Iranian Journal of Veterinary Research, University of Shiraz, Vol. 8, No. 2, Ser. No. 19, 2007

Protective effect of Johne's disease attenuated vaccine in an intensive non-tuberculosis free dairy

Taghipour Bazargani, T.[1*]; Charkhkar, S.[2]; Sadeghi, F.[2]; Khalaj, M.[2]; Rashtibaf, M.[3]; Bagheri, M.[4] and Bazargani, M.[5]

[1]Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; [2]Veterinary Organization, Ministry of Jihad-e-Agriculture, Tehran, Iran; [3]Veterinary Medicine Head Office, Mashhad, Iran; [4]Private Section Veterinarian, Mashhad, Iran; [5]Private Section Veterinarian, Tehran, Iran

[*]**Correspondence:** T. T. Bazargani, Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

(Received 18 Sept 2005; revised version 21 Aug 2006; accepted 23 Aug 2006)

Summary

This study was carried out to control Johne's disease (JD) without interfering with tuberculosis (TB) control programme. A dairy herd (n>1500 heads) was used over a period of 6 years, from 1994 to 1999 for this investigation. The herd had a history of heavy infection with *Mycobacterium avium paratuberculosis* (MAP) with culling rates of 1.5 and 5%, in 1991 and 1995, respectively. The mean annual losses due to the reduction in milk yield, pregnancy rate and carcass weight was about $32000. In addition, the farm has had positive tuberculin test. During this study, 2070 calves within 31 days of age, were inoculated once subcutaneously, with MERIAL JD attenuated vaccine, in the middle of brisket region. The vaccine protected the herd against JD effectively, so that from 1995 up to the end of 1999, the means of JD culling among 2-year-old vaccinated and unvaccinated cattle were 0.52 and 19.4%, respectively. This vaccination had no side-effects on TB control programme that was conducted by the comparative tuberculin test. In the site of inoculation, fibrocaseous inflammation, while increasing in size, was developed. During 2 years, in the majority of animals, this reaction disappeared; only in less than 1.5% of instances the lesion became tumoral and permanent. These animals had no clinical abnormality. While inoculating the vaccine, the insertion of vaccine germ contaminated needles in fingers of vaccinators usually caused temporary, hard and painful inflammation. In one occasion, a progressive granuloma caused by vaccine germ was diagnosed histopathologically and treated with suitable antibiotics.

Key words: Dairy herd, Attenuated vaccine, Johne's disease control, Tuberculosis control

Introduction

In 1895, for the first time, Johne's disease (JD) was noticed in Germany (Whitlock, 2002). Currently, no country in the world can claim that it is free from this disease (Clarke, 1997, Radostits *et al*., 2000). Nowadays, not only economic effects but also zoonotic aspects of JD have very specifically taken the attention of mycobacteriologists (Hutchinson, 1996; Ifrearulundu and Kaneene, 1999). The JD causative agent has a considerable potency to survive for a long time outside and for life inside the body of carrier animals. This mycobacterium is transmitted both horizontally (infected food, water and even air) and/or vertically (either intra-uterine or colostrum and milk of infected dam) (Butler, 1993; Ifrearulundu and Kaneene, 1999; McDonald *et al*., 1999; Whitlock, 2002). Whether an exposed ruminant remains infected forever or becomes affected mainly depends on re-infection (exogenous and/or endogenous) as well as capability of the immune system of the attacked animal (Geissen, 1993; Choidini, 1996). The incubation period of JD is very protracted (2-10 years) and much of spread of this infection takes place in this period (Choidini, 1996; Sweeney, 1996). In addition, there is no treatment for the disease yet (Jean, 1996). Because of all these things, every effort is concentrated on combating

JD (Eddy, 2004).

Materials and Methods

A dairy farm with more than 1500 animals was one of the four farms that had symbolically been under Johne's disease control programme from 1994 to 1999. During the study, calves within 31 days of their birth were inoculated once subcutaneously in the middle of the brisket region with MERIAL JD attenuated vaccine. According to the age of vaccination, inoculated calves were in four classes as 0-7 (G1), 8-14 (G2), 15-21 (G3) and 22-31 (G4) days old.

In the second year of this study, 117 calves (contemporary animals) were selected and assigned randomly into two groups. One group (n = 37) did not receive vaccine and considered as control; the other group (n = 80) received vaccine and considered as vaccinated group.

In order to see if the vaccination makes any problem for TB control plan, at six-month intervals comparative tuberculin tests of this herd had for nine times been checked as well.

In addition, for nine consecutive years (1991-1999), JD culling of the farm and its economical losses were calculated. Meanwhile, accidental self-inoculation was also followed. The data of this study were analysed using chi-square test in SAS (SAS/STAT user's guide, version 6.12 Cary, NC: SAS institute Inc.; 1996).

Results

By the end of this study, 2070 calves were vaccinated. The population of calves in G1, G2, G3 and G4 was 465, 571, 552 and 482 calves, respectively.

During the six years, 240 (19.4%) unvaccinated cows, due to JD affection were culled from the dairy, while in the same period, 11 (0.52%) vaccinated animals showed symptoms similar to that of JD and were culled. The vaccinated culled animals were distributed in the four groups. The mean age of these animals was 4.7 years and about 50% of them were offsprings of JD affected cattle. In the period of this study, 12.9% of calves not inoculated were culled, because of JD affection; none of the contemporary vaccinated ones showed clinical manifestation of JD (P = 0.003). In addition, the rates of total culling and culling due to low milk yield as well as infertility in vaccinated animals were lower than the unvaccinated contemporary ones (P = 0.51, 0.13 and 0.65, respectively) (Fig. 1). Vaccinated calves, at the site of inoculation, showed granulomatous reaction with increasing size for sometime after the injection of the vaccine (Fig. 2). In the majority of the vaccinated animals, the size of the reaction was being reduced at the age of above three years (Fig. 3). In this study, TB comparative test was done on vaccinated animals for nine times; there were no counter-reaction between TB control plan and JD vaccination.

During nine years of follow-up, the JD culling of the farm had a steady increment followed by a steady decrement (Fig. 4) with the highest culling took place at the age of 3.5 to 4.5 years (Fig. 5). While inoculating

Fig. 1: Culling of two groups of vaccinated and unvaccinated contemporary animals of the herd

Fig. 2: Incidence of different reactions in the vaccination site

Fig. 3: Status of the injection site reaction to JD vaccine in different age groups of animals of the herd

Fig. 4: Annual JD culling during nine years in the herd

Fig. 5: Age relevant JD culling during nine years in the herd

the vaccine, the JD causing germ contaminated needle was inserted into vaccinator's fingers several times. These incidences usually resulted in 1 to 2 weeks of hard and painful inflammation. Only in one case, a very progressive and painful granuloma was formed, in whom the clinical diagnosis was not possible, even by a medical specialists; only the histopathologic examination of the biopsied tissue revealed that the lesion was the MAP granuloma. Taking two capsules of 300 mg rifampin daily for 4.5 months resolved the problem.

Discussion

Infection with JD causative agent is quite common in dairy cattle population in Iran, though with various incidences. The herd of this study was a very problematic one (Figs. 4 and 5). In this farm, on the basis of JD culling in 1991 and 1995 and considering the following relative items, the mean annual economic loss was calculated to be $32000. The calculation based on the lowest milk (per litre), meat (per kg) and heifer (per animal) prices.

(a) Reduction of milk yield in each of two lactation before culling; (b) Shortness of life span and relative reduced parity and (c) Lowered carcass weight.

Definitely, in the calculation of the above-mentioned costs the following items are not included:

(1) Low-feed efficacy; (2) Underside calves with high mortality that may be vertically infected (up to 25%), and in this case, being the source of spreading JD causative agent within the herd as well as between the herds; (3) Expenses of diagnosis and treatment of JD cases; (4) Treatment expenses of JD-related mastitis (19%) and infertility (8.4%); (5) Salaries of workers engaged in feeding MAP-infected and JD-affected cows; (6) Animal trade restriction and (7) Above all, human *Mycobacterium avium paratuber-culosis* (MAP) infections and its life-threatening complications

The control plan of JD can have three parts: (Geissen, 1993; Rossiter and Burhans, 1996; Whitlock and Buergelt, 1996; Collins, 1999; McDonold *et al*., 1999).

(a) Identification and culling of the infected animals as early post-infection as possible: in Iran, detection of early infected animals by making the use of DNA-PCR and/or absorbed ELISA and updated modified faecal culture is not yet possible routinely.

(b) Management changes: includes the three

following principals:
- Isolation of the calf from the dam as soon as after birth and raise it in individual or grouped boxes so that it is not being in direct or indirect contact with adult cows at least within the first 6 months of age. This is quite a common but variable practice in most dairies in Iran.
- Diagnosis of JD in cattle with chronic persistent or intermittent diarrhoea, in those with weight lost and/or low milk yield by faecal and/or rectal mucosa smear. Then, cull the positive animals and their latest offspring as soon as possible. This is quite a feasible procedure but with very limited outcome.
- Prevent contamination of feed, water and even air of farm by infected faeces (McDonald *et al*., 1999; Radostits *et al*., 2000).

This is a practical method but is not fully applicable in every intensive dairy in Iran.

Fig. 6: JD culling among ≥2 years-old cows including vaccinated and unvaccinated animals.

(c) Vaccination of the neonate calves: on the basis of above-mentioned limitations, vaccination is the most effective and applicable method to control JD in cattle in Iran. In this study, the effectiveness of vaccination was quite clear (Figs. 1 and 6). This finding is in a good agreement with the statement that the vaccination as a part of the control plan may be the most appropriate for consideration in high prevalence herds (Rossiter and Burhans, 1996). In recent years in Australia, in a 29-month experimental study, the infection rates in vaccinated and unvaccinated animals were 11 and 60%, respectively (McDonald *et al*., 1999). It is a common acceptance that JD vaccine not only remarkably reduces the number of clinical cases, but also it delays the onset of clinical signs (Butler, 1993). In this investigation, the mean age of clinical appearance of the JD in the vaccinated and unvaccinated animals was 4.7 and 3.1 years, respectively. The JD vaccine can act before infection takes place (Radostits *et al*., 2000). In the present work, 50% of the vaccinated cattle affected with JD were born from JD-affected dams. To reduce the post-natal infection rates, the calves were grouped into four classes to find out the best post-natal time for calf vaccination. However, it was not possible to reach an acceptable conclusion. It is worth mentioning that at present, the proper time for neonate vaccination is quite different; i.e., 1-7, 1-31 and 1-35 days after birth (Geissen, 1993; Radostits *et al*., 2000; Eddy, 2004). In this study, vaccinated animals reacted to both mammalian and avian TB, differently so we had no problems to detect cattle infected with TB causative agents. This situation is quite matched with the great discovery that DNA insertion sequences of the two causative agents of avian TB and JD are very close to each other so that the JD causative agent would be named *Mycobacterium avium paratuberculosis* (Geissen, 1993). It is reported that the reaction of JD vaccine injection site is fibro-caseous and it remains for life in the sizes of 2 to 5 cm in diameter (Eddy, 2004). In our study, the reaction showed a tendency for resolution with increasing the age of vaccinated animals and only less than 1.5% of vaccinated animals have had a tumoral mass without making any apparent problem for engaged cattle (Figs. 2 and 3). In one occasion, we excised the mass at the age of four years and the animal is yet quite perfect. Dissection of the removed mass indicated that it was made of bran colored fluid with very much tendency to calcify and abundant connective tissue.

We are going to do some more work in this field too, and to scrutinize the final outcome of it with the two following objectives:
- How well do the operated cows, especially in relation to MAP presence and patho-

genesis?

- What is the result of histopathologic examination of masses to clear the micro-biological and chemical characteristics of the masses?

Finally, whether the events occurred to vaccinators indicate any zoonotic importance of the MAP or not remains for further scientific discoveries (Choidini and Rossiter, 1996; Radostits *et al*., 2000; Whitlock, 2002; Eddy, 2004).

References

Butler, DG (1993). Paratuberculosis in current veterinary therapy. In: Howard, JL (Ed.), *Food animal practice*. (3rd. Edn.), W. B. Saunders Co., PP: 533-534.

Choidini, RG (1996). Immunology resistance to paratuberculosis. Vet. Clin. N. Am. Food Anim. Pract., 12: 313-343.

Choidini, RG and Rossiter, CA (1996). Johne's disease, a potent zoonosis. Vet. Clin. N. Am. Food Anim. Pract., 12: 447-453.

Clarke, CJ (1997). Paratuberculosis and molecular biology. Vet. J., 153: 245.

Collins, MT (1999). Johne's disease in current veterinary therapy. In: Howard, JL and Smith, RA (Eds.), *Food animal practice*. W. B. Sandurs. PP: 344-346.

Eddy, RG (2004). Johne's disease. In: Andrews, AH; Blowey, RW; Boyd, H and Eddy, RG (Eds.), *Bovine medicine*. (2nd. Edn.), Blackwell Science. PP: 857-858.

Geissen, J (1993). A molecular approach to the diagnosis and control of bovine paratuberculosis. A Thesis, Utrecht University, Netherlands. PP: 115-120.

Hutchinson, LJ (1996). Economic impact of paratuberculosis. Vet. Clin. N. Am. Food Anim. Pract., 12: 373-381.

Ifrearulundu, YJ and Kaneene, JB (1999). Distribution and environmental risk factors for paratuberculosis in dairy cattle herd in Michigan. AJVR, 60: 589-596.

Jean, GS (1996). Treatment of clinical paratuberculosis in cattle. Vet. Clin. N. Am. Food Anim. Pract., 12: 417-430.

McDonald, WL; Ridge, SE; Hope, AF and Condron, RJ (1999). Evaluating of diagnostic tests for Johne's disease in young cattle. Aust. Vet. J., 77: 113-119.

Radostits, OM; Gay, CC; Blood, DC and Hinchcliff, KW (2000). *Paratuberculosis (Johne's disease) in veterinary medicine*. 9th. Edn., W. B. Saunders Co., PP: 920-934.

Rossiter, CA and Burhans, WS (1996). Farm-specific approach to paratuberculosis control. Vet. Clin. N. Am. Food Anim. Pract., 12: 383-415.

Sweeney, RW (1996). Transmission of paratuberculosis. Vet. Clin. N. Am. Food Anim. Pract., 12: 305-312.

Whitlock, R (2002). Johne's disease (paratuberculosis). In: Smith, BP (Ed.), *large animal internal medicine*. (3rd. Edn.), Mosby Co., PP: 779-783.

Whitlock, RH and Buergelt, C (1996). Preclinical and clinical manifestation of paratuberculosis. Vet. Clin. N. Am. Food Anim. Pract., 12: 345-346.