AN ENTEROTROPIC AVIAN INFECTIOUS BRONCHITIS VIRUS

R.C. Jones [1], M.El Houadfi [2], Jane K.A. Cook [3], and A. Ambali. [1]

[1] Sub-Department of Avian Medicine, University of Liverpool, Leahurst, Neston, Wirral, L64 7TE, England.

[2] Institute Agromonique et Veterinaire Hassan II, Rabat, Morocco.

[3] Houghton Poultry Research Station, Houghton, Huntingdon, Cambs, PE17 2DA, England.

ABSTRACT

Of six isolates of infectious bronchitis (IB) virus isolated from commercial poultry flocks in Morocco, five (designated D,E,F, H and M) were related serologically to the Massachusetts serotype, while a sixth, (designated G) was found to be different from any previously reported serotype of IB virus. Neutralising antibodies to this virus have been detected in the sera of commercial chicken flocks from a number of regions in Britain. While isolate G, in common with the other 5 isolates, caused respiratory disease typical of IB in 3-week old SPF chicks, it showed a particular predilection for the alimentary tract, and at various times up to 28 days could be isolated from all regions between oesophagus and bursa of Fabricus. Greatest persistence occurred in the anterior regions (oesophagus, proventriculus and duodenum).

INTRODUCTION

As part of an investigation into the nature of viruses involved in respiratory disease in chicken flocks in Morocco, six isolates of IB virus have been characterised (El Houadfi and Jones, 1985). This paper draws particular attention to one of the isolates, designated G which appears to be serologically unusual and enterotropic.

MATERIALS AND METHODS

All isolations were made from chicken flocks in the Rabat-Temara region of Morocco. Five of the agents (D,F,G,H and M) were isolated from respiratory disease in broilers, none of which were vaccinated against IB, and the sixth (E) was from a 25-week old commercial laying flock which was given H52 vaccine in the drinking water at 15 weeks. In 5 cases isolations were made after 1-3 passages of respiratory material in 9 day-old fertile eggs, but isolate G required 5 passages. Isolates were characterised as IB virus based on morphology under the electron microscope, responses to treatment with chloroform, pH 2.9, heating at 56 °C, and demonstration of IB virus group antigen by ELISA. Cross-neutralisation tests were done in tracheal organ cultures to compare the isolates with the major British, American, Australian, Dutch and other European serotypes of IB virus (see Cook 1984 for viruses).

J. B. McFerran et al. (eds.), *Acute Virus Infections of Poultry*

The pathogenicity of the six viruses was tested by intranasal infection of 3-week old SPF chicks, and the virus persistence in trachea, kidney and rectal contents determined by virus isolation in SPF eggs. A second experiment investigated the persistence of virus G in different regions of the alimentary tract over a 28 day period.

RESULTS

Biophysical properties:

All six isolates were characterised as IB viruses by having typical coronavirus morphology, were sensitive to chloroform, resistant to low pH, had variable heat resistance, and possessed the IB virus group antigen.

Serological relationships:

Five of the isolates were broadly related by cross-neutralisation to Massachusetts (M41) serotype and H120 and H52 vaccine viruses. Isolate G was unrelated to the other 5 Moroccan viruses and showed no serological relationship in either direction with any of the reference laboratory or field strains tested.

Incidence of antibodies to virus "G" in Britain:

Neutralising antibodies to isolate "G" were found in 8/30 breeder flocks and 5/16 commercial laying flocks. Serological evidence of infection was widespread, being found as far afield as Scotland (North), Hampshire (South), Norfolk (East) and Wales (West). Antibodies were detected in sera collected in 1978. Of 21 flocks with antibodies to any of the Dutch Serotypes D207, D212, D3128, or D3896, 11 were positive for isolate G also while 10 were negative. None had antibodies to G only.

Pathogenicity experiments:

All 6 isolates induced mild respiratory disease in 3-week old SPF chicks, and microscopic changes in tracheas were typical of IB.

Virus persistence in tissues:

Results of all 6 isolates are shown in Table 1, where the striking feature is the long persistence of isolate G in the lower intestine, although it was not detected in trachea or kidney beyond day 9.

TABLE 1. Persistence of 6 Moroccan IB viruses in the trachea, kidney or lower intestine of SPF chicks infected intranasally at 3-weeks of age.

Virus	Trachea	Kidney	Lower Intestine
D	9a	-	-
E	14	14	
F	14	-	9
G	9	9	28
H	14	-	-
M	14	-	-

a: last day virus isolated between days 7-28

Table 2 shows that isolate G could be detected at all levels of the alimentary tract at various times during the 28 day experiment. Ileum, caecal tonsil and bursa were positive as early as day 3 but the anterior regions (oesophagus, proventriculus and duodenum) showed greater persistence of virus in the later stages.

TABLE 2. Isolation of Moroccan IB virus isolate G from various regions of the alimentary tract, and from trachea and kidney of SPF 3-week old chicks at intervals after infection.

Tissues Examined	Days after infection 3	7	10	14	21	28
Oesophagus	-	-	+	+	+	-
Proventriculus	-	+	+	+	+	-
Duodenum	-	+	-	+	+	+
Jejunum	-	-	+	-	-	-
Ileum	+	+	+	-	-	-
Caecal Tonsils	+	+	+	-	-	-
Bursa of Fabricius	+	+	-	+	-	-
Cloacal swabs	-	+	+	+	+	+
Trachea	+	+	-	-	-	-
Kidney	-	+	+	+	-	-

+: Virus isolated from pooled tissue from 3 birds.
-: no virus isolated.

DISCUSSION

While all 6 Moroccan IB virus isolates induced respiratory disease in SPF chickens typical of IB, virus neutralisation tests separated the five related to the Massachusetts serotype (isolates D,E,F,H and M) from isolate G which is serologically unique, and has a particular predilection for the alimentary tract.

Virus G was initially the most difficult of the agents to isolate, requiring 5 passages in eggs before it caused typical embryo effects. This may perhaps explain why it has not yet been isolated from flocks in Britain despite serological evidence of its presence. The latter fact propably means that viruses related to G are present in other parts of Western Europe. Indeed isolate G might in time prove to be related to one of the IB variants reported elsewhere.

With regard to the enterotropism of virus G, reference has been made by others to persistence of IB viruses in the alimentary tract (Cook, 1968; Alexander and Gough, 1977), although results have usually been based on examination of tissues at the posterior end of the gut or cloacal swabbing. Other evidence supporting the potential susceptibility of different regions of the gut to IB virus has been presented by the in vitro organ culture studies of Darbyshire and others (1976 & 1978). However, no detailed study of the pathogenesis for the alimentary tract of IB viruses such as G has been done, and current experiments are underway to determine which cells of the gut are infected, and whether infection in very young chicks will cause digestive malfunctioning.

At present the significance of isolate G is unknown but a preliminary vaccine trial in young chicks has indicated that H120 vaccination appear to be ineffective (El Houadfi and others, to be published).

REFERENCES

Alexander, D.J. and Gough, R.E. (1977). Isolation of infectious bronchitis virus from experimentally infected chickens. Research in Veterinary Science 23: 344-347.

Cook, J.K.A. (1968). Duration of experimental infectious bronchitis in chickens. Research in Veterinary Science 9: 506-514.

Cook, J.K.A. (1984). The classification of new serotypes of infectious bronchitis virus isolated from poultry flocks in Britain between 1981 and 1983. Avian Pathology 13: 733-741.

Darbyshire, J.H., Cook, J.K.A. and Peters, R.W. (1976). Organ culture studies on the efficiency of infection of chicken tissues with avian infectious bronchitis virus. British Journal of Experimental Pathology 57: 443-454.

Darbyshire, J.H., Cook, J.K.A. and Peters, R.W. (1978). Growth comparisons of avian infectious bronchitis virus strains in organ cultures of chicken tissue. Archives of Virology 56: 317-325.

El Houadfi, M. and Jones, R.C. (1985). Isolation of avian infectious bronchitis viruses in Morocco including an enterotropic variant. Veterinary Record 116: 445.