generally mild and lacked the fibrinous component. Furthermore, pulmonary consolidation and oedema were most severe in Control Group 5. In fact, gross and histopathology could not conclude that a certain type of vaccine preparation can confer the best protection against pneumonic manheimiosis due to insignificant differences between vaccinated Groups 1, 2, 3, and 4.

Nevertheless, based on the severity scores of bronchopneumonia, emphysema, interlobular oedema and the total lung lesions, it is suggested that the freshly prepared

STVac7 vaccine containing preservative and stabiliser is the best vaccine preparation. In fact, adding 0.01% Polysorbate 80 and 2% Benzyl alcohol into the vaccine was found to produce good effects (Wahlgren et al., 2025). Polysorbate 80 is a non-ionic surfactant known for its role in stabilising protein-based biologics (Doost et al., 2018), potentially preventing antigen degradation or aggregation over time. Its inclusion may help maintain antigenic integrity and improve delivery by enhancing solubility and dispersion of the antigen particles, particularly in powder-formed vaccines. Benzyl alcohol, a commonly used antimicrobial preservative in injectable and mucosal formulations, likely contributes to the vaccine's long-term stability and sterility without compromising the immunogenicity (Ruiz et al., 2003). In this case, both freshly prepared and preservative-added vaccines resulted in high normalised BAL count and high antibody levels (Effendy et al., 1998) compared to the challenged non-vaccinated goats throughout the study period. These minimise the development of lung lesions following challenge by live *M. haemolytica*. Although a higher concentration of antigen in the vaccine resulted in a slightly higher lung lesion score and a low normalised BAL count, the antibody pattern was high and impressive.

A newly manufactured STVac7 has shown early indication of an effective vaccine following this preliminary study. However, further studies should focus on large-scale field trials to validate STVac7's efficacy under diverse farm conditions and disease challenges. Exploring the duration of immunity and need for boosters is also important for long-term protection. Optimising the formulation with stabilisers and preservatives to improve shelf-life without reducing efficacy will help advance STVac7 as a practical, scalable solution for controlling pneumonic manheimiosis in small ruminants. Therefore, STVac7 can be an alternative vaccine against pneumonic manheimiosis, especially when reports have suggested low efficacy of the currently available pneumonic manheimia vaccine in some parts of the world (Zamri et al., 2025). Furthermore, this intranasal vaccine is easy to use and user-friendly, especially for the many smallholders.

CONCLUSION

Vaccinating goats with STVac7 vaccine against pneumonic manheimiosis either the freshly prepared, the 21-month-old, the higher dose or those with stabiliser and preservative, resulted in high antibody levels and subsequent mild pathology lesions. However, the freshly prepared STVac7 vaccine containing stabiliser and preservative is the best vaccine preparation to be used against pneumonic manheimiosis. Administration of the freshly prepared vaccine resulted in the least severe macroscopic and microscopic lesions, as well as the highest normalised BAL count.

ACKNOWLEDGEMENT

The authors would like to thank Madam Noor Shazreena Ishak of BioAngle Vacs for the support, and Mr. Aiman Zulkhikim Zulkifli of BioAngle Vacs and Mr. Noraziman Sulaiman of the Faculty of Veterinary Medicine, Universiti Putra Malaysia, for the technical assistance.

REFERENCES

- Abate, F.M., & Fentie Kassa, T. (2023). Isolation and identification of *Mannheimia haemolytica* and *Pasteurella multocida* from symptomatic and asymptomatic sheep and their antibiotic susceptibility patterns in three selected districts of north Gondar zone, Gondar Ethiopia. *Veterinary Medical Science*, 9(4), 1803-1811. <https://doi.org/10.1002/vms3.1166>
- Abera, D., & Mossie, T. (2022). A review on pneumonic pasteurellosis in small ruminants. *Journal of Applied Animal Research*, 51(1), 1-10. <https://doi.org/10.1080/09712119.2022.2146123>
- Annas, S., & Zamri-Saad, M. (2021). Intranasal vaccination strategy to control the COVID-19 pandemic from a veterinary medicine perspective. *Animals*, 11(7), Article 1876. <https://doi.org/10.3390/ani11071876>
- Bkiri, D., Elmejdoub, S., Bamouh, Z., Fihri, O.F., & El-Harrak, M. (2023). Comparative protection of small ruminants against *Mannheimia haemolytica* infection by inactivated bacterin and toxoid vaccines. *Veterinary World*, 16(1), 68-75. <https://doi.org/10.14202/vetworld.2023.68-75>
- Corcoran, G. B., & Ray, S. D. (2014). *Toxicology, encyclopedia*. In S. D. Ray (Ed.), *Encyclopedia of toxicology* (3rd ed., pp. 123-145). Elsevier. <https://doi.org/10.1016/B978-0-12-386454-3.00251-7>
- Doost, A.S., Dewettinck, K., Devlieghere, F., & Van der Meeren, P. (2018). Influence of non-ionic emulsifier type on the stability of cinnamaldehyde nano-emulsions: A comparison of polysorbate 80 and hydrophobically modified inulin. *Food Chemistry*, 258, 237-244. <https://doi.org/10.1016/j.foodchem.2018.03.078>
- Effendy, A.W.M., Zamri-Saad, M., Maswati, M.A., Ismail, M.S., & Jamil, S.M. (1998). Stimulation of the bronchus-associated lymphoid tissue and its effect on *in-vitro* colonisation by *Pasteurella haemolytica*. *Veterinary Research Communication*, 22(3), 147-153. <https://doi.org/10.1023/A:1006064703662>
- Emikpe, B.O., & Akpavie, S.O. (2012). Clinicopathological observations in experimental Peste Des Petit Ruminants virus and *Mannheimia haemolytica* A:2 co-infection in goats. *Nigerian Journal of Physiological Sciences*, 27(2), 129-136.
- Girma, S., Getachew, L., Beyene, A., Tegegne, D.T., Tesgera, T., Debelo, M., Deban, J., Teshome, D., Abdisa, K., Wirtu, A., Tekle, M., Abera, B., Tafess, K., Dandecha, M., Abayneh, K., Gatachew, B., Tufa, T.B., & Tolera, T. S. (2023). Identification of serotypes of *Mannheimia haemolytica* and *Pasteurella multocida* from pneumonic cases of sheep and goats and their antimicrobial sensitivity profiles in Borana and Arsi zones, Ethiopia. *Scientific Reports*, 13(1), 9008. <https://doi.org/10.1038/s41598-023-36026-2>
- Ieven, T., Van Weyebergh, T., Vandebotmermet, M., Devolder, D., Breyeraert, C., & Schrijvers, R. (2021). Tolerability of polysorbate 80-containing COVID-19 vaccines in confirmed polyethylene glycol-allergic patients. *Journal of Allergy and Clinical Immunology: In Practice*, 9(12), 4470-4472. <https://doi.org/10.1016/j.jaip.2021.09.039>

- Poonsuk, K., Kordik, C., Hille, M., Cheng, T.-Y., Crosby, W. B., Woolums, A.R., Clawson, M.L., Chitko-McKown, C., Brodersen, B., & Loy, J.D. (2023). Detection of *Mannheimia haemolytica*-specific IgG, IgM and IgA in sera and their relationship to respiratory disease in cattle. *Animals*, 13(9), Article 1531. <https://doi.org/10.3390/ani13091531>
- Rawat, N., Gilhare, V.R., Kushwaha, K.K., Hattimare, D.D., Khan, F.F., Shende, R.K., & Jolhe, D.K. (2019). Isolation and molecular characterisation of *Mannheimia haemolytica* and *Pasteurella multocida* associated with pneumonia of goats in Chhattisgarh. *Veterinary World*, 12(2), 331-336. <https://doi.org/10.14202/vetworld.2019.331-336>
- Ruiz, L., Reyes, N., Duany, L., Franco, A., Aroche, K., & Rando, E.H. (2003). Long-term stabilisation of recombinant human interferon α 2b in aqueous solution without serum albumin. *International Journal of Pharmaceutics*, 264(1-2), 57-72. [https://doi.org/10.1016/S0378-5173\(03\)00388-0](https://doi.org/10.1016/S0378-5173(03)00388-0)
- Sabri, M.Y., Zamri-Saad, M., Mutalib, A.R., Israf, D.A., & Muniandy, N. (2000). Efficacy of an outer membrane protein of *Pasteurella haemolytica* A2, A7 or A9-enriched vaccine against intratracheal challenge exposure in sheep. *Veterinary Microbiology*, 73(1), 13-23. [https://doi.org/10.1016/S0378-1135\(99\)00205-9](https://doi.org/10.1016/S0378-1135(99)00205-9)
- Sabri, M.Y., Shahrom-Salisi, M., & Emikpe, B.O. (2013). Comparison prior and post vaccination of inactivated recombinant vaccine against manheimiosis in Boer goat farm in Sabah. *Journal of Vaccine and Vaccination*, 4: 173. <https://doi.org/10.4172/2157-7560.1000173>
- Shahudin, M.S., Ghani, A.A.A., Zamri-Saad, M., Zuki, A.B., Abdullah, F.F.J., Wahid, H., & Hassim, H.A. (2018). The necessity of a herd health management programme for dairy goat farms in Malaysia. *Pertanika Journal of Tropical Agricultural Science*, 41(1), 1-18. <http://www.pertanika.upm.edu.my/pjtas/browse/regular-issue?article=JTAS-0849-2016>
- Sharma, R.K., Patil, R.D., Kishtwaria, R.S., & Asrani, R.K. (2011). An outbreak of pneumonic manheimiosis in a livestock farm in sub-temperate region of India. *Haryana Veterinarian*, 50, 89-91. <https://www.luvas.edu.in/haryana-veterinarian/download/har-vet-2011/23.pdf>
- Shiferaw, G., Tariku, S., Ayelet, G., & Abebe, Z. (2006). Contagious caprine pleuropneumonia and *Mannheimia haemolytica*-associated acute respiratory disease of goats and sheep in Afar Region, Ethiopia. *Revue Scientifique et Technique – Office International des Epizooties*, 25(3), 1153-1163. <https://doi.org/10.20506/rst.25.3.1723>
- Smith, J.S., Mochel, J.P., Seo, Y.J., Ahrens, A.P., & Griffith, R.W. (2020). Evaluation of a *Pasteurella multocida* respiratory disease induction model for goats (*Capra aegagrus hircus*). *Comparative Medicine*, 70(5), 323-328. <https://doi.org/10.30802/AALAS-CM-20-000002>
- Stockler, R.M., Stockler, J.W., Shipley, C.F., & Pugh, D.G. (2020). Physical examination, handling, and restraint of sheep, goats, and cervids. In D. G. Pugh, A. N. Baird, M. A. Edmondson, & T. Passler (Eds.), *Sheep, goat, and cervid medicine* (3rd ed., pp. 1-14). Elsevier. 1-14. <https://doi.org/10.1016/B978-0-323-62463-3.00010-4>
- Taunde, P.A., Argenta, F.F., Bianchi, R.M., Cecco, B.S.D., Vielmo, A., Lopes, B.C., & Driemeier, D. (2019). *Mannheimia haemolytica* pleuropneumonia in goats associated with shipping stress. *Ciência Rural*, 49, Article e20180621. <https://doi.org/10.1590/0103-8478cr20180621>

- Tenuche, O.Z. & Emikpe, B.O. (2015). Effect of intranasal recombinant *Mannheimia haemolytica* vaccination on some haematological indices of goats infected with peste des petits ruminants virus. *Sokoto Journal of Veterinary Sciences*, 13(1), 48-52. <https://doi.org/10.4314/sokjvs.v13i1.7>
- Wahlgren, M., Borjesdotter, A-M., Hjalte, J., Martin, J. L., Zhang, L.P., Sjogren, H., & Ulvenlund, S. (2025). Interactions between polysorbate and antimicrobial preservatives in aqueous parenteral products. *Journal of Drug Delivery Science and Technology*, 107, 106765. <https://doi.org/10.1016/j.jddst.2025.106765>
- Wang, Y., Zhen, Z., Yang, Y., Zhang, X., Gao, S., & Cheng, D. (2018). Molecular prevalence and antimicrobial susceptibility of *Mannheimia haemolytica* isolated from fatal sheep and goats cases in Jiangsu, China. *Pakistan Veterinary Journal*, 38(3), 337-340. <https://doi.org/10.29261/pakvetj/2018.056>
- Zamri, Z.A.M., Shahridon, S.A., Jamaluddin, M.J.A., Pakiman, N., & Zamri-Saad, M. (2025). Prevalence of pneumonic manheimiosis of goats and sheep in Asia and Africa: a need for vaccine development. *International Journal of Veterinary Science*, 14(6), 1137-1151. <https://doi.org/10.47278/journl.ijvs/2025.078>
- Zecchinon, L., Fett, T., & Desmecht, D. (2005). How *Mannheimia haemolytica* defeats host defence through a kiss of death mechanism. *Veterinary Research*, 36(2), 133-156. <https://doi.org/10.1051/vetres:2004065>