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## **Control of Abscess Disease of Sheep by Concurrent Vaccination, Zinc Injection and Antiseptic Washing**

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### **ABSTRACT**

Control of abscess disease through vaccination proved to be unsatisfactory in Saudi Arabia. This study was carried out to examine the combined effect of vaccination, zinc injection and antiseptic washing as an effective control method for abscess disease of sheep. Eighty healthy ewes were divided into two groups of 40 animals each. Group 1 ewes were injected subcutaneously with 5 mg kg<sup>-1</sup> zinc, as zinc oxide suspended in olive oil and concurrently vaccinated against abscess disease using a commercial vaccine (Glanvac-6, CSL Limited, Parkville 3052, Victoria, Australia) at its standard dose of 1 mL per ewe. The sheep were then washed with an antiseptic, Chloroxylenol 4.8% (Dettol) at standard dilution. Booster doses of zinc and vaccine were injected one month after the initial treatment and the ewes were re-washed with the antiseptic solution. Group 2 ewes were left as untreated controls. The ewes of both groups were mixed in one pen with five rams heavily infected with abscess disease to act as a source of infection. The ewes were bled before and two days after treatment for the determination of total blood count (CBC) and liver and kidney functions in order to assess the safety of the injected zinc, the vaccine and the antiseptic. Four ewes (10%) in group 1 and 16 (40%) in group 2 developed abscesses on the head during the 12 months course of the experiment. The abscesses appeared on the 10th month post-vaccination in group 1 ewes and on the first month in group 2 animals. Hematological and serum constituents results showed that the injected vaccine and zinc as well as the washed antiseptic were safe to the ewes.

**Key words:** Abscess disease, immunity, vaccine, Dettol, Saudi Arabia

### **INTRODUCTION**

Abscess disease of sheep, commonly known as caseous lymphadenitis CLA, (also known as pseudotuberculosis) is attracting attention because of its worldwide distribution, its contagious nature and of the difficulty of its control. The disease causes great economic losses to the sheep industry and represents a major sheep importation restriction factor. The losses are caused by condemnation and downgrading of carcasses and skin in abattoirs and reduction in wool growth (Paton *et al.*, 1988, 1994).

Abscess disease is essentially a subacute to chronic cellulitis characterized by the development of subcutaneous abscesses in the vicinity of lymph nodes or inside them. The inflammatory process leads to liquefactive necrosis of the affected tissues due to the secretion of an alpha haemolysin by the organism. Alpha haemolysin is lytic to erythrocytes, dermo-necrotic, lethal and exhibits a wide variety of other biological properties (McKean *et al.*, 2007; Kahn *et al.*, 2008).

Zinc has been shown to have strong wound healing and immune stimulating function (Ibs and Rink, 2003). All kinds of immune cells showed decreased functions after zinc depletion (Ibs and

Rink, 2003). Monocyte functions were impaired, natural killer cells cytotoxicity is decreased and phagocytic activity of neutrophils and macrophages were reduced in zinc deficient animals (Ibs and Rink, 2003). Addition of zinc resulted in restored normal functions and resulted in the release of cytokines by immune cells. Microbial growth was diminished in abscesses and it was suggested that a protein in the abscess fluid through its binding effect with zinc inhibited bacterial growth within an abscess (Ibs and Rink, 2003).

Minerals are likely to interact with each other in the animal body, once one mineral concentration is elevated above normal. This interaction can be at the level of absorption, transportation or utilization (Underwood, 1981).

Abscess disease is endemic in Saudi Arabian sheep farms and despite concerted efforts to control it by vaccination and culling, its incidence is increasing annually (Anonymous, 2005) and once it gets into a farm it is difficult to eradicate. A vaccine against the disease is commercially available in Saudi Arabia (Glanvac-6, CSL Limited, Parkville 3052, Victoria, Australia). This vaccine is a bacterin which the manufacturer claims is effective in decreasing the incidence and severity of the disease. However, sheep owners in this country complain of the vaccine as being only partially effective in the control of abscess disease. For this reason, we planned this experiment aiming at finding an effective control measure for abscess disease in sheep farms in the Kingdom, by:

- Increasing immunity of the animals against abscess disease by vaccination
- Boosting the general immunity of sheep by zinc injection Reference(s)
- Killing the bacterium causing the disease in the sheep environment by washing the sheep with Dettol antiseptic (chloroxylene 4.8% diluted 1:100 with water)

The combination of these three methods for the control of abscess disease might be utilized in laying a strategic control program for the disease in the Kingdom of Saudi Arabia.

## **MATERIALS AND METHODS**

**Animals:** The experiment was carried during April 2008 - April 2009 at the experimental station of The College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia. Eighty clinically healthy ewes, obtained from a farm with high incidence of abscess disease, were randomly divided into two equal groups of 40 animals each. The ewes were treated with dectomax (doramectin at 200  $\mu\text{g kg}^{-1}$ -1 mL/50 kg- Pfizer Inc.) prior experimentation to clean them from internal and external parasites.

Group 1 ewes were, ear tagged and injected concurrently with abscess disease vaccine (Glanvac-6, CSL Limited, Parkville, Australia), at its standard dose of 1 mL per ewe and 5 mg  $\text{kg}^{-1}$  zinc as zinc oxide suspended in olive oil, through the subcutaneous route (Mahmoud *et al.*, 1985). They were then washed using a spray pump with the antiseptic Dettol (4.8% Chloroxyleneol) at its standard dilution (1 mL Dettol diluted with 100 mL water). After 4 weeks, a booster dose of the vaccine and zinc was given and the ewes were re-washed with the antiseptic. Group 2 ewes were left as untreated controls. Both groups were mixed with 5 rams highly infected with abscess disease in one pen and allowed to breed freely.

**Methods:** Development of clinical abscesses in every group was observed on daily basis and the month's total was recorded. Five ewes were selected from group 1 and another five from group 2 were randomly selected and were bled from the jugular vein into tubes containing EDTA for routine hematology and into plain tubes to obtain serum for the determination of serum liver and

kidney function tests's, (to monitor the safety of injected vaccine, zinc and Dettol wash). The ewes were bled before and 2 days after the treatments. They were also bled at 6 months post-treatment to determine serum zinc concentration.

Whole blood was analysed for Packed Cell Volume (PCV) and hemoglobin (Hb) concentration using a hematology analyser (Vetscan, Abaxis, Hungary). Serum was analysed for total protein, creatinine, aspartate aminotransferase (AST) activity and Alanine aminotransferase (ALT) activity. Analyses were carried out by automated methods (Refletron plus, Roche Diagnostics, Mannheim, Germany). Zinc concentration in serum was determined by an Atomic Absorption Spectrophotometer (AAS) (Schumatzu Inc., Japan). One milliliter of serum was diluted with distilled water and aspirated into the AAS. Zinc concentration was read from a standard graph prepared by using different dilution of zinc nitrated.

**Statistical analysis:** Results were expressed as means±SD. Significance of differences between means was determined using a computer-adapted statistical program (INSTAT 2).

## RESULTS

Four ewes in group 1 (10%) and 16 in group 2 (40%) developed abscesses on the head during the 12 months course of the experimental period (Table 1). The abscesses appeared on the 10th and the first month postvaccination in groups 1 and 2 ewes, respectively. Abscesses were recognized as swelling that ruptures with time and drain yellow pus.

Hematological and serum constituents' concentrations results showed no significant changes 2 days after vaccination and 2 days post-booster injection (Table 2). Following zinc injection, its concentration rose in serum and was statistically significantly higher for 2 days post injection than pre-injection values which showed that it was well absorbed from the injection site. Its concentration in the serum was normal by the 6th month post-administration (Table 3).

Table 1: Development of abscesses in group 1 and 2 post-vaccination

| Month post treatment | Group 1 (N = 40) | Group 2 (N = 40) |
|----------------------|------------------|------------------|
| 1                    | ---              | 2                |
| 2                    | ---              | 1                |
| 3                    | ---              | 1                |
| 4                    | ---              | 3                |
| 5                    | ---              | 2                |
| 6                    | ---              | 1                |
| 7                    | ---              | 1                |
| 8                    | ---              | 1                |
| 9                    | ---              | ---              |
| 10                   | 1                | 1                |
| 11                   | 1                | 2                |
| 12                   | 2                | 1                |
| Total                | 4 (10%)          | 16 (40%)         |

Table 2: Haematological and serum constituents changes before, 2 days post-treatment and 2 days post-booster (mean±SD)

| Treatments            | PCV (l/l) | Hb (g L <sup>-1</sup> ) | TP (g L <sup>-1</sup> ) | Creatinine (μmol L <sup>-1</sup> ) | AST (UL <sup>-1</sup> ) | ALT (U L <sup>-1</sup> ) |
|-----------------------|-----------|-------------------------|-------------------------|------------------------------------|-------------------------|--------------------------|
| Pre-treatment         | 0.32±2.10 | 112±20                  | 100±14                  | 42.0±1.2                           | 9.7±2.2                 | 2.8±0.8                  |
| 2 days post-treatment | 0.30±0.16 | 103±13                  | 107±10                  | 42.0±1.2                           | 10.7±1.5                | 2.8±0.8                  |
| 2 days post booster   | 0.32±0.23 | 109±16                  | 99±16                   | 38.4±1.6                           | 9.7±1.3                 | 3.2±0.3                  |

p>0.5 (non- significant)

Table 3: Changes in serum zinc concentration ( $\mu\text{g}/100\text{ mL}$ ) in ewes at pre-treatment, 2 days post treatment, 2 days post booster and six months post-vaccination

| Ewe No.       | Pre-treatment               | 2 days post vaccination     | 2 days post booster           | 6 months post vaccination |
|---------------|-----------------------------|-----------------------------|-------------------------------|---------------------------|
| 1             | 164                         | 240                         | 260                           | 182                       |
| 2             | 186                         | 302                         | 288                           | 201                       |
| 3             | 200                         | 268                         | 310                           | 194                       |
| 4             | 148                         | 280                         | 298                           | 158                       |
| 5             | 164                         | 288                         | 288                           | 180                       |
| Mean $\pm$ SD | 172 $\pm$ 20.5 <sup>*</sup> | 275 $\pm$ 23.4 <sup>*</sup> | 288.8 $\pm$ 18.5 <sup>*</sup> | 181 $\pm$ 15.33           |

\*p = 0.001 (highly significant)

## DISCUSSION

The author is not aware of a previous report describing the combined effect of vaccination, zinc injection and antiseptic washing on the incidence of abscess disease of sheep. Extensive research work has been carried in the past two decades to produce effective vaccines for the control of CLA. The vaccines (bactrins, toxoids) reduced the incidence of abscesses in sheep flocks but failed to eliminate the infection or prevent the disease from re-appearing (Dorella *et al.*, 2009; Paton *et al.*, 2003; Eggleton *et al.*, 1991). Locally produced vaccines from indigenous bacterial strains are likely to confer better protection when compared with results obtained from imported vaccines. Using a vaccine produced from virulent UK *C. psuedotuberculosis* isolate, a more potent control of abscess disease was achieved (Fontaine *et al.*, 2006).

The results of this study showed that concurrent vaccination, zinc injection and antiseptic washing protected sheep from abscesses for 9 months and lowered the annual incidence of the disease. Mahmoud *et al.* (2009) reported that concurrent injection of zinc and vaccination produced the longest duration of protection against abscess disease of sheep when compared to vaccination only.

Paton *et al.* (1991) studied the effect of antibody to caseous lymphadenitis in ewes on the efficacy of vaccination in lambs. They showed that vaccinating lambs against abscess disease before weaning was less effective to confer protective immunity against the disease. They also showed that delaying the vaccination to more than 12 weeks might result in animals acquiring natural infection. It would be logical to assume that the best time for vaccinating lambs against abscess disease is after weaning (at 2 months of age), the time where its immune system is very well developed and ready to produce antibodies and its maternal immunity has decreased.

The long term effect of a repeated zinc injection into ewes or lambs should be monitored carefully in a separate experiment to avoid development of a mineral interaction deficiency disease (Underwood, 1978, 1981).

The method of transmission of abscess disease of sheep remained speculative. Dipping, shearing, thorns of Acacia shrubs and feeding of dry barley were just some of the many factors incriminated in the transmission of the disease in different countries (Jones *et al.*, 1997; Anonymous, 2005). We used naturally infected rams to transmit the infection to the experimental ewes and they transmitted the disease effectively well which suggested that direct contact could be an important method of transmission.

In conclusion, concurrent vaccination, zinc injection and antiseptic washing were free from abscesses for 9 months compared to untreated controls. These results can be utilized in formulating a control program for sheep abscesses. In this program, sheep will be vaccinated, zinc injected and washed with antiseptic every 6 months. This efficiency of the program in controlling CLA will be evaluated in future experiment.

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