



CASE REPORT

Detection of Caliciviruses in young pheasants (*Phasianus colchicus*) with enteritis in Italy

Anna Toffan, Luca Bano, Francesco Montesi, Maria Serena Beato,
Roberta De Nardi, Calogero Terregino, Ilaria Capua

Istituto Zooprofilattico Sperimentale delle Venezie. Legnaro (PD), Italy

Corresponding author: Dr. Anna Toffan. Istituto Zooprofilattico delle Venezie. Laboratorio di Microscopia Elettronica. Viale dell'Università 10, 35020 Legnaro (PD), Italy – Tel. +39 049 8084389 – Fax: +39 049 8084360
Email: microscopia@izsvenezie.it

ABSTRACT

During June 2004 a severe enteritis was reported in a farm of 21-28 day old pheasants reared in intensive conditions in North-Eastern Italy. Mortality in the flock had reached 25%. Virological investigations on cell culture of the gut content yielded reoviruses while electron microscopy examination revealed viral particles morphologically related to calicivirus in association with parvovirus-like and rod shaped virus-like particles.

Key Words: Pheasant, Enteritis, Calicivirus.

RIASSUNTO

INFEZIONE DA CALICIVIRUS ASSOCIATA AD UNA SINDROME ENTERICA IN GIOVANI FAGIANI (*PHASIANUS COLCHICUS*)

Il presente lavoro descrive un grave episodio di sindrome enterica osservato in un gruppo di fagiani di allevamento di 21-28 giorni d'età. Oltre all'isolamento di reovirus dal contenuto intestinale, gli esami di microscopia elettronica hanno permesso di evidenziare la presenza di particelle virali morfologicamente riferibili a calicivirus associate a parvovirus-like e rod shaped virus-like particles. Dalla letteratura consultata questo risulta essere il secondo caso di enterite del fagiano associato a infezione da calicivirus, ed in considerazione della grave sintomatologia osservata sarebbe auspicabile un approfondimento della casistica per chiarire il ruolo di questo patogeno nel determinismo delle enteriti dei volatili in generale e del fagiano in particolare.

Parole chiave: Fagiano, Enterite, Calicivirus.

Introduction

Viral enteritis is commonly reported in young birds, and among these, game birds appear to be highly susceptible particularly if reared in intensive farming conditions. Young pheasants (*Phasianus colchicus*) suffer this pathologic condition with mortality rates ranging between 3% to 30% (Gough *et al.*, 1985). Most viral enteric prob-

lems in pheasants are caused by rotavirus (Gough *et al.*, 1985; Reynolds *et al.*, 1986) while parvovirus-like can cause acute hepatitis (Gelmetti *et al.*, 1996). Calicivirus infection is commonly reported in enteric diseases in mammals (human, canine, bovine, porcine) (Murphy *et al.*, 1999), but are less frequently observed in birds (Gough *et al.*, 1992). The present paper reports of the results of laboratory investigations in a flock of pheasants

with acute enteritis, in which the examination of the gut content by electron microscopy resulted in the detection of viruses which are unusually found in this species.

Material and methods

In June 2004, 28 day-old dead pheasant chicks which had experienced severe enteritis were submitted for laboratory investigations. The birds originated from an industrial pheasant farm consisting of 70,000 birds located in northern Italy. Pheasants were kept in 5 sheds and reared on the ground. The clinical condition was characterized by depression, dehydration, severe enteritis and increased mortality rate. Between day 21 to day 28 of age the overall mortality rate reached 25% and then decreased to 3% per day.

The birds had been vaccinated for Newcastle disease and Marble Spleen disease and treated with ampicillin for a mild respiratory disease two weeks prior to the onset of the clinical condition.

Birds were necropsied and bacteriological (aerobic and anaerobic) and parasitological investigations were performed by routine methods. Intestinal contents were processed for attempted virus isolation in chicken embryo liver (CEL) cultures as described (Gough *et al.* 1988) and processed by negative contrast electron microscopy.

The contents of intestine were diluted in phosphate buffered saline (PBS) and centrifuged for 30 minutes at 4500 *g* for clarification. The supernatant obtained was filtered 0,22 μm and ultracentrifuged with Airfuge Beckman for 15 minutes on carbon-coated Formvar copper grids. Negative staining was finally performed using 2% sodium phosphotungstate. Examination was made using a Philips 208 S electron microscope.

Due to the gross findings, the liver was tested for micotoxin detection (aflatoxin B1,B2, G1,G2, 17-epossi-tricotecen and ocratoxin A) with a commercial ELISA kit and fixed in buffered formalin 20% for histological examination.

Results and discussion

On post-mortem examination cloacal pasting, haemorrhagic catarrhal enteritis, undigested food in the lumen, swelling of intestinal loops and thin-

ning of intestinal wall were observed. The liver was enlarged and congested. The bone marrow also exhibited congestion. Blood appeared watery and the kidneys and gizzard exhibited haemorrhages.

Bacteriological and parasitological examinations did not yield any pathogens, except for rare coccidial oocysts, which were found in two birds. Ocratoxin A was detected in an irrelevant amount (4 ppb) in the liver.

Histological examination showed diffuse haemorrhages with necrosis and necrobiosis of hepatocytes, lymphocytic infiltration of the hepatic cords and intranuclear inclusion bodies in the hepatocytes. All these features are considered to be characteristic of viral infection.

Virus isolation attempts yielded reoviruses on first passage in CEL cultures.

Electron microscopy examination revealed three different particles. The most plentiful ones, measuring 30-35 nm, showed a typical cup-shaped depression of capsomeres arranged in icosahedral symmetry typical of Caliciviruses (Wyeth *et al.*, 1981) (Figure 1). The second smaller particles (18-20 nm of diameter) were morphologically related to Parvoviruses (Figure 1). In addition, elongated, striated particles measuring 50-60 nm in length by 18-20 nm in diameter (rod shaped virus-like particles) of unknown significance, but often reported in association with other intestinal viruses (Lavazza *et al.*, 1990) (Figure 2) were also detected. Since no samples of liver tissue were

Figure 1. Electron micrograph of caliciviruses in association with parvovirus-like in the intestinal contents of 28 day old pheasant chicks.

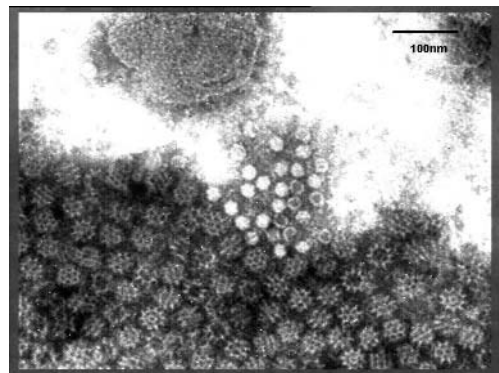
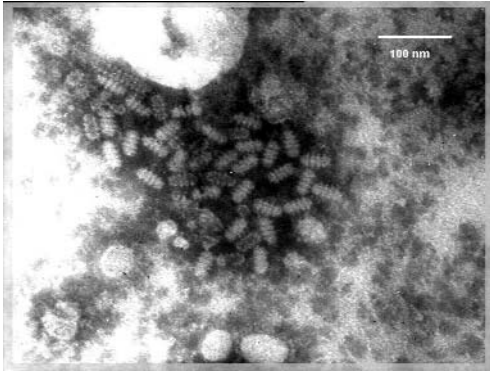


Figure 2. Electron micrograph of rod shaped virus-like particles in the intestinal contents of 28 day old pheasant chicks showing the typical striped aspect.



submitted for electron microscopy, it was not possible to establish whether this organ was also affected.

Conclusions

The present report confirms other findings (Gough, 1992; Gelmetti *et al.*, 1996) in which multiple viral infections are associated with enteritis in game birds. Although it appears extremely difficult to ascertain the role of each virus in the development of the clinical condition, the presence of a great amount of Calicivirus particles in the gut suggest that these viruses could be responsible for at least part of the intestinal lesions and related enteritis observed. Calicivirus infection of pheasants appears to be a rare finding, however, due to the severity of the clinical condition further studies should be carried out in order to establish the role of this virus in the development of enteritis in pheasants.

REFERENCES

- GELMETTI, D., FABBI, M., SIRONI, G., GRILLI, G., LAVAZZA, A., 1996. Identification of parvovirus-like particles associated with three outbreaks of mortality in young pheasants (*Phasianus colchicus*). *J. Vet. Diagn. Invest.* 8:108-112
- GOUGH, R.E., WOOD, G.W., COLLINS, M.S., SPACKMAN, D., KEMP, J., GIBSON, L.A.C., 1985. Rotavirus infection in pheasant poults. *Vet. Rec.* 116:295.
- GOUGH, R.E., ALEXANDER, D.J., COLLINS, M.S., LISTER, S.A., COX, W.J., 1988. Routine virus isolation or detection in the diagnosis of disease in birds. *Avian Pathol.* 17:893-907.
- GOUGH, R.E., DRURY, S.E.D., BYGRAVE, A.C., MECHE, S.C., 1992. Detection of calicivirus from pheasants with enteritis. *Vet. Rec.* 131:209-291.
- LAVAZZA, A., PASCUCCI, S., GELMETTI, D., 1990. Rod shaped virus-like particles in intestinal contents of three avian species. *Vet. Rec.* 9:581.
- MURPHY, F.A., GIBBS, E.P.J., HORZINEK, M.C., STUDDERT, M.J., 1999. *Caliciviridae*. In: *Veterinary Virology*. 3rd ed. Academic Press, San Diego, CA, USA. pp. 533-541.
- REYNOLDS, D.L., THEIL, K.W., SAIF, Y.M., 1986. Demonstration of Rotavirus and Rotavirus-like virus in the intestinal contents of diarrhetic pheasant chicks. *Avian Dis.* 31:376-379.
- WYETH, P.J., CHETTLE, N.J., LABRAM, J., 1981. Avian calicivirus. *Vet. Rec.* 21:109-477.