

PATHOGENESIS OF ROTAVIRUS INFECTION IN CALVES-MORPHOMETRIC STUDIES

N.P. Singh and R.S. Chauhan
College of Veterinary Sciences
G.B. Pant University of Agriculture & Technology
Pantnagar-263145, Nainital, India.

Introduction

Rotaviruses are known to cause acute viral gastroenteritis in neonatal calves. In recent years these viruses were found to be associated with 40-50% cases of diarrhoea in neonatal calves in India. Though the pathogenesis of rotavirus infection has been studied in calves^{5,6}, the information on morphometric's of intestine in rotavirus infected calves is not sufficiently available. The present work therefore was undertaken in order to study the changes in small and large intestine at different time intervals.

Materials and Methods

Pathogenesis of rotavirus infection in 15 SPF colostrum deprived male bovine crossbred calves, isolated immediately after birth and maintained under strict hygienic controls was studied, the calves being divided in 5 groups; with one calf as control and two infected with 5×10^6 TCID₅₀ rotavirus; isolated from natural cases of diarrhoea in calves in India¹. All the calves from respective groups were sacrificed on 2, 4, 6, 8 and 10 day post infection after laparotomy under general anaesthesia. Before sacrificed, the calves were administered 1 mg/kg body weight colchicine intraperitoneally to arrest the cell division in the intestine at Metaphase⁶. Tissues from proximal, mid and distal small intestine and colon were collected at 30, 60, 90 and 120 min after the colchicine administration in carnoy's fixative and⁴ after 1 hr transferred to 70% ethanol and stored till the analysis⁴.

Tissues were processed for morphometrics^{6,10} and 30 villi and 30 crypts were examined at each site. Crypt cell production rate per hour was determined at proximal, mid and distal small intestine and colon using the regression line of the accumulated metaphase blocked cells against time after the inoculation of colchicine⁶.

Results and Discussion

The calves infected with rotavirus isolate, developed diarrhoea after 18-24 hr of inoculation lasting 3-5 days. The rotavirus antigen could be demonstrated in the faeces using ELISA and immunofluorescence tests. Measurements of villus height in the proximal, mid and distal small intestine showed villus height to be significantly decreased in infected calves as compared to controls, the effect being more upto 6 day PI and moderate on 8 and 10 day PI. Crypt depth was increased in all the segments of small intestine and colon while villus crypt ratio decreased in infected calves. The crypt cell production rate per hour was increased in proximal, mid and distal small intestine and in colon due to rotavirus infection. Earlier also workers recorded similar morphometric measurements in rotavirus infected calves. Mebus⁹ opined that after oral infection of rotavirus, the

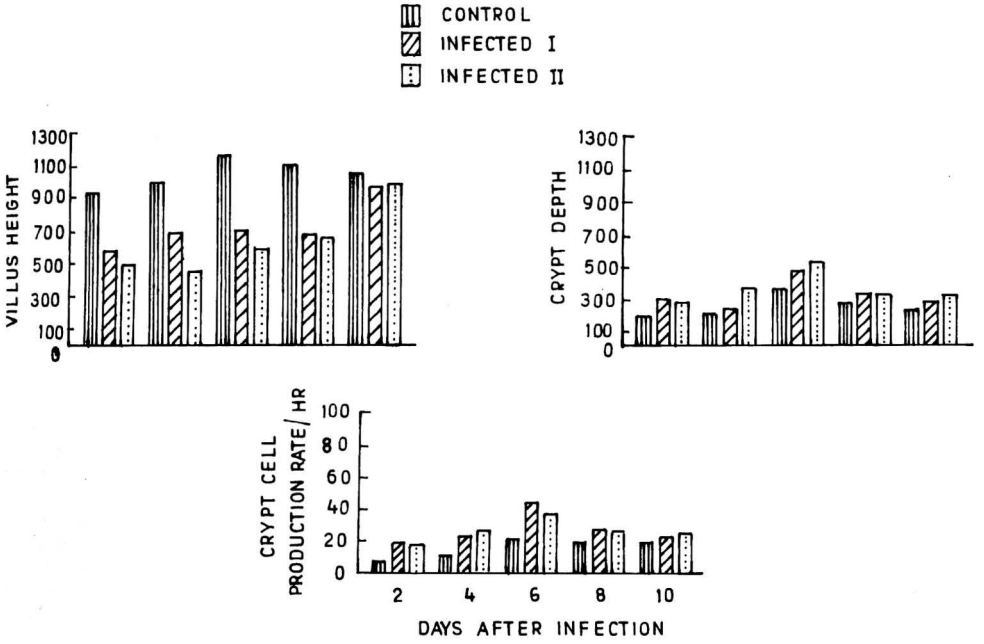


Fig. 1 MORPHOMETRICS : DISTAL SEGMENT OF SMALL INTESTINE

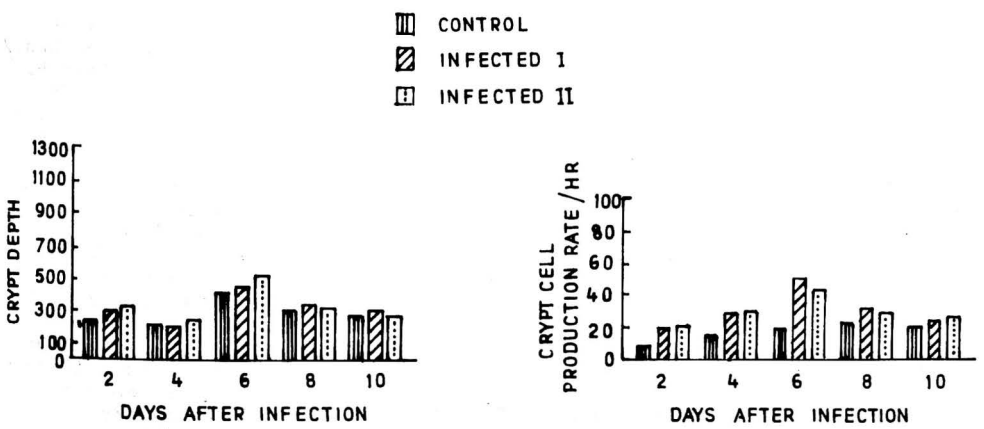


Fig. 2 MORPHOMETRICS: LARGE INTESTINE (COLON)

columnar epithelial cells over villi of small intestine become infected first and the infection then progressed posteriorly. As the infection proceeds, the infected epithelial cells are pushed towards the tip of villi and are lost in lumen and replaced by cuboidal and sometimes by squamous cells causing villus atrophy. The increase in crypt depth and crypt cell production rate is considered as a result of stimulation of rotavirus infection to meet the increased requirement of the cells at villus surface. The production of enterocytes in crypts increased to cover the whole of villus lining and to resume the absorptive capacity⁶. The morphometric observations were further confirmed by histopathological examination of the intestinal tissues and scanning electronmicroscopy³. The presence of rotavirus antigen was observed in the epithelial cells of villi in all the segments of small intestine which was not true for colon. The main site of rotavirus antigen was villus epithelial cells however in some crypt cells it was also demonstrated⁶.

Summary

The morphometric studies including villus height, crypt depth, villus crypt ratio and crypt cell production rate in proximal, mid and distal small intestine and crypt depth and crypt cell production rate in large intestine were carried out in 15 SPF colostrum deprived male bovine calves. On rotavirus infection, decreased villus height and villus crypt ratio, increased crypt depth and crypt cell production rate were recorded in all segments of small intestine as compared to uninfected controls. Morphometrics of colon revealed increased crypt depth and crypt cell production rate in infected calves.

Acknowledgements

Authors are thankful to the Dean, College of Veterinary Sciences and Director, Experiment Station for providing necessary facilities.

References

1. Chauhan, R.S. Immunopathological studies on rotavirus infection in calves. Ph.D. thesis, G.B. Pant Univ. Pantnagar 1991.
2. Chauhan, R.S. and Singh, N.P. Isolation and characterization of filterable agents from pneumoenteritis in calves. *Ind.J.Vet.Path.* 7:53-56, 1983.
3. Chauhan, R.S. and Singh, N.P. Assessment of intestinal damage in rotavirus infected calves by D-xylose malabsorption test. National symposium on Clinical Pathology and Biotechnology in the diagnosis of livestock and poultry diseases 1991.
4. Ferguson, A.; Sutherland, A.; MacDonald, T.T. and Allan, F. Technique for microdissection and measurements in biopsies of human small intestine. *J. Clin. Path.* 30:1068-1073, 1977.
5. Hall, G.A., Bridger, J.C. and Parsons, K.R. Lesions in gnotobiotic calves infected with virulent or avirulent rotavirus. Proceedings of XV World Buiatrics Congress pp 915-920, 1988.
6. Hall, G.A., Parsons, K.R.; Reynolds, D.J. and Morgan, J.H. Detection of enteropathogenic viruses in paraffin embedded intestinal tissues of calves by immunoperoxidase. *J. Med. Microbiol.* 19:14, 1985.
7. MacDonald, T.T. and Ferguson, A. Small intestinal epithelial cell kinetics and protozoal infection in mice. *Gastroenterology.* 74:496-500, 1978.
8. McNulty, M.S. Rotaviruses *J. Gen. Virol.* 40:1-18, 1978.
9. Mebus, C.A., Stair, E.L., Underdahl, N.R. and Twiechus

M.J. Pathology of neonatal calf diarrhoea induced by a reolike virus. Vet. Path. 8:490-495, 1971. 10. Woode, G.N. and Bridger, J.C. Isolation of small viruses resembling artroviruses and calciviruses from acute gastroenteritis of calves. J. Med. Microbiol. 11:441-452, 1978.