Infectious Bovine Rhinotracheitis Virus and Parainfluenza-3 Virus in the Nasal Flora of Dairy Calves

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Introduction

Bovine respiratory tract diseases represent major economic factors in dairy cattle production. The bovine respiratory tract diseases consist of at least three clinical entities: (1) Enzootic pneumonia of calves; (2) "Shipping fever complex"; and (3) Atypical interstitial pneumonia (10).

Infectious agents identified with enzootic pneumonia in calves and shipping fever complex include viruses, bacteria, mycoplasmas, and chlamydia (10). The association of such agents with these diseases has been derived from the isolation and culture of these agents from diseased animals and/or seroconversion (development of specific antibodies). With some agents, but not all, experimental inoculation of susceptible cattle has resulted in disease with signs and lesions, reisolation of inoculated agent, and/or seroconversion.

Viruses among the infectious agents associated with respiratory tract diseases in cattle include: infectious bovine rhinotracheitis virus (IBRV), bovine viral diarrhea virus (BVDV), parainfluenza-3 (PI-3V), respiratory syncytial virus (RSV), adenoviruses, bovine herpesvirus-3 (DN-599 and FTC-2 strains), rhinoviruses, enteroviruses, and reoviruses (12,14,15). The literature contains considerable numbers of publications concerning viral infections in cattle. In addition, more than one virus may be involved simultaneously during respiratory tract disease outbreaks in cattle (16,17). These multiple or mixed viral infections were identified by viral isolation and/or significant increases in serum antibodies during the epizootics.

Viruses, bacteria, and mycoplasmas may be present in normal cattle, possibly in widespread distributions. However, the interaction of these agents may contribute to the disease process resulting in signs and lesions in affected cattle (10,23). Thus the epidemiology of these various infectious agents in healthy cattle is important in understanding their ecology and distrubution.

Therefore, a multidisciplinary study was initiated in the state of Louisiana to identify factors associated with calf mortality and respiratory tract disease in dairy calves. Included in this study was the isolation and identification of viruses, mycoplasmas, bacteria, and chlamydia from nasal swabs of calves in these dairy herds during three collections in a one year interval. This paper reports the distribution of IBRV and PI-3V in the nasal flora of Louisiana dairy calves without signs of respiratory tract disease.

Materials and Methods

Dairy Calves - Twenty dairy farms in southern Louisiana were selected for evaluation (20). Calves varied in age from one- to six-months- old. Calves included in this study were free of clinical signs of respiratory tract disease at the time of sample collections.

Sampling Procedures - Collections were made from two to fifteen calves from each farm in each of three time intervals throughout one year. The sampling periods were: (1) February through May 1979; (2) June through September 1979; and (3) October 1979 through January 1980. Dry cotton swabs were used to swab nasal mucosal surfaces and

were placed in vials containing transport medium with 2% fetal bovine serum and antibiotics (1). The vials were transported on ice to the laboratory and stored at -80°C until tested for viruses. Serums were also collected from each animal for antibody quantitation.

Cell Cultures and Media - Monolayer cultures used included: bovine turbinate (BT), ovine fetal turbinate (OFT), and goat turbinate (GT) (7). The growth conditions and mediums have been described (8). The cultures were used as follows: BT cells for virus isolation; OFT for PI-3V serology; and GT cells for IBRV and BVDV serology.

Viruses used in this study for serology and controls for viral characterization included: IBRV, Cooper strain; BVDV, Singer strain; PI-3V, SF-4 strain; and bovine adenovirus-7 (BAV-7) (7).

Virus Isolation - Portions (0.5 ml) of transport medium containing swab materials were inoculated into each well of 24-well microtitration plates (2 wells/sample) containing 1.5 ml of freshly seeded BT cell suspension (1). Uninfected control inoculums were processed similarly. The plates were incubated at 37°C for 7 days and examined daily for viral cytopathic effects (CPE). When CPE was observed in 75% to 100% of the monolayer, the cells were scraped from the well surface and stored together with the medium at -80°C. Samples negative for CPE on initial passage were subsequently passed a second time in 4-well cell culture slides. Portions (0.2 ml) of each sample were inoculated into each well containing 0.8 ml of freshly seeded BT cells. Cultures negative for CPE at the second passage were examined for noncytopathic BVDV by a direct fluorescent antibody test (FAT) using fluorescein conjugated BVD antiserum. The samples from each calf were considered negative for viruses if the second passage in cell culture was negative for CPE and did not exhibit fluorescence when examined by the direct FAT using labelled BVDV antiserum.

All viral isolates causing CPE were inoculated into 4-well slides as described above and examined by the direct FAT using IBRV, BVDV, or PI-3 labelled conjugates.

Virus Neutralization - A microtitration virus-neutralization (VNT) performed in sterile 96-well microtitration plates with lids was used to identify the viral CPE agents and also to detect virus specific antibodies in serums of calves (7).

In each case, 100 $TCID_{50}$ of the CPE agent or stock virus was incubated with serums diluted twofold. The antibody titer was expressed as the highest final serum dilution completely inhibiting viral CPE in both wells.

The CPE agents in the study were examined for neutralization by monospecific serums to: IBRV, BVDV, PI-3V, and RSV.

Results

Monolayers of BT cells developed CPE when infected with swab materials from 13 calves. These isolates represented swabs from six different herds (Table 1). Nasal swabs from calves in this study did not have more than one virus present at the time of collection. In one herd, number 15, viruses were isolated from 2 successive sampling periods. After the results of the viral isolation attempts and characterization of the isolates were complete, it was learned that calves in herd number 15 had received an intranasal IBRV and PI-3V modified live virus (MLV) vaccine. This vaccine was administered 10 days prior to the collection of nasal swabs and serum.

Table 1. Viral isolates in twenty Louisiana herds during three sampling periods.

Herd Number	Cytopathic Agents Isolated in Each Sampling Period 1*	2	3
1	1/11†(1PI-3V)	0/10	0/8
2	0/10	0/10	0/10
3	0/10	0/10	0/10
4	0/10	0/10	0/10
5	0/10	0/6	0/10
6	0/10	0/10	0/10
7	0/10	0/5	0/10
8	0/10	0/7	0/10
9	0/11	0/2	0/10
10	1/15(1 PI-3V)	0/5	0/10
11	0/10	0/10	0/10
12	0/10	0/8	0/10
13	0/10	0/10	0/10
14	0/10	0/10	0/9
15	0/10	2/9 (2 IBRV)	1/7 (1 PI-3V)
16	0/10	0/9	1/10(1 unclassified)
17	0/10	0/10	4/10(4 unclassified)
18	3/10(3 IBRV)	0/10	0/9
19	0/10	0/10	0/10
20	0/12	0/10	0/10

* Sampling periods: (1) February-May 1979; (2) June-September 1979; and (3) October 1979 - January 1980.

† Number of isolates/number animals tested.

The isolates characterized as IBRV and PI-3V produced CPE in BT cells typical of that for herpesvirus and parainfluenza viruses. The unclassified cytopathic agents developed cell degeneration with cells detaching from the vessel surface by four days post inoculation. The 13 CPE agents were detected on initial passage with no additional agents detected in the second passage.

The isolates designated BRD (Bovine Respiratory Disease Project) -1, -33, and -511 were neutralized by PI-3V antiserum, and BRD -37, -38, -40, -238, and -241 isolates were neutralized by IBRV antiserum. Monolayer cultures infected with BRD -1, -33, or -511 exhibited specific

fluorescence when stained with PI-3V-conjugate. Similarly, monolayers infected with BRD -37, -38, -40, -238, or -241 exhibited fluorescence when stained with IBRV conjugate.

All three calves from which PI-3V was isolated had serum antibodies to PI-3V at the time the nasal swabs were collected. However, the calves from which IBRV was isolated did not have serum antibodies to IBRV.

Discussion

The current study indicated that IBRV and PI-3V may be isolated from apparently healthy dairy calves 1 - 6 months old in Louisiana dairy farms. With few exceptions, the calves sampled in each sampling period were not included in other sampling periods. Although the number of isolates was minimal, the fact that some young calves could harbor the viruses in the nasal tract serves as evidence of a possible source of the viruses in those herds. In this current study, older animals such as replacement yearling heifers or mature breeding animals were not examined. Perhaps in these older animals additional infections would have been detected.

The IBRV isolates, BRD -238 and -241, from herd number 15 during the second sampling period may have represented vaccine virus and/or field strains. The calves were among a group which had received an intranasal IBRV - PI-3V MLV vaccine ten days prior. Other studies indicate that calves may shed IBRV in nasal secretions several days after receiving intranasal MLV IBRV vaccines. Todd *et al.* (1972) reported that the calves shed IBRV 11 days after intranasal vaccination (24). Also Zygraich *et al.* (1974) reported that IBRV was isolated from an animal 13 days after intranasal vaccination (25). In that same study evidence for viral shedding was indicated by the seroconversion in contact animals with those calves vaccinated with an intranasal MLV vaccine strains.

It may be possible to differentiate IBRV vaccine strains from field strains by temperature-sensitive properties of certain vaccines and the analysis of genomes of the various strains subsequent to restriction endonuclease enzyme cleavage (5,13,25). Such studies are being utilized to identify the IBRV isolates from this present study.

Calves from which IBRV was isolated and which were exposed to calves vaccinated with IBRV vaccine did not have serum IBRV antibodies. It would have been expected that the vaccine virus would have induced serum antibodies to IBRV. Todd *et al.* (1972) reported that calves vaccinated with an intranasal MLV IBRV-1 vaccine developed serum IBRV-1 antibodies by 10 days after vaccination (24). It is possible in this study that the calves had an inhibitor such as interferon or antibodies in the nasal secretions which restricted replication of the vaccine strain with minimal resulting antigenic mass to stimulate systemic IBRV antibody production.

All calves in the present study from which PI-3V was isolated had concurrent serum antibodies to PI-3V. Previous studies indicate that PI-3V can be isolated from calves with colostrally acquired maternal PI-3V antibodies subsequent to aerosol exposure to PI-3V (11). Also, calves with serum antibodies to PI-3V resulting from previous infections with PI-3V had PI-3V virus in nasal secretions subsequent to aerosol exposure to PI-3V (6). Thus it is possible to isolate PI-3V from calves with PI-3V serum antibodies resulting from active infection or colostral antibodies from their dams. Reduction in viral isolations after challenge exposure was better reflected by nasal antibody than serum antibody (6,19). In the present study, however, nasal secretions were not collected for assay of viral antibodies.

The number of studies of viral infection in normal healthy calves are limited. Examples of such studies are listed below. Dawson *et al.* (1966) examined 20 calves for 18 weeks by virus isolation attempts from nasal swabs and by serology (4). Viruses were not isolated from nasal swabs, but six calves had serologic evidence (increase in specific antibody levels) of infection when respiratory disease was observed. St. George *et al.* (1972) studied 20 calves for six months with PI-3V isolated from 21 nasal swabs from four calves and there was serologic evidence of BVDV and PI-3V infection (18). Thomas and Collins (1974) reported that a serologic survey of 47 calves during a six month interval detected infections to PI-3V, BVDV, and reovirus (22). Burgess (1977) surveyed six calves over a 14-month interval and reported isolation of PI-3V from one nasal swab and serologic evidence of infection PI-3V also (2). Stott *et al.* (1980) studied seventeen groups of calves during 1972-1975 (21). These calves were brought to a farm by 10 days of age and sample collections were made until up to nine months of age where possible. Seven or eight animals of each group were selected for sample collection. Nasal swabs and serums were collected at regular intervals from 127 normal calves. The PI-3V, RSV, rhinoviruses, adenoviruses, enteroviruses, and untyped viruses were isolated from the normal calves. Also, there was serologic evidence of infection to PI-3V, RSV, rhinovirus, BVDV, and reovirus in the normal healthy calves. Seventeen of 175 calves had PI-3V in the nasal flora soon after they arrived in feedlots without having apparent clinical signs of infection (9). In a closed dairy herd, 7 of 14 calves had serologic evidence of infection (fourfold or more increase in antibodies) to PI-3V between 8 and 13 months of age (3). In that study, clinical respiratory disease was not observed in the calves, nor was PI-3V recovered from nasal swabs from milking cows nor from nasal swabs collected from the calves in the herd during the 13-month study.

Results of the present report and these above reports differ in various aspects. First, several of the above reports deal with longitudinal studies with the same group of calves examined over several months. In the present study, the calves represented a different group of calves in each sampling period. Also the use of the same calves over a longer interval permitted the use of serology to detect infections. In this report only one serum was collected from each calf. Second, the above reports were from various countries around the world. It is possible that under certain conditions

such as geographical and management different viruses may be evident. Thirdly, viral isolation techniques may differ allowing for one study to detect viruses that other investigators may not, using other cultural conditions. In the present study only one bovine monolayer culture was used, BT cells, and these inoculated cultures were held at 37°C in stationary position for two passages only. Other cultural conditions possible to increase viral isolations include: (1) different cell culture types such as fetal bovine kidney or lung, calf testicle, and established cell lines; (2) additional passages of cultures to detect certain viruses such as adenoviruses; (3) different culture temperatures such as 33°C, 34°C, or 37°C; and (4) roller cultures versus stationary cultures.

The results of this study indicate that both IBRV and PI-3V may be found in nasal flora of healthy dairy calves in Louisiana. Similar to the above report untyped isolates were identified (21). Futher studies are in progress to classify these agents.

Summary

Nasal swabs from dairy calves in 20 selected herds were collected once in three sampling periods in one year and examined for viruses. Eight of 573 calves were positive for infectious bovine rhinotracheitis virus (IBRV) or parainfluenza-3 virus (PI-3V). Two herds had calves with PI-3V and one herd had calves with IBRV dring the first collection. A fourth herd had 2 calves with IBRV during the second collection. From this fourth herd PI-3V was isolated from a calf during the third sampling period. There was not evidence of respiratory tract disease at the time of sampling in calves studied.

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References

1. Bech-Nielsen S., Fulton R.W., Cox H.U., et al: Feline respiratory tract disease in Louisiana. Am J Vet Res 41, 1293-1297 (1980). - 2. Burgess G.W.: A study of endemic viruses in a group of calves. N Z Vet J 25, 178-179 (1977). - 3. Burroughs A.L.: Myxovirus parainfluenza-3 respiratory infection in dairy cattle. Am J Vet Res 28, 359-364 (1967). - 4. Dawson P.S., Stuart, P., Darbyshire, J.H., et al: Respiratory disease in a group of intensively reared calves. Vet Rec. 78, 543-546 (1966). - 5. Engels M., Steck F., Wyler, R.: Comparison of the genomes of infectious bovine rhinotracheitis and infectious pustular vulvovaginitis virus strains by restriction endonuclease analysis. Arch Virol 67, 169-174 (1981). - 6. Frank G.H., Marshall, R.G.: Relationship of serum and nasal secretion-neutralizing antibodies in protection of calves against parainfluenza-3 virus. Am J Vet Res 32, 1707-1713 (1971). - 7. Fulton, R.W., Downing, M.M., Hagstad, H.V.: Prevalence of bovine herpesvirus-1, bovine viral diarrhea, parainfluenza-3, bovine adenovirus-3 and -7, and goat respiratory syncytial viral antibodies in goats 43, 1454-1457 (1982). Am J Vet Res. - 8. Fulton, R.W., Pearson, N.J.: Interferon production by bovine tracheal organ cultures infected with bovid herpesvirus-1 strains. Can J Comp Med 44, 447-452 (1980). - 9. Hamdy, A.H., Trapp, A.L.: Investigation of nasal microflora of feedlot calves before and after weaning. Am J Vet Res 28, 1019-1025 (1967). - 10. Lillie, L.E.: The bovine respiratory disease complex. Can Vet J 15, 233-242 (1974). - 11. Marshall, R.G., Frank, G.H.: Clinical and immunologic responses of calves with colostrally acquired maternal antibody against parainfluenze-3 virus in homologous viral infection. Am J Vet Res 36, 1085-1089 (1975). -12. Mohanty, S.B.: Bovine respiratory viruses. Adv Vet Sci Comp Med 22:83-109, (1978). - 13. Pastoret, P.P., Babiuk, L.A., Misra, V., et al: Reactivation of temperature-sensitive and non-temperature-sensitive infectious bovine rhinotracheitis vaccine virus with dexamethasone. Infect Immun 29, 483-488 (1980). - 14. Phillip, J.P., Darbyshire, J.H.: Respiratory viruses in cattle. Adv Vet Sci Comp Med 15, 159-199 (1971). - 15. Potgeiter, L.N.D.: Current concepts on the role of viruses in respiratory tract disease of cattle. Bovine Practitioner 21, 75-81 (1977). - 16. Rosenquist, B.D., Dobson, A.W.: Multiple viral infection in calves with acute bovine respiratory tract disease, Am J. Vet Res 35, 363-365 (1974). - 17. Rosenquist B.D., English, J.E., Johnson, D.W., et al: Mixed viral etiology of a shipping fever epizootic in cattle. Am J Vet Res 31, 989-994 (1970). - 18. St. George, T.D., Hass, C.R., Horsfall, N.: Infections with viruses and bacteria in intensively reared calves in northern Queensland. Aust Vet J 48, 7-11, (1972). - 19. Smith, C.B., Purcell, R.H., Bellanti, J.A., et al: Protective effect of antibody to parainfluenza type 1 virus. New Eng J Med 275, 1145-1152 (1966). - 20. Springer, W.T., Fulton R.W., Hagstad, H.V., et al: Prevalence of Mycoplasma and Chlamydia in the nasal flora of dairy calves. Vet Micro (Accepted for publication, 1982). - 21. Stott, E.J., Thomas, L.H., Collins, A.P., et al: A survey of virus infections of the respiratory tract of cattle and their association with disease. J Hygiene 85, 257-270 (1980). - 22.Thomas, L.H., Collins, A.P.: Virus infection and incidence of respiratory disease: a serological study of four micro-organisms at a large beef-rearing farm. Vet Rec 94, 506-509 (1974). - 23. Thomson, R.G.: Pathology and pathogenesis of the common disease of the respiratory tract of cattle. Can Vet J 15, 249-251 (1974). - 24. Todd, J.D., Volenec, F. J., Paton, I.M.: Interferon in nasal secretions and sera of calves after intranasal administration of avirulent infectious bovine rhinotracheitis virus: association of interferon in nasal secretions with early resistance to challenge with virulent virus. Infect Immun 5:699-706, 1972. - 25. Zygraich, N., Lobmann, M., Vascobinic, E., et al: In vivo and in vitro properties of a temperature sensitive mutant of infectious bovine rhinotracheitis. Res. Vet Sci 16:328-335, 1974.