TRANSMISSION OF BOVINE LEUKEMIA VIRUS BY RECTAL PALPATION - CONTROL AND EPIDEMIOLOGIC STUDIES

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Introduction

Bovine Leukemia Virus (BLV), a C-type retrovirus, is horizontally spread in cattle by infected lymphocytes.[1] Infection with the BLV is lifelong.

Several routine management procedures have been implicated in the transmission of BLV, especially manual dehorning and use of contaminated needles.[2,3] There are both questions and concern regarding the probability of transmitting BLV infections in cattle during rectal palpation.[4] This concern seems especially pertinent since almost all breeding-age cattle receive multiple rectal examinations to evaluate the reproductive tract, and rectal sleeves are often not changed when cattle in the same herd are being palpated. There have also been experiments which have shown that BLV infection in cattle can occur by transferring infected blood into the rectum.[5,6,7] In these studies, infective blood was either purposely infused into the rectum of cows and calves or was inoculated onto the rectal sleeve prior to palpating calves.

The intent of this study was to determine if bovine leukemia virus could be transmitted by more routine rectal examination of BLV-negative, breeding age, cattle.

Materials & Methods

Animals

Thirty dairy heifers and cows (all greater than 15 mo. of age) were purchased from 2 herds, both of which had previously been found to be serologically negative for BLV. All BLV sero-negative cattle were housed individually so that there was no contact with other cows. Fourteen BLV-negative animals were used in Experiment 1 and 16 in Experiment 2 which was conducted 1 year later. All 30 cattle were confirmed to be seronegative for BLV on 3 separate serum samples prior to starting the trial; the first sample was collected on the farm, the second at the initiation of isolation and the third, 30 days later immediately prior to the start of the trial. All serologic testing for the detection of BLV antibodies was done using the radioimmunoassay (RIA) with the virion envelope glycoprotein as antigen[8] and/or by an enzyme-linked immunoassay (ELISA) of similar sensitivity and specificity.

Two positive Jersey cows, one with and one without persistent lymphocytosis, were used as positive cows in Experiment 1. In Experiment 2, 16 BLV-positive Holstein or Jersey cows were used, 8 of which had lymphocytosis.

Experimental Design

Experiment 1

Fourteen BLV-negative cattle were randomly assigned into 1 of 2 two equal-sized groups: A (1-7) and B (8-14). Individual housing and isolation of each animal was maintained. For 4 weeks, 1 of the 2 positive cows (on a daily rotating basis) was transported to the separate locations of one cow in Group A and one cow in Group B. The positive cow (either #351 or #315) was rectally palpated by an experienced bovine clinician in a manner considered to be routine for reproductive examination. During this exam, the cervix, both uterine horns, and both ovaries were gently palpated. Following daily palpation of one of the 2 positive cows, the rectal sleeve was discarded and a new sleeve used for palpation of a cow in Group A. For the cows in Group B, palpation of the positive cow was repeated and the same rectal sleeve was then used to palpate a cow in Group B. The palpation of each negative cow occurred within 1 minute of the palpation of the positive cows. Cows in each group were palpated in a reversing sequence over the 28 days such that the design difference between the 2 groups of negative cattle was the absence or presence of a changed rectal sleeve (see Fig. 1).

Experiment 2

Sixteen BLV-negative Guernsey heifers (15-18 mo. of age) were randomly assigned into 2 equal size experimental groups: C (1-8) and D (9-16). Each animal was individually housed and maintained in isolation. Each BLV-negative heifer was randomly assigned (by design but not physically) to a BLV-positive cow (N = 16). Each heifer in Group C was paired with a BLV-positive cow with lymphocytosis and each heifer in Group D was paired with a BLV-positive cows without lymphocytosis. Each BLV-negative heifer in both groups was palpated on two occasions, one month apart, immediately after their assigned positive cow had been palpated and without a change in sleeve. The paired positive cow was rectally palpated by an experienced bovine clinician in a manner considered to be routine for reproductive examination as described in Experiment 1.

On the days of palpation, each positive cow in both Experiment 1 and 2 was transported to the location of the corresponding negative heifer but was not allowed physical contact.

Following the palpation period, all negative cows or heifers remained in individual isolation for an additional 90 days. Blood was collected every month after the start of the palpation and during the 90 day post-palpation isolation period. The serum of each animal was tested using either RIA or ELISA for determination of BLV antibodies. All aspects of Experiments 1 and 2, including pre-palpation and post-palpation testing, were performed during the months of November through April in order to lower any possibility of insect transmission.

Results

In Experiment 1, 3 animals in group B (no sleeve change) developed antibodies to BLV. All three were positive on the first post-palpation serologic test (28, 30, 31 days after their first palpation had occurred). No further positives were detected at 2 later test dates, 1 month apart. All animals in group A (sleeve change) remained negative.

In Experiment 2, 1 heifer in Group C (palpation after a cow with lymphocytosis) was serologically positive when tested 30 days after the initial palpation. No other heifers in that

group or any in Group D (heifers palpated after BLV-positive cows with normal lymphocyte counts) became positive during the experiment.

Discussion

Experiments 1 and 2 confirmed that BLV can be transmitted in breeding age or mature dairy cows by rectal palpation. The use of BLV-negative cattle from BLV-seronegative herds, repeated testing of these cattle for BLV antibodies using either RIA or ELISA, strict isolation of all cattle, and conduction of the entire experiment during the insect-free season should have ensured that any transmission that occurred in these studies was from rectal palpation. The incidence of infection from Experiment 1 cannot be readily applied to the general bovine population since the 2 positive cows were palpated with considerably more frequency than would normally occur in practice. Although no red discoloration of any rectal sleeve was noted, the frequent palpation of the 2 positive cows was likely to have caused irritation of the rectal mucosa which might increase the number of lymphocytes on the rectal sleeve. The 16 cattle in Experiment 1, who were negative at the beginning of the study, were only palpated weekly; it is unlikely that they received rectal irritation significantly greater than that which might occur in practice. The efficiency of BLV transmission by rectal palpation was increased with rectal trauma in one study[7] and the abnormally frequent palpation of cows in a university teaching herd was associated with an increased incidence of BLV infection when rectal sleeves were not changed.[9] In the teaching herd study, both positive and negative cows may have received rectal irritation from abnormally frequent palpation.

Experiment 2 was performed after the completion of the first experiment and for the purpose of determining if rectal palpation, performed with a frequency similar to that which might occur in practice, could result in the transmission of BLV. Experiment 2 confirmed that BLV transmission could occur from rectal palpation when palpation of both positive cows and negative cows was performed only once per month and without known trauma. Nevertheless, the incidence of infection was low (6%) and occurred only in a single heifer that was palpated after palpation of a positive cow with lymphocytosis. The failure of any (7) of the heifers which were palpated immediately after a BLV-positive cow without lymphocytosis to become infected, was not surprising since non-PL cows are not generally as infective as PL cows.[10] There is a positive correlation between viral expression, percentage of provirus-infected lymphocytes, and infectiveness and age-related absolute lymphocyte number.[10]

The low incidence of infection in Experiment 2 would be in agreement with the results in two field studies which could not find evidence for appreciable transmission of BLV by rectal palpation.[9,11] There are many factors which must be considered when making the decision for the appropriate method of palpation within a herd. The prevalence of BLV-positive cows and PL cows in a herd, the frequency of lymphosarcoma in a herd, any potential sale of valuable genetic stock and the expected increase in cost associated with changing sleeves should all be considered. The routine changing of sleeves was recommended as part of a program that was successful in decreasing the prevalence of BLV in a commercial dairy herd.[12]

Although there is considerable information on the infectivity of individual cows and PL cows, there is scant information on the susceptibility of negative cows to infection. In these 2 experiments, there did not appear to be an increased incidence of infection in association with the number of palpations. The only cattle that became infected were all likely to have become infected on either the first or second exposure. The 3 heifers that became infected in Experiment 1 were all seropositive by less than 3 days after their first palpation and since 14

or more days would be required for seroconversion,[13] it is probable that they became infected on either the first or second exposure. The only heifer to become infected in Experiment 2 became infected on the first palpation exposure. Although no conclusions can be drawn from such a small number, it is conceivable that these animals may have been more susceptible to infection.

Summary

Bovine Leukemia Virus (BLV) was transmitted by rectal palpation in breeding-age or adult dairy animals in two separate studies. In the initial study, three of seven (43%) animals became infected after being palpated immediately after 1 of 2 BLV-positive cows had been palpated without a change of sleeve. Two BLV-positive cows, 1 with persistent lymphocytosis and 1 without, were used on an alternating daily basis. Seven control animals remained negative after being palpated in the exact manner but with a change of sleeve. Rectal palpation was repeated weekly in the 14 BLV-negative cattle for four weeks. In the second study, 16 breeding-age, BLV-negative heifers obtained from herds without BLV-serologically-positive cattle were randomly paired by design (not physically paired) with 1 of 16 BLV-positive animals. Of the 16 BLV-positive cows, eight had persistent lymphocytosis (PL) and eight had normal lymphocyte counts. The 16 positive animals had not been palpated for one month prior to initiation of the study. All 16 negative animals were palpated on two occasions, immediately after palpation of their assigned positive cow, one month apart, without a change of rectal sleeve. One heifer, that had been palpated immediately after a BLV-positive PL-cow became infected.

Both studies were conducted during an insect-free season and all negative animals were housed individually, in isolation, for one month prior to initiating the studies, and for three months after the last palpation. Serologic testing was performed by either radioimmunoassay or enzyme-linked immunosorbent assay monthly, beginning 2 months prior to palpation, and for 3 months after the last palpation. All palpations were performed by experienced palpators who were instructed to routinely identify and examine both ovaries, uterine horns and cervix.

These studies confirm that BLV can be transmitted via rectal palpation, especially if BLV-positive, PL-cows are present in the herd, and/or if palpation frequency is great. The heifer in experiment 2 became positive after only one exposure, yet the other 7 remained negative after 2 exposures. Also, all 3 animals in experiment 1 were positive 30 days after the palpation was begun. Since it required 10 days for development of antibodies after experimental inoculation with almost certainly higher numbers of infecting lymphocytes than occurred in this study, it is possible that all 3 of these animals were infected on either the first or second rectal palpations. Although certainly no conclusions can be made from such a small number, it is interesting to speculate that these animals may have been more susceptible to infection. Although there is considerable information on infectivity of certain cows, there is no information on difference in susceptibility of negative cows to infection.

PALPATION SCHEDULE

Positive Cow #351	Day	Negative Cattle #	Positive Cow #315	Day	Negative Cattle #
	1	1,8		2	2,9
	3	3,10		4	4,11
	5	5,12		6	6,13
	7	7,14		8	8,1
	9	9,2		10	10,3
	11	11,4		12	12,5
	13	13,6		14	7,14
	15	8,1		16	9,2
	17	10,3		18	11,4
	19	12,5		20	13,6
	21	14,7		22	1,8
	23	2,9		24	3,10
	25	4,11		26	5,12
	27	6,13		28	14,7

Zusammenfassung

In zwei voneinander unabhaengigen Studien wurde das Bovine Leukaemie Virus (BLV) durch rektale Palpation bei Faersen und Milchkuehen uebertragen. Im ersten Versuch wurden drei von sieben (43%) Rindern infiziert nachdem sie sofort nach einer von zwei BLV-positiven Kuehen ohne Handschuhwechsel rektalisiert worden waren. Verwendet wurden zwei BLV-positive Kuehe (eine ohne und eine mit persistierender Lymphozytose) im taeglichen Wechsel. Sieben Kontrolltiere wurden auf die gleiche Art und Weise, jedoch mit Handschuhwechsel, rektalisiert und blieben negativ. Die rektale Untersuchung wurde bei den 14 BLV-negativen Rindern vier Wochen lang im woechentlichen Abstand durchgefuehrt.

Im zweiten Versuch wurden 16 BLV-negative Faersen aus Herden ohne BLV-serologisch positiven Rindern jeweils mit einem von 16 BLV-positiven Tieren rektalisiert (Paarbildung). Von den 16 BLV-positiven Kuehen hatten acht Tiere eine persistierende Lymphozytose (PL) und acht wiesen normale L ymphozytenzahlen auf. Die 16 positiven Tiere waren fuer einen Monat vor Versuchsbeginn nicht palpiert worden. Alle 16 negativen Rinder wurden zweimal im Abstand von einem Monat jeweils nach dem positiven Partner ohne Handschuhwechsel rektalisiert. Eine Faerse, die nach einer BLV-positiven PL-Kuh rektalisiert worden war, wurde infiziert.

Beide Studien wurden in einer insektenfreien Jahreszeit durchgefuehrt, alle negativen Tiere waren fuer einen Monat vor Versuchsbeginn und fuer drei Monate nach der letzten Rektaluntersuchung einzeln (in Isolation) aufgestallt. Serologische Tests wurden entweder durch Radioimmunoassay oder Enzyme-linked immunosorbent assay in monatlichen Abstaenden durchgefuehrt, beginnend zwei Monate vor der ersten und drei Monate nach der letzten Rektaluntersuchung. Das Rektalisieren wurde von erfahrenen Untersuchern durchgefuehrt; sie waren angewiesen routinemaessig beide Ovarien, Uterushoerner und die Zervix aufzufinden und zu untersuchen.

Die beiden Studien zeigen, dass BLV via Rektaluntersuchung uebertragen werden kann; BLV-positive PL Kuehe in der Herde und/oder haeufiges Rektalisieren erhoehen das Infektionsrisiko.

Résumé

Le Virus de la Leucémie bovine (V.L.B.) a été transmis par palpation transrectale à des bovins laitiers en âge de reproduction et adultes au cours de deux études distinctes. Dans l'étude initiale, trois animaux sur sept (43%) sont devenus infectés après avoir été palpés immédiatement après qu'une ou deux vaches V.L.B. positives eurent été palpées sans changement de gant de fouille. Deux vaches V.L.B. positives, une avec Lymphocytose Persistante (L.P.) et une sans, ont été utilisées en jours alternés. 7 animaux contrôles restèrent négatifs après avoir été palpés de la même façon mais avec changement de gant de fouille. La palpation transrectale a été répetée de façon hebdomadaire chez les 14 bovins négatifs pendant quatre semaines.

Dans la deuxième étude 16 génisses en âge de reproduction et V.L.B. négatives, obtenues de troupeaux sans bovins sérologiquement B.L.V. positifs, ont été fictivement et aléatoirement appariées à un animal appartenant à un groupe de 16 animaux V.L.B. positifs. Parmi les 16 vaches V.L.B. positives 8 avaient une lymphocytose persistante (L.P.) et 8 avaient une numération lymphocytaire normale. Les 16 animaux positifs n'avaient pas été palpés durant le mois précédant le début de l'étude. Chacun des 16 animaux négatifs fut fouillé immédiatement après qu'ait été palpée la vache positive à laquelle il était assigné, sans changement de gant de fouille et ce par deux reprises à un mois d'intervalle. Une génisse fouillée immédiatement après une vache V.L.B. positive et L.P. est devenue infectée.

Les deux études ont été conduites pendant une saison sans insecte et tous les animaux négatifs étaient logés individuellement et isolement depuis le mois précédant le début de l'étude et durant trois mois après la dernière palpation. Les tests sérologiques ont été effectués par R.I.A. (Radioimmunoassay) et E.L.I.S.A. (Enzyme-linked immunosorbant assay) tous les mois à compter de deux mois précédant la première palpation et pendant trois mois suivant la dernière palpation. Toutes les palpations ont été réalisées par des praticiens expérimentés formés à identifier et examiner en routine les ovaires, cornes utérines et col de l'utérus.

Ces études confirment que le V.L.B. peut être transmis par palpation transrectale, particulièrement lorsque des vaches V.L.B. positives et L.P. sont présentes dans le troupeau et/ou si la fréquence des palpations est élevée.

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