## Key Highlights

- Endotoxin concentration of Bovilis J-5 was the lowest per milliliter of vaccine and per label-directed vaccination volume compared with three other commercially available Gram-negative, lipopolysaccharide core-antigen vaccines used to control bovine mastitis.
- The endotoxin load in a Bovilis J-5 immunization was 15-fold below the U.S. Pharmacopeia (USP) recommendation for the upper limit of endotoxin in an immunization for a 1,500-lb. cow.

# Comparison of Endotoxin Concentrations in Bovilis® J-5 with Those in Three Commercially Available Gram-negative, Lipopolysaccharide Core-antigen Vaccines

## SUMMARY

Bovilis J-5 is an *Escherichia coli* J-5 bacterin distributed by Merck Animal Health and intended for use in healthy dairy cattle as an aid in the reduction of mastitis due to *E. coli*. The purpose of this study[1] was to compare the endotoxin concentrations in Bovilis J-5 to those in three commercially available Gram-negative, lipopolysaccharide core-antigen vaccines used to control bovine mastitis. Nine bottles of each vaccine, each bottle from a unique lot and serial number, were tested for endotoxin concentration by the *Limulus* amebocyte lysate assay using kinetic turbidimetric detection. Pair-wise comparisons of means indicated that all vaccines differed in endotoxin concentration. Mean endotoxin concentration for each vaccine was Bovilis J-5 44 EU/mL, Endovac-Dairy® (Endovac Animal Health) 85,156 EU/mL, Enviracor® J-5 (Zoetis) 5,936 EU/mL and J-VAC® (Boehringer Ingelheim) 351,636 EU/mL. The endotoxin concentration of Bovilis J-5 was lowest per milliliter of vaccine and per label-directed vaccination volume compared with the other three vaccines tested.

## INTRODUCTION

The effectiveness of a Gram-negative core-antigen mastitis vaccine is due in part to the bacterial surface exposure of the conserved portion of lipopolysaccharide (endotoxin) common among coliform bacteria, thereby providing immune protection against a wide variety of coliform strains. While the exposure of cows to endotoxin during vaccination creates a desired immune response, excessive endotoxin could result in adverse effects such as fever, loss of appetite, loss of pregnancy, decreased milk production and sometimes even death. Therefore, a goal in formulation of Gram-negative core-antigen vaccines is to provide adequate antigen exposure to elicit an effective immune response while minimizing the potential adverse endotoxin load a cow must endure. The purpose of this study was to compare the endotoxin concentrations in Bovilis J-5 to three commercially available Gram-negative core-antigen vaccines used to control bovine mastitis.

## PROCEDURES

Nine bottles of each vaccine, each bottle from a unique lot and serial number, were tested for endotoxin units (EU) per milliliter of vaccine by the *Limulus* amebocyte lysate assay using kinetic turbidimetric detection. Data were analyzed using a linear mixed model for a complete block study design. A single sample from each vaccine lot and serial number was the experimental unit. A random intercept term was included in the model to account for the lack of independence among samples within replicates.

## RESULTS

Endotoxin concentrations differed among vaccine products (Table 1). Pair-wise comparisons of all vaccine products indicated that all means differed significantly ($P<0.01$). Mean for Bovilis J-5 was 44 EU/mL (range 3-100 EU/mL), Endovac-Dairy 85,156 EU/mL (range 44,700-156,000 EU/mL), Enviracor J-5 5,936 EU/mL (4,100- 8,000 EU/mL) and J-VAC 351,636 EU/mL (range 223,000-459,000 EU/mL).

## CONCLUSIONS

Endotoxin concentrations in Bovilis J-5 were significantly lower than in the other three commercially available Gram-negative core-antigen bacterins. The concentration of endotoxin in a vaccine is a consequence of the purification process during manufacturing of product.[2] While no regulatory standards for endotoxin limit in vaccines currently exist, the USP recommends a manufacturing limit of 5 EU/kg of the animal for preclinical evaluation. Therefore, the USP-recommended upper limit of endotoxin in an immunization for an average 1,500-lb. Holstein dairy cow would be 3,400 EU.

Average concentration of endotoxin in Bovilis J-5 in the study was 44 EU/mL of vaccine. Label directions for Bovilis J-5 are 5 mL per injection, thus the average endotoxin concentration would be 220 EU per immunization (Table 1). These results indicate the endotoxin load in a Bovilis J-5 immunization was below the USP-recommended upper limit of endotoxin in an immunization for an animal the weight of an average Holstein. Each lot of the other three vaccines exceeded this recommendation with endotoxin load per immunization ranging from 6-fold to 270-fold above this limit.

**Table 1.** Comparison of endotoxin units (EU) per milliliter (total product label directed vaccination volume for four Gram-negative core-polysaccharide-antigen bacterins.)

| | BOVILIS® J-5 | ENDOVAC-DAIRY® | ENVIRACOR™ J-5 | J-VAC® |
|---|---|---|---|---|
| **Dose** | 5 mL | 2 mL | 5 mL | 2 mL |
| **Mean EU/mL*** | 44[a] | 85,156[b] | 5,936[c] | 351,636[d] |
| **Range EU/mL** (rounded to nearest 100) | 3 – 100 | 44,700 – 156,000 | 4,100 – 8,000 | 223,000 – 459,000 |
| **Total Product Label Mean EU/vaccination**** | 220 | 170,312 | 29,680 | 703,272 |

*USP recommends a limit of 5 EU/kg of the animal being vaccinated or 3,400 EU for an average 1,500-lb. Holstein.

**Calculated by multiplying dose by mean EU/mL.

[a,b,c,d] Means with different superscripts differ ($P<0.05$).

[1]Data on file, Merck Animal Health.

[2]Brito LA, Singh M. Acceptable levels of endotoxin in vaccine formulations during preclinical research. *J Pharm Sci.* 2011;100:34-37.

**Bovilis J-5 Warning:** This product contains oil adjuvant. In the event of accidental self-injection, seek medical attention immediately. For additional information, please see the product label.






MERCK Animal Health