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EVALUATION OF FACTORS AFFECTING THE RESPONSE OF LLAMAS TO VACCINATION WITH *CLOSTRIDIUM PERFRINGENS* C TOXOID

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La vaccination des Lamas contre la toxine de Clostridium perfringens (C) fait partie des plans de prévention dans de nombreuses fermes de Lamas. Les Lamas atteints d'un Syndrome de Déficience Immunitaire du Jeune Lama (SDIJL) ne répondent pas à la vaccination par une augmentation du taux d'anticorps. Le but de cette étude est de caractériser la réponse de Lamas en bonne santé à cette vaccination, et d'identifier les facteurs pouvant affecter l'aptitude des Lamas à répondre positivement à cette vaccination.

A chacune des quatre périodes de prélèvements sanguins, réalisés à 6 mois d'intervalle, la vaccination a été administrée, et un second prélèvement effectué environ deux semaines plus tard. Les titres plasmatiques mesurés par un test ELISA variaient de 100 à 12.800. Une réponse positive était définie comme une augmentation d'au moins deux dilutions après un titre initial d'au moins 100 à 800. Quand le titre initial était de 1.600 ou plus, un résultat était considéré comme positif pour une augmentation d'une seule dilution ou plus. Les données ont été soumises à un traitement de statistique descriptive et à une régression logistique. Les données suivantes ont été retenues dans le modèle : élevage d'origine, âge, sexe, poids, état d'engraissement, concentration plasmatique en immunoglobulines G, titre initial en C. perfringens de type C, intervalle de prélèvement, statut vaccinal antérieur, période d'étude, et toutes les interactions de premier ordre.

Un total de 797 Lamas ont été étudiés. Le modèle final comprenait les variables suivantes : ferme d'origine, période d'étude, statut vaccinal antérieur, état d'engraissement, titre initial et âge.

Nos résultats suggèrent l'utilité du test comme devant faire partie de l'évaluation du statut du système immunitaire des Lamas, en démontrant des résultats d'épreuve dans une large population de Lamas de différents âges et de différentes provenances.

INTRODUCTION

Assessment of the immune system is indicated when an immunodeficiency condition is suspected.(Virella *et al.*, 1993a; Virella *et al.*, 1993b) Quantitation of antibodies after antigenic challenge is suggested as a means of evaluation of humoral immunity.(Virella *et al.*, 1993a) An immunodeficiency condition affecting juvenile llamas (Juvenile Llama Immunodeficiency Syndrome or JLIDS)(Hutchison *et al.*, 1992; Hutchison and Garry, 1994; Hutchison *et al.*, 1995) has been described. The condition is characterized by wasting and opportunistic infections. Vaccination of llamas with *Clostridium (C.) perfringens* C toxoid is part of routine herd health management on many llama farms.(Long, 1989) Llamas affected with JLIDS have pre-vaccination titers $\leq$ 100, and fail to respond to vaccination with a measurable increase in titer;(Hutchison *et al.*, 1995) however, the nature of the response has not been studied in populations of healthy llamas in their natural settings. The purpose of this study was to characterize the response of healthy llamas to this test, and to identify factors that affect the ability of a llama to respond positively to challenge with *C. perfringens* C toxoid.

MATERIALS AND METHODS

This study was part of a larger project in which llamas from 5 farms were sampled on 4 occasions, each 6 months apart. All llamas from each farm were sampled during the 1st sampling period. Subsequently, llamas born since previous sampling period, and new additions to the herd that were aged < 2.5 years (y) (2nd sampling period) or 3 y (3rd and 4th sampling periods), were enrolled. A llama was included in this study on the first occasion on which it was sampled in the project. At each sampling period, llamas were identified and weighed. A body condition score(Johnson, 1994) (BCS) between 1(emaciated) and 10 (extremely obese), with 5 being ideal, was assigned. Age, gender, previous vaccination status, and sampling period in which llama was evaluated, were recorded. Llamas were vaccinated with a product containing *C. perfringens* C toxoid, and blood was collected into lithium heparin vacutainer tubes via jugular venipuncture. A second blood sample was collected approximately two weeks later. Plasma was harvested via centrifugation and samples were maintained at -20º C until analysis.

Pre- and post-vaccination titers between 100 and 12,800 were measured using an ELISA test.(Hutchison *et al.*, 1995) A positive response to vaccination was defined as a twofold or greater increase in post- over pre-vaccination titer for llamas that had pre-vaccination titers < 1,600, and as a onefold or greater increase for llamas that had pre-vaccination titers of > 800. Plasma immunoglobulin G (IgG) was measured by single radial immunodiffusion kit (Llama IgG Test Kit, Triple J Farms, Redmond, Washington, USA).

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Multivariable logistic regression procedures(Hosmer, Jr. and Lemeshow, 1989) were used to model the positive response of a llama to vaccination with *C. perfringens* C toxoid. The *p* values for entry to and removal from the model 0.10 and 0.15, respectively. Factors considered for inclusion in the model were farm of origin, age, gender, body weight, BCS, plasma IgG concentration, previous vaccination status, days between collection of pre- and post-vaccination samples, sampling period in which evaluated, season of year, and all first order interaction terms of these variables. The criterion for statistical significance (other than for entry and removal of terms from the logistic regression model) was $p \leq 0.05$.

RESULTS AND DISCUSSION

The five participating llama farms varied in location, llama population, llama purpose, and management styles. A total of 907 llamas were evaluated; however, complete information was available for only 797 of these. Five hundred thirty-two (66.7%) had history of previous vaccination. A total of 450 llamas (56.5%) responded to vaccination with *C. perfringens* C toxoid. The proportion of llamas previously vaccinated increased with age, and the percentage of previously-vaccinated llamas that responded ranged from approximately 65 to 90% in llamas greater than 4 m of age. Factors identified by the multivariable logistic regression model as affecting the response of llamas to vaccination were farm, sampling period in which llama entered the study, BCS, previous vaccination status, age, and pre-vaccination titer. Age was found not to be linear in the logit, and was modeled as a categorical variable with 10 age groups. Gender, body weight, plasma IgG concentration, number of days between collection of pre- and post-vaccination samples, season of year, and all first-order interaction terms were not statistically-significant predictors of a positive response to vaccination. No llama aged > 4 m recorded pre- and post-vaccination titers of 100.

This study has provided previously-lacking information concerning the responses of healthy llamas to vaccination with *C. perfringens* C toxoid. A wider range of responsiveness and pre-vaccination titers was observed in these llamas than previously was noted in control llamas used in an earlier study.(Hutchison *et al.*, 1995) Nevertheless, confidence in the ability of this test to identify JLIDS llamas is enhanced because no llamas aged greater than 4 m recorded responses consistent with those previously identified in JLIDS llamas. Use of the multiple logistic regression model allowed identification of factors that affect the ability of a llama to respond to vaccination.

CONCLUSION

Assessment of response to vaccination with *C. perfringens* C toxoid is an easily-performed and inexpensive means of evaluating llama humoral immunity and has particular application in the diagnosis of JLIDS.

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