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# Study on Efficiency of Blackleg Vaccine in Cattle in Khuzestan. Province, Iran.

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**ABSTRACT**

Blackleg is a fatal acute disease of young cattle between 6 months to 2 years old. It produces an acute local infection resulting in inflammation of muscles and blood poisoning leading to rapid death. Blackleg has been diagnosed in Iran at Razi Institute since 1938 and the causative agent was isolated. The main object of this study is to detect and evaluate the efficiency of blackleg vaccine in calves that were vaccinated against this disease in the field. The vaccination mainly was carried out in area which outbreak of the disease had been recognized previously. The vaccine were injected twice at interval of two weeks in 394 out of 1913 calves and the rest of animals were unvaccinated and kept as control and were injected with saline. Vaccination of calves were carried out at the age of 3 - 6 months old during the period between May 2004 to April 2006. After the second injection, vaccinated and unvaccinated animals were observed for 9 months. Thirty nine out of 394 calves were removed from vaccinated calves due to lack of co-operation of the owners with the project executive. No case of blackleg disease was observed in vaccinated calves whereas 25 out of 1519 unvaccinated re affected, and 10 out of them died eventually. The results also indicated that there was a significant difference between vaccinated and unvaccinated calves, $X^2 = 0.008 (P < 0.05)$. The study showed that efficiency of the vaccine was positive without any case of blackleg in vaccinated animals. However, the presence of the disease in some animals after vaccination might be due to incorrect consumption of vaccine by animal husbandry and also due to lack of booster injection specifically in area where there was sudden changes of weather from warm to cold.

**Keywords:** *Clostridium chauvoei*, Blackleg, Vaccination, Efficiency

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## INTRODUCTION

*Clostridium chauvoei* is the pathogenic agent of blackleg causing inflammation of muscles, serious toxemia and high mortality in cattle, sheep and many other domestic and wild animals. It is considered that *Clostridium chauvoei* is the most important cause of economic loss in livestock [1]. Most cases of blackleg occur in the warm months of the year. Outbreaks can occur following excavation of soil, which can expose and activate latent spores. Also the disease is enzootic in areas with a history of flooding and it is usually common that a number of animals to be affected within a small time frame. In cattle the disease is mostly confined to animals that are rapidly growing and are on a high nutritional plan [2]. The control of the disease is done by commercial vaccines consisting of whole formulized cultures, generally presented as polyvalent formulation [3]. Blackleg in cattle has been recognized since 1938 in Iran which mostly affects cattle in enzootic farm. In 1968 a severe and extensive outbreak occurred in a vast area among cattle flocks in fifteen villages in south part of Iran which killed 400 cattle [4]. In the retrospective study of soil-born disease of cattle in Zambia, malignant edema and blackleg were widespread, a total of 103 cases of blackleg were reported between 1985-1997. It was found that specific soil-conditions associate emergence of the soil-borne disease [5]. For the first time reported that *Clostridium chauvoei* strain Jakari produces neuraminidase enzyme in vitro that provide a clear understanding on the pathogenesis of blackleg disease [6]. The only effective means of controlling blackleg is by vaccination. Several formulated made vaccines are available commercially and care should be taken to follow the manufacturer's instructions [7]. The blackleg anaculture vaccine has been prepared for immunization of cattle and sheep against the disease at Razi Institute using traditional manner [8] and in fermenter [9]. The present study was therefore carried out to evaluate the efficiency of this vaccine in those cities of Khuzestan province in Iran.

## MATERIALS AND METHODS

Thirty bottles of formulized whole cell blackleg vaccine, strain *C.N.701, batch number. 499 from head office of veterinary organization in Khuzestan province at Ahvaz city was used. Experimental concentrated blackleg vaccine was prepared according to the method described by **FAO [10]. The medium (modified medium for production of experimental *Clostridium chauvoei* vaccine by fermenter) consisting of peptone, glucose, sodium chloride, cysteine hydrochloride and yeast extract was prepared by fermenter and inoculated by *Clostridium chauvoei* strain for preparation of blackleg vaccine. Aluminum hydroxide gel adjuvant was added to the high yield vaccine. The vaccine was also concentrated by the method of precipitation. he data of outbreak of the disease were identified from May 1999 to April 2004 in Khuzestan cities such as Ahvaz, Dashte Azadegan, Shush, Shadegan and Ramhormouz veterinary centers. Two injections consisting of 2- 3 ml dose at interval of two weeks were inoculated subcutaneously near shoulder at the age of 3-6 months old in 394 out of 1913 calves and the rest animals were unvaccinated and kept as control and were injected with saline from May 2004 to April 2006. A tag of identification was attached to each vaccinated calf. A specific form to register data for animal husbandry and the address subsequently can be referred to were prepared. Also an application form concerning recording of the probable presence of the disease and information about the plan of execution was prepared. After the second injection all animals, vaccinated and

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unvaccinated were observed for 9 months and any abnormality such as death was registered. Some factors such as type of strain used in vaccine, storage conditions, dose, vaccination schedule and interval between two njections were strictly observed. The degree of correlation between vaccinated and unvaccinated calves were assessed by Fisher's exact test and also for calculation of number of vaccinated calves the statistical formula $n=z^2 p(1-p)/d^2$ was used.

* Collection Number
**Food & Agriculture Organization

## RESULTS AND DISCUSSIONS

Some outbreaks of blackleg disease were reported in some cities of Khuzestan province from May 1999 to Apr. 2004. The present study was carried out in those cities to evaluate the efficiency of a whole culture formulized vaccine in calves. Thirty nine out of 394 calves were removed from vaccinated calves due to lack of co-operation of the owners. No case of the blackleg disease was observed in vaccinated calves as it is shown in Table 1, whereas out of 1519 calves which were not vaccinated 25 were affected and 10 out of them eventually died as it is shown in Table 2. Comparison between vaccinated and unvaccinated calves is shown in Table 3.In this study some factors such as type of strain used in vaccine, storage conditions, dose, vaccination schedule and interval between two injections were strictly observed according to standard. The result showed that no case of blackleg disease was observed in vaccinated herd. At the same time by using Fisher's exact test $X^2 = 0.008 (p < 0.05)$, it can be concluded that evaluation of the vaccine was positive.

weather from warm to cold.

**Table 1.** Vaccinated calves (experimental group) in different cities of Khuzestan province from May 2004 to Apr.2006 (n=394)

| City name | *No. of vaccinated calves | No. of affected calves |
|---|---|---|
| Ahvaz | 82 | 0 |
| Dashte Azadegan | 68 | 0 |
| Shush | 76 | 0 |
| Shadegan | 74 | 0 |
| Ramhormouz | 55 | 0 |
| Out | 39 | 0 |
| Overall | 394 | 0 |

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**Table 2.** Unvaccinated calves (control group) in different cities of Khuzestan province from May 2004 to Apr.2006 (n=1519).

| City name | No. of unvaccinated calves | No. of affected calves (No. of mortality) |
|---|---|---|
| Ahvaz | 445 | 11(4) |
| Dashte Azadegan | 301 | 5(1) |
| Shush | 324 | 6(2) |
| Shadegan | 259 | 1(1) |
| Ramhormouz | 190 | 2(2) |
| Overall | 1519 | 25(10) |

**Table3.** Comparison between vaccinated calves (experimental group) and unvaccinated Calves (control group) in different cities of Khuzestan province from May 2004 to Apr.2006

| City name | No. of vaccinated calves (No. of affected) | No. of unvaccinated calves (No. of affected) |
|---|---|---|
| Ahvaz | 82(0) | 445(11) |
| Dashte Azadegan | 68(0) | 301(5) |
| Shush | 76(0) | 324(6) |
| Shadegan | 74(0) | 259(1) |
| Ramhormouz | 55(0) | 190(2) |
| Out | 39(0) | 0(0) |
| Overall | 394(0) | 1519(25) |

$X^2 = 0.008 (p < 0.05)$

The best vaccine was the one made from the local strain isolated from that area. Thus it is necessary that the local strains should be recognized [2]. According to the statement of Kerry in 1967 from the nine different strains isolated from different parts of the world there were difference in immunology between the *Clostridium chauvoei* strains and immunity of animals under field condition is important and therefore various local strains must be used in vaccine production [11]. For this reason, since the evaluation of monovalent vaccine is positive, there is no need to recognize local strains and then used for production of blackleg vaccine [12]. In a study on evaluation of two types of vaccine A-7 and

UB-7 against clostridia diseases concluded that one injection of vaccine UB-7, does not provided immunity against clostridia diseases unless second injection is carried out. Also in this study it is clear for creation of an active immunity by vaccination, the maternal antibody level must decreased and the vaccination of newborn calves must not be carried out in the early stage of age. Therefore the best method and interval time of two injections between 2 - 4 weeks at the age of 3 – 6 months is important [13] Also in areas where the peak of the diseases is high, one vaccination has been proposed at the age of 3 weeks while other two injections before the age of 6 months is necessary. One injection as a booster dose has also been recommended for expensive cattle after one year old [14]. Occurrence of the disease after vaccination might be due to incorrect consumption of vaccine by animal husbandry, indeed other minor factors such as storage conditions of vaccine, method and site of injection, dose and exact schedule of vaccination were strictly observed according to standard. And other reason the lack of booster injection specifically in areas where there is a sudden changes of

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## REFERENCES

[1] Smith LD, Williams BL. The pathogenic anaerobic Bacteria. 3rd edn. Charles C. Thomas Springfield, Illionis, 1984, pp.164-179.
[2] Radostitis OM, Blood DC, Gay CC, Hinchcliff KW. Blackleg. Veterinary Medicine: A textbook of the disease of cattle, sheep, goats and Horses. 9th edn. Bailliere Tindall, Oxford, 2000, pp. 760-763.
[3] Crichton R, Solomon J, Barton AM. Biologicals 1990; 18: 49-54.
[4] Ardehali M, Derakhshan H. Arch Razi Inst 1975; 27: 37-41.
[5] Hang' ombe BM, Isogai E, Lungu J, Mubita C, Nambota A, Kirisawa R, Kimura K, Isogai H. Comp mmunol Microbial Infect Dis 2000; 23(4): 277-284.
[6] Useh NM, Nok AJ, Ajanus OJ, Balogun EO, Oladele SB, Esievo KAN. Veterinarski Arhiv 2004; 74 (4): 289-298.
[7] Walker PD. Veter Med Vacc 1992; 10 (14): 977.
[8] Moosavi M, Ardehali M, Pilehchian R. Arch Razi Inst 1992; 42/43: 91-94.
[9] Pilehchian R, Moosavi M, Ardehali M. Large Scale Cultivation of *Clostridium chauvoei* (blackleg) vaccine by Fermenter. The 3rd International Iran and Russia Conference, Moscow, Abstracts Book, 2002, pp. 18-20.
[10] http://www.fao.org/DOCREP/004/T0278E/T0278E02.html.
[11] Kerry JB. Res Vet Sci 1967; 8: 89-97.
[12] http://cnrit.tamu.edu/cgrm/whatzhot/newadams.html.

[13] Troxel TR, Gadberry MS, Wallace WT, Kreider DL,Shockey JD, Colburn EA, Widel P, Nicholson I. J Anim Sci 2001; 79(10): 2558- 2564.
[14] Noble J. Cattle Diseases, Blackleg. Animal and Plant Health Service © the State of Queensland, Department of Primary Industries 2001.