
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Pergamon

*Comp. Immun. Microbiol. infect. Dis.* Vol. 20, No. 3, pp. 205–210, 1997

Printed in Great Britain
PII: S0147-9571(97)00005-2
0147-9571/97 $17.00 + 0.00

# ISOLATION AND IDENTIFICATION OF AVIAN ROTAVIRUS FROM PHEASANT CHICKS WITH SIGNS OF CLINICAL ENTERITIS

R. LEGROTTAGLIE, V. RIZZI and P. AGRIMI

Dipartimento di Patologia Animale, Profilassi e Igiene degli Alimenti–Università degli Studi di Pisa–Viale delle Piagge 2, 56124 Pisa, Italy

*(Received for publication 3 March 1997)*

**Abstract**—Three rotaviruses were isolated from intestinal contents obtained from a flock of 6–8-day-old pheasant chicks showing diarrhoea and increased mortality. The isolates were characterized as avian group A rotavirus by immunoenzymatic technique (ELISA) and polyacrylamide gel electrophoresis (PAGE). © 1997 Elsevier Science Ltd

*Key words*: Pheasant chicks, rotavirus, viral diarrhoea.

**Résumé**—Trois souches de rotavirus ont été isolées, en culture cellulaire de la ligne Ma 104, à partir de contenus intestinaux de faisandeaux avec diarrhée entraînant une mortalité grave. Les souches ont été classées comme rotavirus aviaires du group A en utilisant une techique ELISA et l'analyse du génome viral par électrophorése en gel de polyacrilamide. Copyright © 1997 Elsevier Science Ltd

*Mots clefs*: Faisandeaux, rotavirus, diarrhée virale.

## INTRODUCTION

Rotaviruses are now recognized as a common cause of enteric disorders in a wide range of mammalian species, including humans [2]. Avian rotaviruses were first detected in the United States by Bergeland *et al.* [1] in diarrheic turkey poults. In recent years, avian rotaviruses have been detected and/or isolated from a variety of diseased birds, including turkeys, chickens, guinea fowls and pheasants, with diarrhoea and increased mortality, and from the faeces of apparently healthy ducks and pigeons, as reported by McNulty [9].

The demonstration and the isolation from diseased pheasant poults of rotaviruses antigenically related to mammalian group A rotavirus have been reported in the United States by Yason and Schat [12], Reynolds *et al.* [10] and in Italy by Foni *et al.* [4].

Limited serological investigations as regards the spread of rotavirus infection in pheasants have been reported.

An epidemiological survey on rotavirus infection in some avian species, carried out in Tuscany (Italy) between 1985 and 1988, showed a high prevalence (90%) of antibody-positive game farms with 29.44% positive reactors to bovine group A rotavirus antigen among 489 samples of pheasant parent sera from ten different flocks [8].

Foni *et al.* [4] found neutralizing antibodies to group A rotavirus in about 50% of parent sera and egg yolks examined, during the observation of the enteric syndrome in a rearing unit of pheasant poults in Lombardy (Italy).

We report the isolation, on a continuous cell line (MA-104), of rotaviruses from pheasant chicks, with clinical signs of enteritis, and their characterization by means of ELISA and by analysis of viral genomic dsRNA.

## MATERIALS AND METHODS

### *Flock history*

The game farm, located in the Province of Lucca (Tuscany, Italy), had 92 breeding families, each consisting of seven females and one male. During the breeding season of 1989, a total of approximately 44 000 eggs were laid. About 36 000 were fertile (82%), 27 000 poults were hatched (76% of the fertile eggs) and 25 000 poults were raised (94% of the number hatched).

In normal conditions the mortality rate of poults in a complete reproductive cycle is about 8%, of which 2.5% occurs in the first week and 3.5% in the period from the 8th to the 49th day; a further 2% can be expected to die after transfer to a flying-pen.

An outbreak of diarrhoea and increased mortality (12.50%) occurred in the summer of 1989 in some rearing units, in 6–10-day-old pheasants, in absence of specifically bacteriological etiology.

### *Samples for virological research*

The specimens, in the form of complete intestine, were from 6–10-day-old dead pheasants with signs of clinical enteritis.

For diagnostic purposes the enzyme-linked immunosorbent assay (ELISA) was performed, from the intestinal contents, according to the manifacturer's instructions using a commercial kit (IDEIA Rotavirus Test, Celltech Diagnostic Ltd, Cambridge, U.K.).

### *Cell culture and virus isolation*

For virus isolation, intestinal contents were suspended 1/10 in phosphate-buffered saline (PBS, pH 7.4) with antibiotics.

The suspensions were centrifuged at 3000 rpm for 10 min at 4°C to remove faecal debris.

The supernatants were mixed with trypsin at a final concentration of 10 μg/ml and the mixtures were incubated at 37°C for 30 min, then filtered through a 45 nm syringe filter and stored at − 70°C until further use.

The preparation of the MA-104 cell line and virus isolation were performed as described previously by Legrottaglie *et al.* [7].

### *Identification of isolated viruses*

*ELISA test.* The virus isolates were tested by ELISA with the above-mentioned commercial kit. Non-infected MA-104 cell lisate was used in order to check test specificity.

*Analysis of viral genomic dsRNA.* As previously described by Legrottaglie *et al.* [7], viral dsRNAs were extracted from the infected MA-104 cell lysate by the phenol–cloroform extraction method. Segmented double-stranded dsRNAs were separated in a 7.5% polyacrylamide slab gel with 3.5% stacking gel, at a constant voltage of 80 volts and variable amperage, for 18 h. The gel was stained with ethidium bromide and photographed under a UV light transilluminator.

## RESULTS

As the rearing season proceeded, an increase in mortality levels was noted in some rearing units. Many sick birds showed seediness, stopped feeding and suffered watery diarrhoea and dehydration; death occurred within 36–48 h of the onset of diarrhoea.

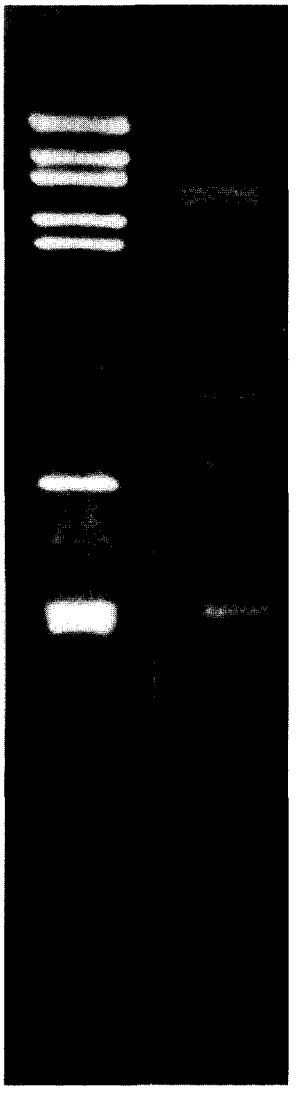


Fig. 1. Electrophoretic analysis of viral genome dsRNAs from pheasant origin group A rotavirus (155/89/LU, 175/89/LU, 178/89/LU isolates: Lane A) and from bovine rotavirus (NCDV isolate: Lane B) in polyacrylamide gel stained with ethidium bromide.

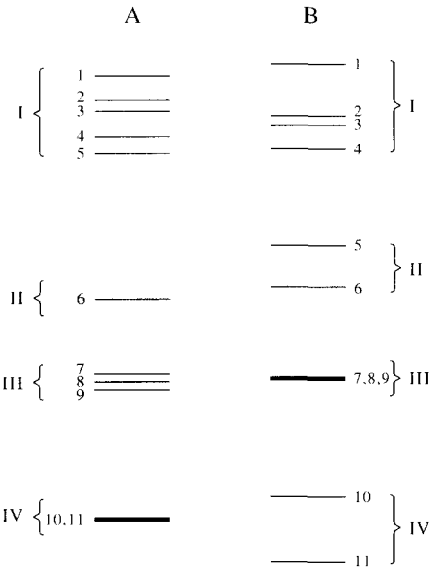


Fig. 2. Diagrammatic representation of the genome profiles of pheasant origin rotavirus (155/89/LU, 175/89/LU, 178/89/LU isolates: Lane A) and of bovine rotavirus (NCDV isolate: Lane B).

Losses started at 3–4 days and reached a peak at 5–8 days. Stunting of survivors followed the initial mortality.

Post-mortem examinations revealed abnormally distended caeca with fluid material and gas. The diagnostic ELISA tests, carried out on five samples of intestinal contents, were negative, whereas cytopathic viruses were isolated on the MA-104 cell line from the 155/89/LU, 176/89/LU and 178/89/LU samples.

The cytopathic effect (CPE) was noted, for all isolates, at the third passage and was characterized by rounded swollen cells containing granular cytoplasma. There was a progressive detaching of infected cells that, for some time, remained attached to the plastic by a slender bridge of cytoplasma. Finally the entire monolayer was destroyed.

The three isolates, when tested with ELISA, gave positive results for group A rotaviral antigen, while non-infected cell lysate turned out to be negative.

The dsRNAs extracted from infected cell cultures were sufficient to produce a fairly distinct electropherotype pattern in ethidium bromide-stained polyacrylamide gels.

Lane A in Figs 1 and 2 illustrates the PAGE pattern of dsRNA of the three isolates. In comparison with the mammalian Nebraska calf diarrhoea virus (NCDV) in Lane B, the segment 5 migrated with class I size and only one segment (6) migrated in the second size class. The three segments (7, 8 and 9) in the third size class migrated with a discrete separation (triplet). Segments 10 and 11 in the fourth size class migrated close together.

## DISCUSSION

An enteric syndrome, characterized by anorexia, diarrhoea, severe dehydration and increased mortality, occurred in 6–8-day-old pheasants in a game-rearing farm located in Tuscany (Italy).

Although the faecal samples were negative to ELISA rotavirus diagnostics, three rotavirus strains were directly isolated in MA-104 cells from the diluted and trypsinized intestinal contents of pheasant chicks showing clinical signs, in the absence of common pathogenic bacteria.

The inocula did not have cytotoxic effects and, at the third passage, the infected monolayers showed cellular changes morphologically similar to those reported for MA-104 cells infected with mammalian rotaviruses by Legrottaglie *et al.* [7].

Each of the three isolates shared a common antigen with group A mammalian rotaviruses and possessed a genome electropherotype with characteristics similar to those reported for other avian rotaviruses by Yason and Schat [12], Todd and McNulty [11] and Reynolds *et al.* [10].

The three isolates showed the same dsRNA migration pattern, similar to that of electropherotype 'e', within electropherogroup 1, recognized by Todd and McNulty [11] from chickens.

Rotavirus-only infections and simultaneous rotavirus and other enteric virus infections, associated with diarrhoeal disease in pheasant poults, has been reported by Gough *et al.* [5, 6], Yason and Schat [12], Reynolds *et al.* [10] and Foni *et al.* [4]; nevertheless, the pathological significance of rotavirus remains to be determined.

Foni *et al.* [4] report that the clinical signs, following experimental infection in pheasant poults with rotavirus isolate alone, were very mild or absent, whereas the infection with filtered stool from sick birds, containing rotavirus, parvovirus- and coronavirus-like viral particles, was able to cause a clinically manifested disease.

On the other hand, Gough *et al.* [6] have reported that the experimental infection with an 'atypical' rotavirus alone, may cause disease and high mortality in 2–3-day-old pheasants.

In our outbreak, the isolation of a group A rotavirus from diseased birds is not a surprising finding, since a serological survey has demonstrated that 90% of ten game-rearing farms investigated by Magnani [8] in Tuscany have experienced this infection.

In our observation, since a rotavirus was isolated three out of five times in intestinal contents from sick birds, in the absence of common pathogenic bacteria, it might speculated that this virus played an important role in causing the enteric syndrome observed.

We think that, besides the genuine difference in virulence of avian rotavirus strains, as has been shown for bovine rotavirus by Bridger and Pocock [3], the interaction of rotavirus with other factors, such as other infectious agents or environmental stress, can condition the variations in the severity of the disease.

The high prevalence of antibodies to group A rotavirus, pointed out in pheasant flocks from several Tuscany districts, even if it implies an high endemic virus circulation, is not, in our opinion, incompatible with the presence of acute disease symptoms. Such behaviour can often be observed also in other receptive species, including the avian ones.

Further investigations on the pathogenicity of rotavirus isolates in experimentally infected chicks could strenghten the hypothesis that the observed mortality is indeed consistent with rotavirus infection.

Finally, the ability to characterize individual virus isolates by genetic analysis of their dsRNA migration pattern is of potential importance in epidemiological investigations.

*Acknowledgements*—We thank Dr A. Mannelli for sending us the records of the flock history and the specimens for virus isolation. The financial support for this research was provided by the University of Pisa.

## REFERENCES

1. Bergeland, M. E., McAdaragh, J. P. and Stotz, I. (1977) In: Rotaviral enteritis in turkey poults. *Proceedings of the 26th West Poultry Diseases Conference*, 129–130.
2. Bonaduce, A., Martore, F. and Iovane, G. (1989) Gastroenteriti virali degli animali domestici. Nota V. Retroviridae—Enteriti da rotavirus. *Acta Medica Veterinaria* **35,** 261–321.
3. Bridger, J. C. and Pocock, D. H. (1986). Variation in virulence of bovine rotaviruses. *Journal of Hygiene* **96,** 257–264.
4. Foni, E., Gelmetti, D., Nigrelli, A. D., Gatti, R. and Carra, V. (1989) Sindrome riferibile ad enterite trasmissibile in fagiani da ripopolamento: riproduzione sperimentale della malattia, isolamento di rotavirus. *Selezione Veterinaria* **30,** 879–888.
5. Gough, R. E., Wood, G. W., Collins, M. S., Spackman, D., Kemp, J. and Gibson, L. A. C. (1985) Rotavirus infection in pheasant poults. *Veterinary Record* **116,** 295.
6. Gough, R. E., Wood, G. W. and Spackman, D. (1986) Studies with an atypical avian rotavirus from pheasants. *Veterinary Record*, **118,** 611–612.
7. Legrottaglie, R., Volpe, A., Rizzi, V. and Agrimi, P. (1993) Isolation and identification of rotavirus as aetiological agents of neonatal diarrhoea in kids. Electrophoretical characterization by PAGE. *Microbiologica*, **16,** 227–236
8. Magnani, P., (1988) Le infezioni da rotavirus nei conigli e nei volatili. Ricerche sierologiche e virologiche in allevamenti di aclune province toscane. Thesis. Faculty of Veterinary Medicine. University of Pisa, 1988.
9. McNulty, M. S., (1990). Rotavirus infections. In *Diseases of Poultry*, 9 edn, B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, and M. W. Yoder, Editors. Iowa State University Press, Ames, Iowa, pp. 628–635.
10. Reynolds, D. L., Theil, K. W. and Saif, Y. M. (1987) Demonstration of rotavirus and rotavirus-like virus in the intestinal contents of diarrheic pheasant chicks. *Avian Diseases* **31,** 376–379
11. Todd, D. and McNulty, M. S. (1986) Electrophoretical variation of avian rotavirus RNA in polyacrylamide gels. *Avian Pathology*, **15,** 149–159.
12. Yason, C. V. and Schat, K. A. (1985) Isolation and characterization of avian rotaviruses. *Avian Diseases* **29,** 499–508.