Factors Associated with Adverse Events Following Administration of *Histophilus somni* Vaccine to Calves

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ABSTRACT

To analyze the factors associated with an appearance of adverse events in calves following Histophilus somni vaccination, we investigated changes in rectal temperature, tumor necrosis factor-alpha (TNF-α) levels, and clinical signs in vaccinated Japanese Black calves (n=19) . The average rectal temperature 24 hr post-vaccination was significantly higher in male calves compared with pre-vaccination. In contrast, the rectal temperature of female calves remained constant during the observation period. Febrile male calves aged over 9 months had inappetence, while the appetite of calves aged less than 8 months was normal. These results suggest that sex and age are associated with adverse effects elicited by H. somni vaccination. The amount of TNF-α in the peripheral blood derived from male calves was significantly lower than that derived from steer and female calves. Sex

and/or age-related effects were not identified in reported cases of adverse events. Acute shock was the most common presentation in reported cases, typically when the vaccine was administered with another multivalent vaccine. Therefore, H. somni vaccination is considered safe when administered according to the manufacturer's instructions.

INTRODUCTION

Severe adverse events following administration of vaccines containing inactivated Gram-negative bacteria have been reported in Japan (http://www.maff.go.jp/nval/). In particular, most cases have been related to cattle vaccines containing inactivated Histophilus somni. It has been speculated that this is due to endotoxins in the vaccines (Usui et al., 2013). As part of general risk management for vaccination, the manufacturer's directions instruct veterinarians to avoid vaccinating sick cattle. Administration of the vaccine is also prohibited in combination with other vaccines, and observation of animals post-vaccine for adverse effects is required. Although there have been concerns about the safety of the H. somni vaccine, risk factors associated with adverse events have not been determined, making prediction of adverse events difficult.

Generally, immunological responses to endotoxin, a common component of Gram-negative bacteria, activate leukocytes and produce proinflammatory cytokines (Van Amersfoort et al., 2003). Proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), produced by many types of cells are responsible for early immunological responses, including a thermostatic set point increase at the level of the hypothalamus via prostaglandin E2 (Van Deventer et al., 1990; Suffredini et al., 1999; Günther et al., 2011). Proinflammatory cytokine signals are further transmitted to the brain, where complex thermoregulatory mechanisms are triggered to increase the body temperature (Dinarello et al., 1984; Saper and Breder, 1994; Luheshi and Rothwell, 1996; Dinarello, 1999). The inflammatory cascade triggered by proinflammatory cytokines can eventually cause toxic shock and death (Günther et al., 2011). Measurement of rectal temperature and quantification of proinflammatory cytokines are therefore useful to monitor progression of inflammatory responses in cattle.

In this study, we therefore measured rectal temperatures and TNF-α levels in the peripheral blood of Japanese Black calves following vaccination, as well as hematological and biochemical changes and clinical signs to investigate any adverse events.

MATERIALS AND METHODS

Farm and Animals

This study was conducted between June 3rd and 7th 2013 on the Sumiyoshi Livestock Science Station of Miyazaki University, Miyazaki, Japan. A total of 19 clinically healthy Japanese Black calves (5 male calves, 6 steers, and 8 female calves) aged 5–11 months (average: 8.34 months) were included in the study, as adverse effects had been reported in this age group. Calves were fed twice daily (9am and 4pm) with 2.0 kg of concentrate diet, with access to fresh water, orchard grass hay, and mineral salt blocks ad libitum during the observation period. For male calves, the concentrate diet contained 68–70 % total digestible nutrients (TDN) and 15–15.5 % crude protein (CP) on a dry matter (DM) basis. For female calves, the diet contained 72–73 % TDN and 12–13 % CP on a DM basis. All protocols were approved by the Institutional Review Board for Animal Experiments of the University of Miyazaki (Approval number: 2013-015).

Vaccination

A commercial inactivated H. somni vaccine was purchased from KyotoBiken Laboratories, Inc. (Kyoto, Japan). The vaccine was administered intramuscularly and needles were changed after each injection.

Animal Monitoring

Rectal temperatures were measured using the Matsuda veterinary thermometer (Asahi Glass Co., Ltd., Chiba, Japan). Animals were examined by three veterinarians at 0, 1, 3, 24, and 72 h post-vaccination (hpv).

Table 1. Rectal temperatures following vaccinations. [a]

Hours post-vaccination	0		1		3		24		72	
Groups	Avg	(±SD)	Avg	(±SD)	Avg	(±SD)	Avg	(±SD)	Avg	(±SD)
Calves (n=19)	39.28	±0.33	39.18	±0.31	39.22	±0.32	39.86**	±0.76	38.94	±0.23
Females (n=8)	39.31	±0.43	39.28	±0.36	39.28	±0.42	39.27	±0.47	38.98	±0.15
Intact males (n=5)	39.38	±0.15	39.20	±0.24	39.22	±0.22	40.16*	±0.65	38.73	±0.22
Steers (n=6)	39.15	±0.26	38.98	±0.20	39.12	±0.23	40.58**	±0.40	38.73	±0.18

[a] *Annotations denote p-values less than 0.01 (**) and 0.05 (*) obtained via Mann-Whitney U-test*

Quantification of TNF-α, Biochemical, and Hematological Analyses

Blood from the jugular vein of each calf was collected into 4-ml Vacuette tubes without an anticoagulant (Greiner Bio-one GmbHy, Kremsmunster, Austria). Serum was harvested after centrifugation at 1,400 g for 5 min. TNF-α serum levels were determined using the GSI Bovine TNF-α ELISA kit (Genorise Scientific, Inc., Paoli, USA) as per the manufacturer's instructions. Total protein (TP), total cholesterol (T. chol.), creatinine (Cre), triglyceride (TG), total bilirubin (T. bil.), uric acid (UA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), gamma-glutamyl transpeptidase (γ-GTP), and cholinesterase (ChoE) in sera were measured using an autoanalyzer (Hitachi 7600-110S, Tokyo, Japan) at 0, 1, 3, 24, and 72 hpv.

Blood from the jugular vein of each calf was collected into 4-ml Vacuette EDTA tubes (Greiner Bio-one GmbHy). The total white blood cells (WBCs), total red blood cells (RBCs), hemoglobin level (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and blood platelet count were measured by using an autoanalyzer (IDEXX laboratories, Tokyo, Japan) at 0, 1, 3, 24, and 72 hpv.

Previously Reported Adverse Events with H. somni Vaccination

Reports of previous adverse events were obtained from the Japanese online adverse effect database (http://www.nval.go.jp/asp/se_search.asp). The name of the vaccine was used as a search key to determine the number of previously reported adverse events.

Statistics

Statistically significant differences were determined using the Mann-Whitney U test.

RESULTS

Clinical Findings in Vaccinated Calves

The changes in rectal temperatures in vaccinated calves are shown in Table 1. The rectal temperature in vaccinated male and steer calves at 24 hpv was significantly increased compared with prevaccination. In contrast, the rectal temperature in female calves remained stable

Table 2. TNF-α (ng/mL) levels following vaccination

Hours post-vaccination	0		1		3		24		72	
Groups	Avg	(±SD)	Avg	(±SD)	Avg	(±SD)	Avg	(±SD)	Avg	(±SD)
Calves (n=19)	9.55	±7.63	11.20	±9.61	9.41	±8.73	10.11	±11.13	10.24	±8.60
Females (n=8)	11.01	±7.11	13.44	±11.38	11.09	±9.60	13.12	±14.43	12.83	±10.56
Intact males (n=5)	5.39	±1.57	4.71	±2.65	3.82	±2.32	4.52	±2.11	5.82	±2.91
Steers (n=6)	10.79	±11.82	13.19	±8.82	13.07	±10.32	11.07	±9.59	10.61	±8.53

Table 3. *Numbers of adverse events reported*

Japanese black calves	39
Females	23
Steers	16
Dairy calves	7
Females	6
Males	1
Total	46

throughout the observation period. Febrile male and steer calves aged over 9 months had inappetence once febrile, while no appetite changes were detected in calves aged less than 8 months and female calves (data not shown). All calves showing clinical signs recovered within 72 hpv.

TNF-α Levels in Calves Following Vaccination

The amount of TNF-α in peripheral blood derived from male, steer and female calves remained constant during the observation period (Table 2). No significant differences were observed during the pre- and post-vaccination. However the average of TNF-α in peripheral blood differed in each group (male, steer and female calves: 4.9, 11.7 and 12.3 ng/mL, on average, respectively); in addition, the average of TNF-α in peripheral blood derived from male calves were significantly lower than that derived from steer and female calves ($p<0.01$).

Hematological and Biochemical Changes in Vaccinated Calves

Total WBC count was significantly increased at 24 hpv compared with that of pre-vaccination in all calves (0 and 24 hpv: 8980 and 9908 WBC/μL, on average, respectively, $p<0.05$). However, there was no significant difference between male and female calves. No significant differences were detected in total RBC count, Hb level, MCV, MCH, MCHC, and platelet count (data not shown). The amount of Cre in male calves was significantly increased at 24 hpv compared with pre-vaccination (0 and 24 hpv: 0.792 and 0.87 mg/dL, in average, respectively, $p<0.01$). No significant changes were detected in the other parameters (TP, T. chol., TG, T. bil., UA, AST, ALT, LDH, γ-GTP, and ChoE).

Analyses of Previously Reported Adverse Events

According to the National Veterinary Assay Laboratory (NVAL) database, 46 adverse effects have been previously reported (Table 3). Twenty-three of these died because of shock. However, specific factors associated with appearance of adverse effects in cattle following administration of H. somni vaccine were not determined.

DISCUSSION

Gender-based differences in immune system function have long been observed in humans. Several factors contribute to this immunological dimorphism, including sex hormones, genetic makeup, environmental causes, and, more recently microchimerism (Ghazeeri et al., 2011). Thus, lack of fever in female calves may be explained by hormonal differences (Mouihate et al., 2003). While the mechanism behind sexual dimorphism is still unclear, the risk of the adverse effect associated with the vaccination seems to be higher in male calves, particularly those aged over 9 months. However, hematological and biochemical changes in vaccinated calves reveal that the early systemic effects of the vaccination are minor.

Detection of a vaccine-induced inflammatory response using peripheral blood examination was difficult. In humans, men generally have higher blood levels of several proinflammatory cytokines (such as interleukin-6 and TNF-α) typically produced by monocytes and macrophages, suggesting sex-based differences in innate immunity (Asai et al., 2001; Bouman et al., 2004; Bouman et al., 2005). One manifestation of this is that women are more resistant to bacterial infections (Bouman et al., 2005). These reports also suggest that sex must be considered in examination of adverse effects following vaccination. In the present study, TNF-α levels in the peripheral blood from male calves were lower than those from steer and female calves. On the other hand, the rectal temperature at 24 hpv was higher in male and steer calves than in female calves as compared with that at pre-vaccination period. These findings suggest that only TNF-α in the peripheral blood do not appear to directly correlate to increased rectal temperature. However, when severer adverse effects such as shock once developed, TNF-α) levels may be increased in local site as well as systemic site including the peripheral blood, resulting in aggravation of inflammation.

Field cases of fatal shock were reported in both steers and female calves. The discrepancy between these historical cases and the results of the current study is likely multifactorial. In the historical cases, 31 out of 46 calves had other vaccines administered simultaneously with the H. somni vaccine. Furthermore, these vaccinations were often administered on the farm without direct examination of cattle by veterinarians. These situations may increase the risk of severe shock following vaccination.

CONCLUSION

Further studies are necessary to determine factors that will ensure vaccines are used safely. While sex and age are associated with mild adverse effects of H. somni vaccination, the vaccine is generally safe when administered according to the manufacturer's directions.

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