Molecular Epidemiology and Subgroup Analysis of Bovine Group A Rotaviruses Associated with Diarrhea in South African Calves

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Rotavirus-positive specimens were recovered from 143 Afrikander calves on two farms in the northeastern Cape of South Africa during late 1988 and 1989. The rotavirus strains were analyzed by polyacrylamide gel electrophoresis of the RNA genome, and four rotavirus RNA electrophoretypes, each with a long profile, were identified. A distinct RNA profile was identified on the farms during 1988, but by early 1989, two patterns existed, one unique to each farm. Over the next 8 months a new electrophoretic pattern emerged on one farm, whereas the pattern on the other farm remained unchanged. The rotavirus subgroup I antigen was detected in all specimens examined with subgroup-specific monoclonal antibodies. Non-group A rotaviruses were not identified by RNA genome analysis of 82 specimens from calves with diarrhea negative for group A rotaviruses by an enzyme-linked immunosorbent assay.

Infectious diarrheal illness of newborn calves is a major disease problem in both dairy and beef herds. However, very little is known of rotavirus infection in South African cattle (14, 15). Analysis of the electrophoretic mobilities of the 11 segments of double-stranded RNA by polyacrylamide gel electrophoresis (PAGE) yields a pattern which is both constant and characteristic for a particular rotavirus isolate (1). This technique has been widely used for epidemiological studies of human rotavirus infection, although only relatively few studies have examined the molecular epidemiology of bovine rotaviruses (2, 8–12, 17). None have been reported from southern Africa.

Recently, non-group A rotaviruses with both the group B and the group C antigen have been described in bovine species (11, 16). These viruses lack the common group A rotavirus antigen and have a clustering pattern of the double-stranded RNA genome segments on PAGE that is different from the 4,2,3,2 pattern of group A rotaviruses (7). PAGE remains the standard method of differentiating the group-specific rotavirus strains.

The group A rotaviruses can be further characterized antigenically by the presence of a subgroup antigen which forms part of the inner capsid of the virion and which is encoded by gene segment 6 (6). Very little is known of the subgroup specificity of bovine rotaviruses, with the few strains typed having subgroup I specificity (4, 12, 13).

In this study, we report the characterization of bovine rotaviruses from two Afrikander stud farms in the northeastern Cape of South Africa. Their RNA electrophoretypes and VP6 subgroup-specific antigen were analyzed.

Stool specimens were obtained from 6-week-old calves with diarrheal illness on the two farms. A single stool specimen was collected by one of us from each calf with diarrhea in November of 1988 (17 calves) and in February (27 calves), August (17 calves), and November of 1989 (82 calves). The presence of rotavirus was diagnosed by direct

negative staining electron microscopy. The rotaviruses were identified as group A rotaviruses by enzyme-linked immunosorbent assay (ELISA) (Rotavirus EIA; International Diagnostic Laboratories, Jerusalem, Israel), which reacts only with the common rotavirus group A antigen. All diarrheal stool specimens were subjected to PAGE after standard phenol-chloroform extraction and ethanol precipitation to enable the detection of group B or group C RNA electrophoretypes. Representative rotavirus strains from each RNA electrophoretype and from each farm were selected for subgroup analysis. The selected rotavirus specimens were analyzed by a VP6 subgroup-specific ELISA using the monoclonal antibodies developed by Greenberg et al. (3, 4).

Four different RNA electrophoretypes were identified in 143 stools with rotavirus (Fig. 1). The RNA profiles were all typical of those for group A rotaviruses with a long profile. One electrophoretype was identified in the stools collected from both farms during 1988. In early 1989, two electrophoretypes differing in the migration of gene segments 2, 5, 8, and 9 were seen, with one profile specific to each farm (Fig. 2). Similarly, at the end of 1989, two patterns were seen in the two herds. On farm 1 (from which fewer specimens were collected), the RNA profile remained the same. However, on farm 2 the profile had changed, the migration of gene segments 2 and 9 differing from that of the previous strain (data not shown).

Although extensive genomic variation of bovine rotaviruses has been reported to occur in different herds within a geographically small, defined area (13), most smaller studies, investigating isolated herds, have reflected results similar to those described here, where only one RNA electrophoretic strain circulates within the herd at any one time (2, 13, 17).

Rotaviruses can also be antigenically characterized by a common or group antigen, of which group A rotaviruses are the most prevalent and most studied (7). The group B and C rotaviruses have been distinguished on the basis of their RNA electrophoretypes (7). However, in this study only rotavirus strains with the typical group A rotavirus gene
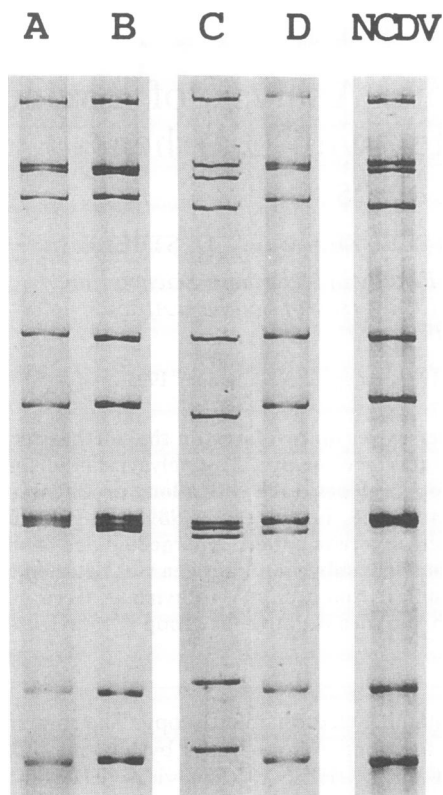
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FIG. 1. RNA profiles of the rotavirus electrophoretypes identified from calves on two farms in the Vryburg District, northeastern Cape of South Africa, during 1988 and 1989. The figure was compiled from portions of photographs taken of different gels. Migration was from the top to the bottom. Lane A shows the rotavirus strain which was identified on farm 1 during 1989. Lanes B and C show the strains identified on farm 2 during late (B) and early (C) 1989. Lane D is the common strain found on both farms during 1988. NCDV is the Lincoln strain, which was used as a reference.
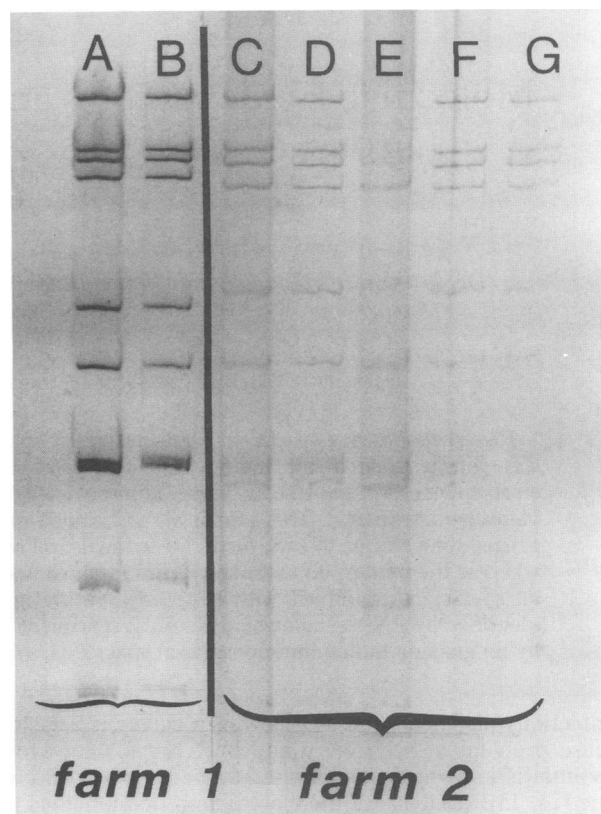

FIG. 2. Comparison of the RNA electrophoretic patterns from the two farms during February 1989. Migration was from the top to the bottom. Lanes A and B are strains isolated from farm 1, and lanes C to G are strains isolated from farm 2.

distribution (i.e., gene segment migration arranged in the overall pattern of 4,2,3,2) (7) were identified.

The group A rotaviruses may be further characterized on the basis of their subgroup (VP6) antigen, which is encoded by gene segment 6 (4, 6). Very few studies have investigated the subgroup specificities of bovine rotaviruses (4, 12, 13), and none have been conducted on African bovine isolates. In this study, as in the few others, all typeable bovine rotavirus strains were identified as being subgroup I specific. Two strains could not be typed with the monoclonal antibodies used, although this may have been due to a lack of sufficient antigen present in the stool. Both of these stools reacted as weakly positives with the commercial ELISA kit.

In conclusion, the genome electrophoretyping method is a useful technique to gain important epidemiological data on rotavirus disease outbreaks and to help in monitoring modes of disease transmission. However, the RNA electrophoretype, although allowing differentiation between strains of virus, does not give an indication of the serotype antigenicity of the strains, which would be important for the effective use of a rotavirus vaccine. Reports of the failure of the bovine rotavirus vaccine (13) to protect against symptomatic infection may well be due to a difference in antigenicity of the VP7 serotype. Further studies are needed to determine the VP7, and possibly VP4 (5), serotype specificity of South African isolates if an effective vaccine strategy is to be implemented.

REFERENCES

1. Estes, M. K., D. Y. Graham, and D. H. Dimitrov. 1984. The molecular epidemiology of rotavirus gastroenteritis. Prog. Med. Virol. 29:1–22.
2. Fijtman, N. L., M. E. Barrandeguy, E. M. Cornaglia, and A. A. Schudel. 1987. Variations and persistency of electrophoretypes of bovine rotavirus field isolates. Arch. Virol. 96:275–281.
3. Greenberg, H. B., J. Flores, A. R. Kalica, R. G. Wyatt, and R. Jones. 1983. Gene coding assignments for growth restriction, neutralization and subgroup specificities of the W and DS-1 strains of human rotavirus. J. Gen. Virol. 64:313–320.
4. Greenberg, H. B., V. McAuliffe, J. Valdesuso, R. Wyatt, J. Flores, A. Kalica, Y. Hoshino, and N. Singh. 1983. Serological analysis of the subgroup antigen of rotavirus using monoclonal antibodies. Infect. Immun. 39:91–99.
5. Hardy, M. E., G. N. Woode, Z. Xu, and M. Gorziglia. 1991. Comparative amino acid sequence analysis of VP4 for VP7 serotype 6 bovine rotavirus strains NCDV, B641 and UK. Virology 65:5535–5538.
6. Kalica, A. R., H. B. Greenberg, R. G. Wyatt, J. Flores, M. M. Sereno, A. Z. Kapikian, and R. M. Chanock. 1981. Genes of human (Wa) and bovine (UK) rotaviruses that code for neutralization and subgroup antigens. Virology 112:385–390.
7. Pedley, S., J. C. Bridger, J. F. Brown, and M. A. McCrae. 1983.

Molecular characterisation of rotaviruses with distinct group antigens. J. Gen. Virol. **64**:2093–2101.

8. **Pocock, D. H.** 1987. Characterization of rotavirus isolates from sub-clinically infected calves by genome profile analysis. Vet. Microbiol. **13**:27–34.

9. **Rodger, S. M., and I. H. Holmes.** 1979. Comparison of the genomes of simian, bovine and human rotaviruses by gel electrophoresis and detection of genomic variation among bovine isolates. J. Virol. **30**:839–846.

10. **Sabara, M., D. Deregt, L. A. Babiuk, and V. Misra.** 1982. Genetic heterogeneity within individual bovine rotavirus isolates. J. Virol. **44**:813–822.

11. **Snodgrass, D. R., A. J. Herring, I. Campbell, J. M. Inglis, and F. D. Hargreaves.** 1984. Comparison of atypical rotaviruses from calves, piglets, lambs and man. J. Gen. Virol. **65**:909–914.

12. **Theil, K. W., and C. M. McCloskey.** 1988. Partial characterization of a bovine group A rotavirus with a short genome electrophoretype. J. Clin. Microbiol. **26**:1094–1099.

13. **Theil, K. W., and C. M. McCloskey.** 1989. Molecular epidemiology and subgroup determination of bovine group A rotaviruses associated with diarrhea in dairy and beef calves. J. Clin. Microbiol. **27**:126–131.

14. **Theodoridis, A., L. Prozesky, and H. J. Els.** 1979. The isolation and cultivation of calf rotavirus in the Republic of South Africa. Onderstepoort J. Vet. Res. **46**:65–69.

15. **Theodoridis, A., L. Prozesky, and H. J. Els.** 1980. Studies on neonatal calf diarrhoea caused by rotavirus: transmission of the disease and attempted vaccination of colostrum deprived calves. Onderstepoort J. Vet. Res. **47**:31–34.

16. **Tsunemitsu, H., L. J. Saif, B. Jiang, M. Shimizu, M. Hiro, H. Yamaguchi, T. Ishiyama, and T. Hirai.** 1991. Isolation, characterization, and serial propagation of a bovine group C rotavirus in a monkey kidney cell line (MA104). J. Clin. Microbiol. **29**:2609–2613.

17. **Verly, E., and J. Cohen.** 1977. Demonstration of size variation of RNA segments between different isolates of calf rotavirus. J. Gen. Virol. **35**:583–586.