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Comparison of the Efficacy of Bar-Vac® 10 Ways Con Retigen® and Ultrachoice ™8 Vaccines in Sheep in Field Conditions

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Abstract: The purpose of this study was to compare the efficacy of Bar-Vac® 10 ways con Retigen® and Ultrachoice ™ 8 vaccines in field conditions and determine the effect of both vaccines on commercial sheep production, as measured by average daily gain. Healthy *Awassi* sheep (lambs and adults; n = 299) were enrolled in a blinded, clinical field study under commercial conditions. The number of animals with diseases and health conditions was significantly lower in the Bar-Vac group as compared with that of the Ultrachoice group. This effect was evidenced by a reduction in the number of animals off-feed (i.e., experiencing post-vaccine reaction) and of those with upper respiratory tract infection/pneumonia and enteritis (P=0.003). Furthermore, the average daily gain (P=0.004) and immune response (serum anti-alpha toxin levels) (P=0.002) were significantly higher in the Bar-Vac versus the Ultrachoice group. These findings suggest the Bar-Vac® 10 ways con Retigen® vaccine provides greater protection in sheep than Ultrachoice ™ 8.

Key words: Clostridial Diseases · Sheep · Average Daily Gain · Pneumonia · Vaccine

INTRODUCTION

Clostridial diseases are a widespread and important group of pathologies first described in the early 1800s [1]. These diseases are generally divided into four classes, depending on the affected organ or system and can affect the gastrointestinal system, the parenchymatous organs, cause myonecrosis and toxemia, or give rise to neurologic disorders [1]. Vaccination is the primary means of protecting against clostridial diseases, given their acute nature, rapid progression and poor response to treatment [2]. Most currently available clostridial vaccines contain inactivated toxoids, although exceptions include *Clostridium chauvoei* vaccines, which include some cellular material [1]. Respiratory diseases are another common cause of significant losses in sheep production [3-5]. Combining clostridial vaccines with important respiratory pathogens may add value to vaccination programs, minimizing the need to capture animals and hence improving sheep health and welfare. Several currently available clostridial vaccines contain other, non-clostridial bacteria, such as *Mannheimia haemolytica* [2].

While several commercial combination vaccines are available in Jordan, clinical field trials comparing their efficacy are scarce in the veterinary literature and little data is available to farmers and veterinarians seeking to select the most efficacious vaccine. Bar-Vac® 10 ways con Retigen® (Boehringer Ingelheim, Germany) and Ultrachoice ™ 8 (Zoetis Inc, US) are both widely used to protect sheep and goats against blackleg, malignant edema, black disease, gas-gangrene, enterotoxemia and enteritis. Unlike Ultrachoice ™ 8, Bar-Vac® 10 ways con Retigen® also protects against diseases caused by *Pasteurella multocida* Types A-1 and D and *Mannheimia haemolytica* type 1.

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The purpose of this study was to compare the efficacy of Bar-Vac® 10 ways con Retigen® and Ultrachoice ™ 8 vaccines in commercial sheep production under field conditions. The efficacy of each vaccine was determined based on the number of sick and dead sheep, as well as the average daily gain in each experimental group.

MATERIALS AND METHODS

Study Design: A clinical field study of 8 weeks duration was carried out under commercial conditions.

Study Animals: Sheep selected for the trial were carefully chosen to be representative of normal healthy sheep and to avoid those with endemic disease. Participating animals were selected from three regions in Jordan; Al Zarqa'a, Al Mafraq and Al Badiah. All underwent a general health inspection and evaluation. Adults, including pregnant ewes, had been vaccinated with Ultrachoice ™ 8 on 2 occasions: 8 months and 9 weeks before the start of the experiment. Sheep that were bright, alert and responsive, with normal appearance and behavior, were enrolled in the study. Special attention was paid to the respiratory system to rule out the presence of any abnormalities, including increased respiratory rate, nasal discharge, sneezing and coughing. In total, 299 healthy *Awassi* sheep were enrolled in the study. The *Awassi* breed is the most common breed raised in Jordan and other countries of the Middle East.

Procedures: Selected animals were individually weighed, identified with ear tags and randomly assigned for vaccination with either Bar-Vac® 10 ways con Retigen® (n=150) or Ultrachoice ™ 8 (n=149). Each group consisted of lambs of 3-6 weeks of age (Bar-Vac, n=75; Ultrachoice, n=75) and adult sheep of 2-7years of age (Bar-Vac, n=75; Ultrachoice, n=74). Animals in both groups received 1 ml of the corresponding vaccine, administered subcutaneously behind the left shoulder on Day 0, followed by a booster dose 4 weeks later (D 28). Subsequently, animals from the two experimental groups were housed together. Weighing, tagging and vaccine administration was performed by veterinarians. The evaluating veterinarian, farmer and workers were blinded to vaccination groups.

Health Evaluation and Follow up: Sheep were monitored daily and observed closely. The flock was visited once a week by a veterinarian and observations recorded. In addition, the farmer and workers were contacted daily by the veterinarian to register any abnormalities or signs of sickness, including fever, increased respiratory rate, nasal discharge, coughing and sneezing and diarrhea. Animals that became sick were examined by the veterinarian to determine the cause of illness and the treatment required. Those diagnosed with similar diseases were treated using the same protocol selected by the supervising veterinarian. A detailed description of the tentative diagnosis, physical examination findings and treatment was recorded. Animals that died during the course of the study underwent post-mortem examinations to determine the cause of death.

Measurement of Average Daily Gain: Lambs (n=149 in total) were weighed twice during the course of the study (Days 0 and 56). Average daily weight gain (ADG) was calculated as the difference in weight divided by the number of days.

Immunological Testing: To evaluate the immune response in each experimental group, serum samples were obtained randomly from a representative number of animals from each group (15 lambs and 10 adults per group) and tested for the presence of antibodies against alpha and epsilon toxins using an ELISA kit for serodiagnosis of *Clostridium perfringens* alpha and epsilon toxins (Bio-X Diagnostics, Jemelle, Belgium).

Table 1: Bar-Vac® 10 ways con Retigen® versus Ultrachoice ™ 8 vaccines: comparison of efficacy in Awassi sheep in field conditions

Day 0	Day 28	Day 56
Weighing (Lambs; n=149)		Weighing (Lambs; n=144)
Ear Tagging		
Assignment to experimental groups		
Vaccine administration	Administration of vaccine booster dose	
Serum Collection		
(30 lambs; 20 adults)	Serum Collection	
(30 lambs; 20 adults)	Serum Collection	
(30 lambs; 20 adults)		

Serum samples were tested 3 times: before the first vaccination (Day 0); before the booster vaccination (Day 28); and 4 weeks after the second vaccination (Day 56). On Day 0, from each experimental group were randomly sampled.

An overview of the study design is provided in Table 1.

Statistical Analysis and Data Management: SPSS software Version 21 (SPSS Inc., Chicago, IL, USA) was used for descriptive statistical analyses. A Student's T-test was used to compare serum antibody titers and ADG between groups. The numbers of sick animals in each group were compared using Pearson's Chi-Square test, with P-values = 0.05 considered significant.

RESULTS

Of the 299 animals enrolled, 294 completed the study (Bar-Vac group n= 148; Ultrachoice group n=146). Two animals (one from each group) died on Day 1. The field diagnoses based on post-mortem examination were pneumonia (Ultrachoice) and enterotoxemia (Bar-Vac group). Another two animals (one from each experimental group) were deemed "chronic" after repeated antibiotic treatment for arthritis and were excluded from the study. One animal from the Ultrachoice group died on Day 12 and was diagnosed with pneumonia after post-mortem examination.

General Health Conditions and Diseases: Health conditions and diseases observed in the two experimental groups are summarized in Table 2. In total, 29 animals became ill during the course of the study; 7 from the Bar-Vac group and 22 from the Ultrachoice group. In the Ultrachoice group, 5 animals were off-feed on Day 1; 4 had gingivitis on Day 2; 10 had upper respiratory tract infections on Day 7; 2 had enteritis on Day 25; and one had arthritis on Day 25. In the Bar-Vac group, 1 animal was off-feed on Day 1; 1 had gingivitis on Day 2; 4 had upper respiratory tract infections on Day 7; and one had arthritis on Day 22.

Table 2: Comparison of the incidence of health conditions/disease in the two experimental groups

Health Condition/Disease	Ultrachoice Group: No. of Animals Affected	Bar-Vac Group: No. of Animals Affected	Comparison (P value)
Off-Feed	5	1	0.097
Gingivitis	4	1	0.174
Upper Respiratory Tract Infection/Pneumonia	10	4	0.098
Enteritis	2	0	0.155
Arthritis	2	2	0.996
Total	22	7	0.003

Table 3: Comparison of average daily gain between the two experimental groups after sorting by sex

Experimental Group	No. of Males	No. of Females	Mean ADG (kg/day): Males	Mean ADG (kg/day): Females
Bar-Vac	45	28	0.26	0.21
Ultrachoice	41	32	0.23	0.19
Comparison (P value)			0.020	0.118

Table 4: Comparison of average daily gain between the 2 experimental groups after sorting by initial weight

Weight Category		VAC	N	Mean	Std. Deviation	Std. Error	P-value
Low (≤ 7.0 kg)	ADG	Bar	11	0.1891	0.04460	0.01345	0.722
		UT	20	0.1815	0.06150	0.01375	
Medium (7.1-9.0 kg)	ADG	Bar	24	0.2438	0.04906	0.01001	0.012
		UT	36	0.2119	0.04465	0.00744	
High (> 9.0 kg)	ADG	Bar	38	0.2561	0.07398	0.01200	0.666
		UT	17	0.2471	0.06381	0.01548	

Table 5: Comparison of percentage inhibition of alpha toxin antibodies between the two experimental groups

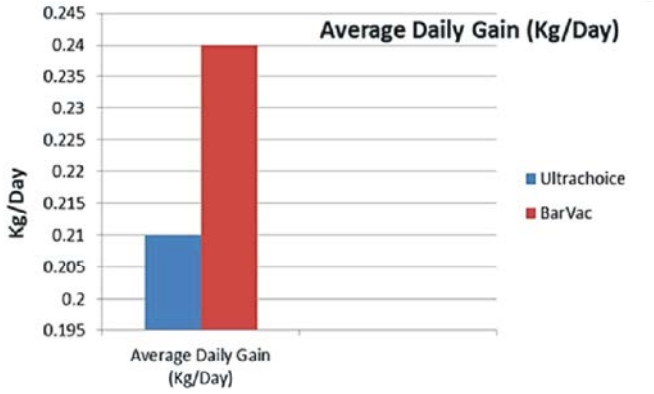
Interval	VAC	N	Mean	Std. Deviation	Std. Error Mean	P-value
*Day0_28	UT	24	-2.3558	13.64249	2.78476	0.002
	BR	25	8.6408	9.14339	1.82868	
*Day0_56	UT	24	4.0029	15.03610	3.06923	0.327
	BR	25	7.5116	9.14671	1.82934	

* Day0_28 = Percentage inhibition on Day 28 minus percentage inhibition on Day 0; Day0_56 = Percentage inhibition on Day 56 minus percentage inhibition on Day 0.

Table 6: Comparison of the percentage inhibition of epsilon toxin antibodies between the two experimental groups

Interval	VAC	N	Mean	Std. Deviation	Std. Error Mean	P value
*Day0_28	UT	24	-1.0500	8.30630	1.69552	0.331
	BR	25	-3.3084	7.79289	1.55858	
*Day0_56	UT	24	-.5442	7.96254	1.62535	0.034
	BR	25	-6.2528	10.12249	2.02450	

* Day0_28 = Percentage inhibition on Day 28 minus percentage inhibition on Day 0; Day0_56 = Percentage inhibition on Day 56 minus percentage inhibition on Day 0.



	Group	N	Mean	Std. Deviation	Std. Error Mean	Comparison Sig. (2-tailed)
ADG	Bar	73	.2419	.06626	.00776	
	UT	73	.2118	.05848	.00685	P=0.004

Fig. 1: Comparison of average daily gain in the two experimental groups

Average Daily Gain: The average daily gain was 0.24 kg/day and 0.21 kg/day for the Bar-Vac and Ultrachoice groups, respectively (P=0.004; Figure 1). To investigate the influence of sex, ADG was compared between the 2 groups after lambs were sorted by sex (Table 3). ADG values were also compared after lambs were sorted into 3 categories according to initial weight; Low (≤ 7.0 kg), Medium (7.1- 9.0 kg) and High (> 9.0 kg), (Table 4).

Immunological Testing: The percentage inhibition of toxin antibodies positively correlates with the degree of positivity of the serum sample tested. For accurate comparison between the two experimental groups, percentage inhibition of alpha and epsilon toxin antibodies (Tables 5 and 6, respectively) was calculated for two intervals during the course of the study; Days 0-28 and Days 28-56.

DISCUSSION

The results of this study demonstrate a decreased incidence of disease (P=0.003), higher ADG in 3-6 week-old lambs (P=0.004) and an enhanced greater immune response (as measured by anti-alpha toxin levels; P=0.002) in lambs and adults vaccinated with Bar-Vac® 10 ways con Retigen® as compared with those treated with Ultrachoice ™ 8. While Bar-Vac® 10 ways con Retigen® has a wider spectrum of protection; these two vaccines are often considered substitutes, since farmers seem to allocate more importance to the clostridial protection.

Several factors may contribute to the reduced incidence of disease in the Bar-Vac group. Given its wider spectrum of protection against pathogens Bar-Vac protects lambs against diseases caused by *Pasteurella multocida* Types A-1 and D and *Mannheimia haemolytica* type 1. In line with this profile, the number of animals affected with upper respiratory tract infection and pneumonia in the Bar-Vac group was lower than that recorded in the Ultrachoice group. Another factor that may have contributed to better vaccine performance is the number of off-feed animals (i.e., those experiencing post-vaccination reaction), which was lower in the Bar-Vac group. Both vaccines protect against enteric clostridial diseases, which are important causative agents of enteritis in sheep. While no cases of enteritis were observed in the Bar-Vac group, 2 lambs in the Ultrachoice groups were affected. Unfortunately, this was purely a field study and the cause of enteritis in the affected animals was not determined.

Average daily gain is an important production parameter which can influence the choice of vaccine used in routine vaccination programs. There was a significant difference in ADG between the two groups, which persisted between the male groups when lambs were stratified according to sex and between the medium groups when lambs were stratified according to weight, suggesting that the observed differences were not influenced by these factors and may be attributable to the better efficacy of the vaccine.

CONCLUSION

Our findings suggest that Bar-Vac® 10 ways con Retigen® provides greater protection than Ultrachoice ™ 8 in 3-6 week-old lambs, as evidenced by a decreased incidence of disease, increased ADG and augmented immune response (anti-alpha toxin levels).

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