Efficacy of *Mannheimia haemolytica* A2, A7, and A2 and A7 combined expressing iron regulated outer membrane protein as a vaccine against intratracheal challenge exposure in sheep

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This experimental study was done on a total of 40 male lambs with the objectives of developing experimental vaccines from *Mannheimia haemolytica* serotypes A2 and A7 that express iron regulated outer membrane protein and *in vivo* evaluation of their efficacy. Lambs were categorized in to four experimental groups and vaccinated with 1 ml of vaccine containing \(5 \times 10^8\) CFU/ml. Group 1 was vaccinated with *M. haemolytica* A2, group 2 with A7, group 3 with serotype A2 and A7 combination, and group 4 received saline as control. They were challenged intratracheally by the respective homologous serotype after 35 days of vaccination. Post challenge clinical investigation showed that significant higher rate of morbidity was seen in control group which was demonstrated by raised rectal temperature (by 0.5-1°C) and respiratory signs. From the total of 26 lambs challenged with live *M. haemolytica* A2 and A7, 6 (23.1%) and 4 (15.3%) lambs were found dead and sick, respectively. Higher mortality and morbidity were observed in unvaccinated control group; however, lesser was recorded in combined vaccinated group. Lung lesions of variable severity were observed in 13 (50.0%) lambs following challenge. From vaccinated groups, 5 (27.8%) lambs were found to have a +1 lung lesion score. All of the lambs in unvaccinated control group had scores between +2 and +3. There was a statistically significant difference (\(p < 0.05\)) between control and vaccinated groups, while no statistically significant difference (\(p > 0.05\)) was seen among vaccinated groups concerning lung lesion scores. Furthermore, the respective serotypes of *M. haemolytica* were successfully re-isolated from pneumonic lungs at a mean titre range of \(10^{2.2} – 10^{8.1}\) CFU/g. In conclusion, lambs which received combined vaccine confer relatively good protective efficacy than *M. haemolytica* A2 or A7 vaccinated groups. Therefore, further study should be done on evaluation of antibody titer at different time points.

**Key words:** Challenge, efficacy, lambs, lung lesion score, *Mannheimia haemolytica*, morbidity, mortality.

**INTRODUCTION**

Pneumonia is a major cause of economic losses in sheep industry, and *Mannheimia haemolytica* is one of the infectious agents most frequently associated with pathologic damage of ovine respiratory tract that causes fibrinous and necrotizing lobar pneumonia and pleurapneumonia (Haziroglu et al., 1994). *M. haemolytica* is an opportune-stic pathogen that inhabits the nasopharynx and tonsils of cattle and sheep (Radostits et al., 2006) and is capable of...
causing infection when the body’s defense mechanisms are impaired (Haig, 2011). Environmental stress factors like inclement weather, shipment, weaning, overcrowding and complex interactions among several infectious agents can serve as cofactors for pathogenesis of pneumonic pasteurellosis (Kraabel and Miller, 1997; Ganheim et al., 2005).

A major problem in the control of pneumonic pasteurellosis is the lack of vaccine which consistently induces protective immunity against M. haemolytica (Dyer, 1982). A number of live and killed vaccines have been developed and used, but their efficacy in field trials has been variable, ranging from no effect to reduced or even increased morbidity and mortality (Catt et al., 1985; Chengappa et al., 1988; Confer et al., 1988).

The composition of the bacterial surface is an influential factor in the interactions between pathogens and host defences (Costerton, 1988). The availability of iron appears to modify the surface composition of many pathogens (Neilands, 1982; Brown and Williams, 1985; Ikeda and Hirsh, 1988) including M. haemolytica (Deneer and Potter, 1989; Ogunnariwo and Schryvers, 1990) and these surface alterations may have a consequence in the pathogenesis of the respective disease. A variety of antigens, which may serve as potential immunogens, have been isolated from M. haemolytica. Outer membrane proteins (OMPs) of M. haemolytica seemed to be most important for stimulating immunity (Mosier et al., 1989a) and could be used in vaccine preparations (Gatewood et al., 1994; Pati et al., 1996). M. haemolytica A2 is the most common isolate from pneumonic lungs of sheep and goats throughout the world (Bahaman et al., 1991; Gilmour et al., 1991a; Davies and Donachie, 1996), and most research activities on the development of Mannheimia vaccine have focused on incorporating either a suitable isolate of M. haemolytica A2 or immunogenic antigens extracted from the serotype (Bahaman et al., 1991; Mosier, 1993). The outer membrane proteins of M. haemolytica A7 were effective in protecting animals against homologous and heterologous infection of live M. haemolytica A2, A7 and A9 (Sabri et al., 2000).

Despite annual vaccination programs against pasteurellosis using killed P. multocida biotype A containing vaccine (Ovine pasteurella vaccine), produced at the National Veterinary Institute, Ethiopia, high mortality and morbidity has been reported by farmers and veterinarians. Currently there is no effective vaccine that protects sheep from strains of M. haemolytica derived from different geographical origins. However, given the significant economic losses due to sheep pneumonia caused by this bacterium, it is necessary to develop a vaccine for the benefits of sheep industry. Therefore, the objectives of this experimental study are to develop M. haemolytica serotypes A2, A7, and A2 and A7 combined experimental vaccine that express iron regulated outer membrane protein and to conduct in vivo evaluation of their efficacy by challenge protection, lung lesion score and bacterial count.

**MATERIALS AND METHODS**

**Experiment Animals**

This experimental study was conducted at National Veterinary Institute (NVI), Debre Zeit, Ethiopia. A total of 40 male conventionally reared lambs, aged 6 – 9 months, with no history of vaccination against pneumonic pasteurellosis and free from clinical signs of pneumonia were selected for this study. They were individually identified with ear tags and dewormed with albendazole (7.5 mg/kg body weight) upon arrival at the experimental station. The trial was started only when all lambs were kept for two weeks of adaptation period and were screened for M. haemolytica A2 and A7 by indirect haemagglutination test. Animals were randomly assigned into four experimental groups and they were kept in identical environmental and management facilities in the same paddock.

**Experimental vaccines and adjuvant preparation**

The experimental vaccine used in this study was prepared at NVI Bacteriology Laboratory, Ethiopia. M. haemolytica serotypes A2 and A7 were selected and used as candidate vaccine strains based on their higher rate of isolation from different parts of Ethiopia (Zelege, 1998; Tefere, 2000; Sisay and Zerihun, 2003; Ayelet et al., 2004; Mulate, 2007). These strains were isolated and identified from different outbreaks of pneumonic pasteurellosis in the country based on sugar fermentation (arabinose and trehalose) and the organisms were serotyped based on capsular antigens using indirect haemagglutination test with M. haemolytica known serotype antisera and stored at NVI germ bank in a lyophilized form at -20°C.

The lyophilized form of M. haemolytica was cultured in tryptose soy broth (TSB) enriched with 10% horse serum. The tubes were pre-incubated for 48 h to check its sterility and the tubes with no contamination were used for bacterial growth. Serotype A2 and A7 were inoculated into the media separately and incubated at 37°C for 18 h. Bacterial growth was checked by examination of smears, turbidity and pH of the media.

Bacterial growth suspension that was harvested from TSB was cultured on tryptose soy agar (TSA), sheep blood agar and MacConkey agar. Purity, colony characteristics, haemolytic effect, oxidase test and growth on MacConkey agar were used as primary identification procedure. Analytical profile index (API) 20NE were used for biochemical identification. Finally, the identity of the isolates was confirmed serologically using IHA.

Iron restricted growth was achieved by growing the bacteria in 100 ml brain heart infusion (BHI) broth supplemented with iron chelator (2,2’- Dipyridyl) at a concentration of 150 µM. The culture was incubated at 37°C with rotatory shaking at 80 oscillations per minute for 18 hr as described by Gilmour et al. (1991b) and Confer et al. (1995).
Table 1. Summary of the experimental design for vaccination and challenge infection.

<table>
<thead>
<tr>
<th>Group</th>
<th>Vaccine type</th>
<th>No. of lambs</th>
<th>Vaccine dose (ml)</th>
<th>Type of challenge and no. of lambs</th>
<th>Inoculum Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A2</td>
<td>10</td>
<td>1</td>
<td>Live M. haemolytica A2 (5 lambs)</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>A7</td>
<td>10</td>
<td>1</td>
<td>Live M. haemolytica A7 (5 lambs)</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>Bivalent (A2 and A7)</td>
<td>10</td>
<td>1</td>
<td>Live M. haemolytica A2 (4 lambs) and A7 (4 lambs)</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>Sterile saline water</td>
<td>10</td>
<td>1</td>
<td>Live M. haemolytica A2 (4 lambs) and A7 (4 lambs)</td>
<td>4</td>
</tr>
</tbody>
</table>

The bacterial mass in the vaccine preparation was determined using titration and standard plate count method as described in Quinn et al. (2002). Counts were made on titrations that yield 30 to 300 colonies per plate. The viable count for each serotype used in the vaccine was 5 x 10^6 CFU/ml.

Inactivation of the culture was done using 0.5% formaldehyde and aluminum potassium sulfate (Alum) (10%) was used as an adjuvant.

Vaccination experimental design

At the start of the experiment, animals from group 1-3 were injected subcutaneously with 1 ml 5x10^5 CFU/ml of M. haemolytica, grown under iron restriction, and formalin-killed, with aluminum potassium sulfate adjuvant. Group 1 received the vaccine containing M. haemolytica serotype A2; group 2 received the vaccine containing M. haemolytica serotype A7; and group 3 received M. haemolytica serotype A2 and A7 combined vaccine. Group 4 was injected in the same manner using sterile saline water and used as unvaccinated controls (Table 1).

Challenge infection

Animals were immunosuppressed using dexamethason at a dose of 0.04 mg/kg body weight for three consecutive days (Charley, 1990) before challenge. According to Sabri et al. (2000), five weeks after vaccination, 5 animals from group 1 were challenged intra-tracheal with 4 ml inoculum containing 5.2 x 10^9 CFU/ml of live M. haemolytica A2. Similarly, 5 animals from group 2 were challenged intra-tracheal with 4 ml inoculum containing 5 x 10^8 CFU/ml of live M. haemolytica serotype A7. Group 3 was split into two groups: each containing 5 animals and one of the group was challenged with A2 and the other group by A7. Finally, half of the control group (5 animals each) was challenged with A2 and the other half with A7 as shown in Table 1.

Following the intratracheal challenge, all lambs were observed daily for signs of respiratory infection and death before the surviving lambs were slaughtered on day 10 post-challenge. Lambs were considered febrile when the mean rectal temperature was >39.1 according to Robertshaw (2004). The entire respiratory tracts were examined and the extent of the lung lesions was determined according to the method described by Akan et al. (2006).

Vaccine efficacy evaluation

The efficacy of the vaccine was determined based on the resistance of vaccinated lambs against M. haemolytica infection, or severity of pneumonia post challenge, the lung lesion score and dose of the bacterial titer from lung lesions.

Clinical investigation and necropsy following challenge

Rectal temperatures were recorded twice a day for 10 consecutive days and lambs were observed closely for clinical symptoms mainly related to respiratory signs including death if present. Necropsy was performed following 10 days post challenge and lungs were examined for M. haemolytica-induced lesions and scored accordingly. The time of death was used to categorize the infection as peracute (< 48 hrs), acute (3-7 days), sub acute (> 8 days) according to Shafarin (2009). Based on the percentage of consolidated lung masses, lung lesions were scored as follows: 0 (lungs without lesions), +1 (1 – 4% consolidation), +2 (5 – 14% consolidation), and +3 (≥15% consolidation) according to Akan et al. (2006).

Bacterial isolations and colony count

Samples like heart, blood, lung, liver, thoracic fluid and mediastinal lymph nodes were collected immediately after slaughter. The specimens were processed for M. haemolytica re-isolation. Suspected colonies were identified as M. haemolytica by Gram staining and biochemical tests. Samples from which M. haemolytica could not be isolated were re-cultured for a maximum of three times before they were considered negative and discarded.

From each sample, serial ten-fold dilution in sterile BHI broth was made, and 0.1 ml volume of appropriate dilution were inoculated on two plates (blood agar plate and tryptose soy agar plate) per dilution. After overnight incubation at 37°C, plates that yield between 30 and 300 colonies were counted and the average of the two plates were taken. Plates that show absence of growth was re-incubated for 24 h. Representative colonies were serotyped based on capsular antigens using IHA test with M. haemolytica serotype antisera as described by Quinn et al. (2002).

Statistical analysis

Data generated from this experiment was recorded and stored in MS Excel 2007. Results were statistically analyzed using SPSS software versions 19.0 for MS windows. Descriptive statistics were used to analyze the clinical parameters post challenge. Pair wise comparison using Wilcoxon rank test was used for comparing lung lesion score among the different control and vaccinated groups.

RESULTS

Prior to vaccination, all experimental lambs were screened for the presence of antibody against M. haemolytica serotypes A2 and A7 using indirect haemagglutination test and all were found negative. All
lambs were daily monitored and clinical signs were recorded following challenge. The mean rectal temperature was raised from 0.5 – 1°C starting from day 1 post challenge in all vaccinated groups up to day 3 post challenge, but the rectal temperature remained raised for 10 days post challenge in the control group as shown in Figure 1.

The prominent clinical signs revealed in very sick lambs were high rectal temperatures (≥ 40°C) and tachypnoea or dyspnoea and death of the animals within 48 h post challenge. Mildly affected lambs showed anorexia, coughing, mucoid nasal discharge and dullness. All unvaccinated controls had signs of respiratory tract infection of variable severity and raised rectal body temperature throughout the period of observation following challenge.

From the total of 26 lambs challenged with live M. haemolytica serotypes A2 and A7, 6 (23.1%) and 4 (15.3%) lambs were found dead and sick, respectively. In this study, in unvaccinated control group, death was seen within 48 h post challenge, while deaths from vaccinated groups were found within 3-7 days post challenge. No deaths of lambs were observed after 8 days post challenge. Higher mortality and morbidity were observed in the unvaccinated control group and lesser were recorded in lambs which received A2 and A7 M. haemolytica combined vaccine as illustrated in Table 2. From the total of 26 lambs challenged with live M. haemolytica A2 and A7, 13 (50.0%) lambs had lung lesions as shown in Table 2.

### Lung lesions score

M. haemolytica serotype A2 vaccinated group had two (40%) lambs with +1 lung lesions score as shown in Figure 2, while M. haemolytica serotype A7 had one

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Table 2. Summary of animals vaccinated with M. haemolytica serotypes, challenge serotypes, number of dead and sick animals, and number of lambs with lung lesions.

<table>
<thead>
<tr>
<th>Vaccine used</th>
<th>Challenge serotype</th>
<th>No. of lambs challenged</th>
<th>No. of dead lambs</th>
<th>No. of sick lambs</th>
<th>No. of lambs with lung lesions</th>
<th>Mean counts of M. haemolytica in lungs (log_{10} per g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. haemolytica A2</td>
<td>A2</td>
<td>5</td>
<td>1(20%)</td>
<td>1(20%)</td>
<td>2(40)</td>
<td>10^{3.5}</td>
</tr>
<tr>
<td>M. haemolytica A7</td>
<td>A7</td>
<td>5</td>
<td>1(20%)</td>
<td>0</td>
<td>1(20%)</td>
<td>10^{3.1}</td>
</tr>
<tr>
<td>M. haemolytica A2-A7</td>
<td>A2</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1(25%)</td>
<td>10^{3.8}</td>
</tr>
<tr>
<td></td>
<td>A7</td>
<td>4</td>
<td>1(25%)</td>
<td>0</td>
<td>1(25%)</td>
<td>10^{2.2}</td>
</tr>
<tr>
<td>Unvaccinated control</td>
<td>A2</td>
<td>4</td>
<td>1(25%)</td>
<td>2(50%)</td>
<td>4(100%)</td>
<td>10^{8.1}</td>
</tr>
<tr>
<td></td>
<td>A7</td>
<td>4</td>
<td>2(50%)</td>
<td>1(25%)</td>
<td>4(100%)</td>
<td>10^{7.6}</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>26</td>
<td>6(23.1%)</td>
<td>4(15.3%)</td>
<td>13(50.0%)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Mean rectal temperature for ten consecutive days post challenge.
(20%) lamb. When the necropsy findings were evaluated comparatively, there was a statistically significant difference (p < 0.05) between the control and the vaccinated groups with regards to lung lesion scores, while there was no statistically significant difference (p > 0.05) among M. haemolytica A2, M. haemolytica A7, and A2-A7 M. haemolytica combined vaccinated groups.

Severe lung lesions ranging from +2 to +3 were observed in all 8 (100%) unvaccinated control group challenged by live M. haemolytica serotype A2 and A7 while 5 (27.8%) lambs were found as +1 lung lesion score from vaccinated groups. Statistically significant difference (p < 0.05) in lung lesion scores was observed between the vaccinated and unvaccinated control group as shown in Table 3. A strong statistically significant difference (p = 0.015) was observed between A2 and A7 M. haemolytica combined vaccinated and the unvaccinated control group. However, there was no statistically significant difference (p > 0.05) among vaccinated groups. The less extensive lung lesions was found in M. haemolytica A2 and A7 combined vaccinated groups when compared with separate serotype A2 and A7 vaccinated groups.

Microbiological isolations

Tissue and fluid samples including heart blood, lung, liver, thoracic fluid and mediastinal lymph nodes were collected from each lamb immediately after necropsy and were processed for re-isolation of M. haemolytica. The respective serotypes of M. haemolytica were successfully re-isolated from all lungs showing pneumonic lesions, but none of the lungs without pneumonic lesions yielded M. haemolytica. The organisms were re-isolated from all pneumatic lungs in both vaccinated and unvaccinated control groups, but were isolated from all tissue specimens in unvaccinated control group. M. haemolytica were isolated from the lung lesions at a mean titre range of $10^{2.2} - 10^{8.1}$ CFU/g as shown in Table 2.

DISCUSSION

In this study, an experimental vaccine efficacy against M. haemolytica infection in lambs was investigated based on development of pneumonia, lung lesion score and bacteriological findings following challenge. The study
was performed by using experimental *M. haemolytica* serotype A2, A7, and A2-A7 combined vaccine, grown under iron restriction, in four trial groups of lambs followed by challenge with homologous serotype of *M. haemolytica*. The *Mannheimia* serotypes contained in this experimental vaccine appear to be appropriate for Ethiopian conditions, because they have been shown to occur at a high frequency in different parts of the country (Zeleke, 1998; Teferi, 2000; Sisay and Zerihun, 2003; Ayelet et al., 2004; Abera, 2005; Mulate, 2007) and did not have a vaccine produced in Ethiopia against these serotype. The most important result of this study was that vaccination with A2 and A7 *M. haemolytica* combined vaccine confer relatively good protective efficacy than *M. haemolytica* A7 or A2 vaccinated groups.

Lambs were monitored daily, from days 0 through 10, post challenge for the presence of clinical signs related to respiratory problems. The mean rectal temperature was found raised by 0.5 – 1°C for 3 days post challenge in all the unvaccinated control and vaccinated groups. This is in agreement with the study of Akan et al. (2006) who demonstrated that rectal temperature had been elevated up to 1°C in the treatment groups for the first three days post challenge. The rectal temperature declined after three days of post challenge in the vaccinated groups, but failed to reduce in the control group in this study. Mekonnen (2012) reported a 1.5°C rise of rectal temperature in the control group than all vaccinated lambs with capsular antigen of *M. haemolytica*.

The clinical signs were evaluated and revealed that very sick lambs had high rectal temperatures (≥ 40°C) and tachypnoea or dyspnoea and died within 48 h post challenge; mildly affected lambs showed signs like anorexia, coughing, oculo-nasal discharge and dulness, and survived until necropsy. Less severe clinical signs related to respiratory problems and fewer deaths were recorded in vaccinated groups than unvaccinated control group. This result is in agreement with the work of Odugbo et al. (2006) who reported that the prominent clinical signs were anorexia, mucoid nasal discharge, coughing for lambs infected with *P. multocida*. This variable clinical signs might be due to the protective effect of the vaccine against *M. haemolytica* infection.

From the total of 26 lambs challenged with live *M. haemolytica* serotype A2 and A7, 6 (23.1%) and 4 (15.3%) lambs were found dead and sick, respectively. In unvaccinated control group, death was seen within 48 h post challenge, while deaths were found within 3-7days post challenge from vaccinated groups. High mortality was observed in the unvaccinated control group than vaccinated groups. This result demonstrated that unvaccinated lambs have less protection against *M. haemolytica* infection. That might be due to lack of protective antibody against *M. haemolytica* serotypes.

When the necropsy findings were evaluated comparatively, there was a statistically significant difference (p < 0.05) between control and vaccinated groups concerning lung lesion scores, while there was no statistically significant difference (p > 0.05) among vaccinated groups. Severe lung lesions ranging from +2 to +3 were observed in all unvaccinated control group challenged with live *M. haemolytica* serotypes A2 and A7. Significant decrease in lung lesions after challenge with *M. haemolytica* strains following vaccination showed that protection using iron regulated outer membrane protein showed that vaccine of *M. haemolytica* strains is an effective method against an infection with homologous strains; however, no comments was made on cross-protection with heterologous strains because challenging was not performed with other serotypes in this study. A strong statistically significant difference (p = 0.015) were observed between *M. haemolytica* A2 and A7 combined

### Table 3. Pair wise comparisons of lung lesions score for vaccinated and control groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Compared groups</th>
<th>Standard deviation</th>
<th>Mean rank</th>
<th>Sum of rank</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Positive rank</td>
<td>Negative rank</td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>A2 and A7 combined</td>
<td>0.548</td>
<td>0.00</td>
<td>1.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Control</td>
<td>A2</td>
<td>0.447</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>A7</td>
<td>A2 and A7 combined</td>
<td>0.535</td>
<td>3.00</td>
<td>0.00</td>
<td>15.0</td>
</tr>
<tr>
<td>Control</td>
<td>A2</td>
<td>0.447</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
</tr>
<tr>
<td>A7</td>
<td>A2 and A7 combined</td>
<td>0.535</td>
<td>3.00</td>
<td>0.00</td>
<td>15.0</td>
</tr>
<tr>
<td>Control</td>
<td>A7</td>
<td>0.535</td>
<td>0.00</td>
<td>3.00</td>
<td>0.00</td>
</tr>
<tr>
<td>A2 and A7 combined</td>
<td>0.447</td>
<td>4.00</td>
<td>2.00</td>
<td>4.00</td>
<td>2.00</td>
</tr>
</tbody>
</table>
vaccinated group and unvaccinated control group. This indicated that less extensive lung lesions were found relatively in *M. haemolytica* A2 and A7 combined vaccinated group as compared to separate serotype A2 and A7 *M. haemolytica* vaccinated groups. This finding was similar to the work of Mekonnen (2012) who reported that vaccine prepared from the capsular antigen of combined *M. haemolytica* A2 and A7 strains provide significantly less extensive lung lesion score than the control, *M. haemolytica* A2, and A7 vaccinated groups.

The respective serotypes of *M. haemolytica* were successfully re-isolated from all pneumatic lung lesions. *M. haemolytica* was re-isolated from all samples (heart, blood, lung, liver, thoracic fluid, and mediastinal lymph nodes) in unvaccinated control groups, but they were re-isolated only from the lung tissue sample in vaccinated groups. This failing of isolation of this organism from other tissues might be due to the protective effect of the vaccine evidenced by limited bacterial population. This finding is supported by the work of Quinn et al. (2002).

High mean bacterial titer was found in unvaccinated control group challenged by live *M. haemolytica* A2 (10^9.1 CFU/g) and *M. haemolytica* A7 (10^7.6 CFU/g) where as the least was recorded in *M. haemolytica* A2 and A7 combined vaccinated group (10^2.2 CFU/g). This high bacterial titer has demonstrated that the unvaccinated control group has failed to protect against challenge. Gilmour (1980) revealed that in sub-acute cases which have not been treated with antibiotics *M. haemolytica* counts in excess of log10^7 CFU/g of lung lesion are usually obtained.

In conclusion, vaccine containing iron regulated outer membrane protein expressed *M. haemolytica* A2, A7, and A2 and A7 combined vaccine significantly provide protection against homologous strains.

**Conflict of Interests**

The author(s) have not declared any conflict of interests.

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