A five year randomized controlled trial to assess the efficacy and antibody responses to a commercial and autogenous vaccine for the prevention of infectious bovine keratoconjunctivitis

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Introduction

Infectious bovine keratoconjunctivitis (IBK) is the most commonly diagnosed ocular disease of cattle and presents with clinical signs including corneal ulceration, epiphora, blepharospasm, conjunctival swelling, photophobia, and/or buphthalmos among others.[1] *Moraxella bovis* (*M. bovis*) is the only infectious agent with a demonstrated causal relationship with IBK.[2,3] Other infectious agents are often found in IBK lesions, but attempts to reproduce IBK-like disease using other infectious agents have been unsuccessful thus far.[4,5] Vaccinating calves with either commercially available or autogenous vaccine products is commonly used as a prevention strategy by producers and veterinarians. However, studies that have examined the efficacy of these products have historically had mixed results at best.[6] The objective of this study was two-fold. A randomized controlled trial was performed to assess the efficacy of a commercial and autogenous vaccine formulation for the prevention of IBK over five years (2016 – 2020) in a herd that has historically had a relatively high annual incidence of IBK. Antimicrobial treatment success and adjusted weaning weights were also compared between the vaccine groups. Secondly, we developed a novel ELISA to assess the serum IgG levels of calves between the different vaccines. The target antigen for the ELISA was a recombinant full-length (157 amino acids in length) protein identical to the type IV pilus protein of Epp-63 (300) strain of *M. bovis* which represents a known virulence factor for this strain.[7] The ELISA allowed us to examine whether the level of anti-*M. bovis* IgG antibodies had an effect on the cumulative incidence of IBK.

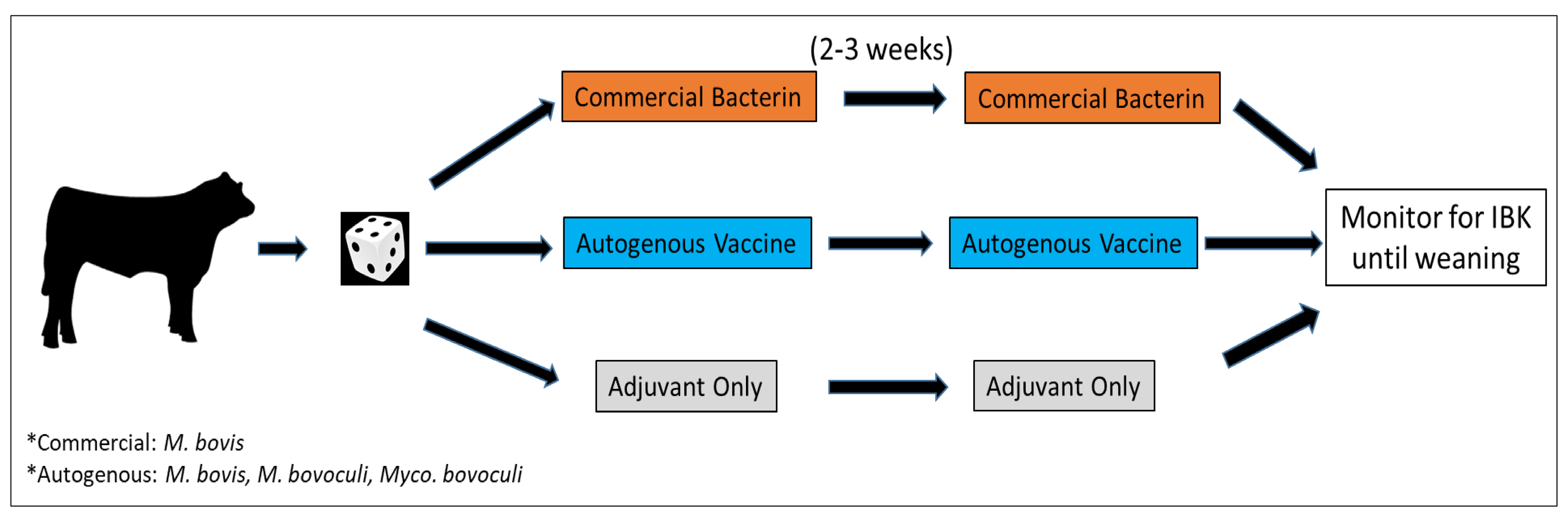

Figure 1: Graphical abstract of the randomized controlled study design.

Materials & Methods

- The annual offspring of a beef teaching herd at the Eastern Nebraska Research, Extension, and Education Center (ENREEC) were randomly assigned to one of the three vaccine treatment groups each year. The study included 1198 calves from 2016 through 2020.
- Calves were vaccinated twice, 2-3 weeks apart prior to turnout.
- Age at initial vaccination ranged from 10-98 days.
- Calves were monitored for clinical signs of IBK throughout the grazing period until weaning in early October.
- Data collected included calf ID, sex, hide color, date of birth, weaning weight, incidence(s) of IBK, and response to treatment.
- Commercial *M. bovis* bacterin used was Ocu-guard MB-1 (Boehringer Ingelheim Vetmedica).
- Autogenous formulation included isolates of *M. bovis*, *Moraxella bovoculi*, and *Mycoplasma bovoculi* (Phibro Animal Health).
- Adjuvant only treatment consisted of 3 mL of the oil-in-water adjuvant formulation Emulsigen-D developed by the autogenous manufacturer (Phibro Animal Health).
- Pre and post vaccination serum were obtained from each calf prior to initial vaccination and 2-3 weeks after the second vaccination respectively.
- The anti-*M. bovis* IgG ELISA utilized 1 µg of recombinant antigen per well with serum dilutoins of 1:400 and secondary rabbit anti-bovine IgG antbody (H+L) diluted 1:5,000. Detailed ELISA methods are available in the published manuscript: doi: 10.3390/vaccines10060916

Materials and Methods Cont'd

- Analysis of the treatment effect on incidence of IBK used SAS and a logistic regression model with a logit link function and symbols defined as follows: η is the overall intercept, τ_i is the effect of the i^{th} treatment, h_j is the effect of the j^{th} hide color, s_k is the effect of the k^{th} sex, $b(h)_{jl}$ is the effect of the l^{th} management group nested with hide color, and t_m is the effect of the m^{th} year. Year, treatment, hide color, sex, and breed were treated as fixed. For analysis on treatment effect on retreatment rates, the binomial model was used and only calves that developed IBK were analyzed.

$$y_{ijklm} \sim Binomial(p_{ijklm})$$

$$\eta_{ijklm} = \log\left(\frac{p_{ijklm}}{1-p_{ijklm}}\right) = \eta + \tau_i + h_j + s_k + b(h)_{jl} + t_m$$

- Relative risk of IBK by vaccine treatment group was analyzed using the following formula:

$$Relative\ Risk = \frac{1-P_2}{1-P_1} \times Odds\ Ratio$$

Results

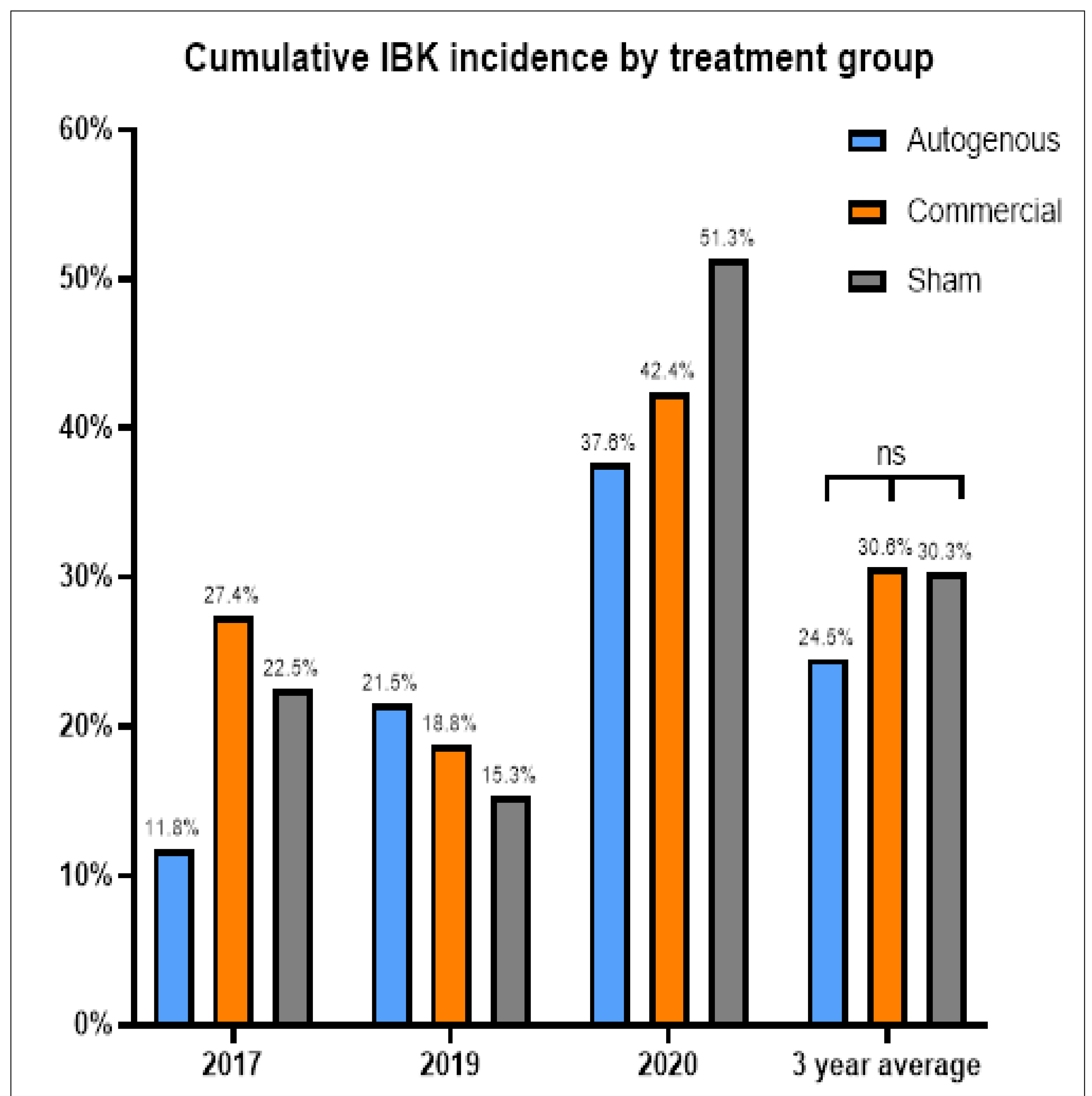

Figure 2: Annual and 3-year average incidence of IBK diagnosis by vaccine treatment group. Percentages calculated as the number of IBK diagnoses per calves enrolled each year. Vaccine treatment did not significantly affect IBK incidence. ns = not significant (p = 0.25). Years 2016 and 2018 were omitted from analysis due to an exceptionally low disease burden.

Results Cont'd

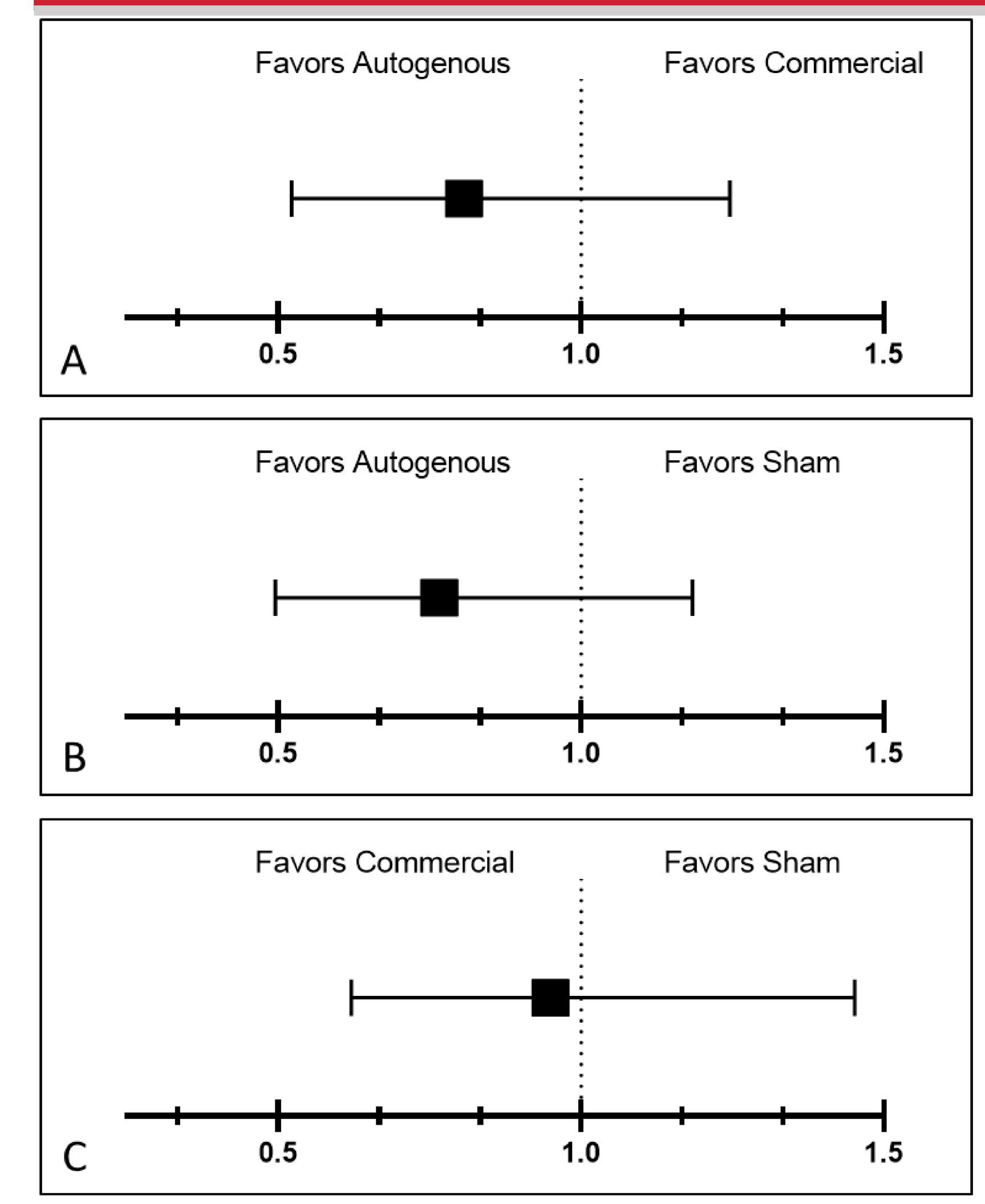

Figure 3: Risk ratio for the effect of treatment on IBK incidence. There were no significant differences in IBK incidence among any of the treatment groups.

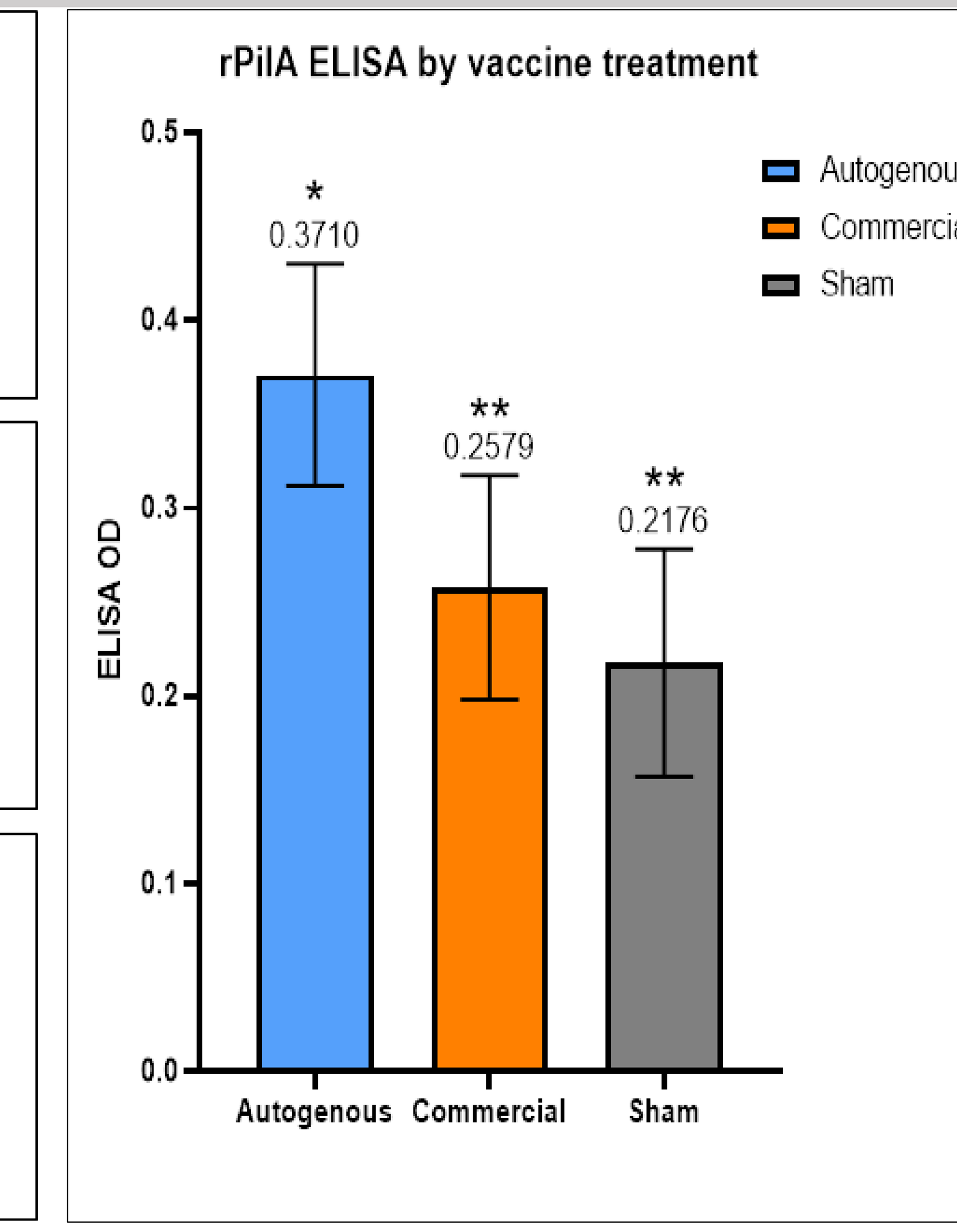

Figure 4: Relative ELISA OD readings by vaccine treatment group. Error bars indicate 95% confidence limits. The autogenous vaccinates averaged significantly higher anti-*M. bovis* antibody levels.

Conclusions

There were no significant differences observed in cumulative incidence of IBK between the commercial bacterin, autogenous, or sham vaccine treatment groups in this long term study. Additionally, there were no significant differences observed regarding the need for more than one antimicrobial treatment per case of IBK among the vaccine treatment groups (data presented in manuscript, p = 0.15). The autogenous group had significantly higher levels of anti-*M. bovis* IgG than both the commercial and sham treatment groups. Animals that developed IBK had lower average serum anti-*M. bovis* antibody levels than non-IBK calves, but this difference was insignificant (data presented in manuscript, p = 0.37). Since IBK cases had lower average antibody levels, humoral immunity may still play at least a partial role in IBK protection and future attempts to increase this response may be warranted.

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