Types of Avian Infectious Bronchitis Strains Isolated in Quebec

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ABSTRACT

Between 1976 and 1980, 24 isolates of infectious bronchitis virus were obtained from Quebec flocks. The serological classification of these isolates was demonstrated by cross neutralization tests using antisera to 13 different reference virus strains. Of the 24 isolates, ten were identified as Connecticut, six Holland and one SE-17 types. Seven strains did not react with any of the specific antisera.

RÉSUMÉ

Vingt-quatre souches de virus de bronchite infectieuse aviaire ont été isolées au Québec entre 1976 et 1980 et identifiées sérologiquement. Ces 24 isolats ont été comparés avec 13 souches obtenues de différents laboratoires de référence, en utilisant leur sérum homologue, dans une épreuve de séroneutralisation croisée. Les résultats de ce travail démontrent que dix isolats sont de type Connecticut, six de type Holland, un de type SE-17 et que les sept autres ne réagissent avec aucun des sérums utilisés.

INTRODUCTION

In 1969 (9), it was reported that of ten strains of avian infectious bronchitis virus (IBV) isolated in Quebec, nine were classified as Massachusetts and one as Connecticut serotypes. Subsequent to use of vaccines containing these two types, good protection was obtained against the natural disease.

However, five years later immunized flocks exhibited little resistance to infectious bronchitis and a survey was undertaken to determine if new serological types of IBV were responsible for these outbreaks.

MATERIALS AND METHODS

VIRUSES

Thirteen different reference serological types of IBV were obtained from various sources (Table I). They were passed twice in specific pathogen free (SPF)[1] embryonated chicken eggs for the production of antigen needed for the serum neutralization (SN) tests.

Twenty-four strains of IBV were chosen randomly from field isolates obtained between 1976 and 1980. These isolates were recovered from the respiratory tract and/or kidneys by inoculation into the allantoic sac of 11 day old SPF embryonated chicken eggs. Four days later, the allantoic fluid was collected and serially reinoculated

TABLE I. Origin of Infectious Bronchitis Virus Used in this Study

Virus Strains	Level of Passage in Chick Embryo	Sources
Arkansas 99	7th	Dr. R.B. Johnson
Clark 333	8th	University of Maryland
JMK	10th	College Park, MD 20740, USA
Florida 18288		
Australian T	?	Dr. R.C. Chubb University of New England Armidale, N.S.W. 2351 Australia
Holland	52nd	Dr. H.N. Lasher Sterwin Laboratories Inc. P.O. Box 537, Millsboro DE 19966, USA
Massachusetts	5th	American Type Culture Collection 12301 Parklawn Drive Parkville, MD 20852, USA
SE-17	?	Dr. M.S. Hofstad College of Veterinary Medicine Iowa State University Ames, IA 50011, USA
Connecticut	?	Dr. G. Lang
Holte	?	University of Guelph
Gray	?	Guelph, Ontario
Iowa 97	?	N1G 2W1, Canada
Iowa 609	?	

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four times in embryonated eggs. The presence of IBV was then identified by electron microscopy and by observing the atrophy and/or death of the embryos. Each positive isolate was subsequently subcultured five times before typing.

ANTISERA

Specific antisera against the reference strains were produced in groups of ten SPF birds, four weeks of age. Strict environmental procedures were taken to prevent contamination from outside sources. All the birds were bled one week prior to inoculation with the strains of viruses and all of them were proven to be free of antibodies against all these viruses. The birds were infected with a virus suspension containing $10.^{5.5}$ to $10^{7.5}$ EID_{50}/0.1 mL and administered as follows: two drops intraocularly, 0.25 mL intratracheally and 2 mL subcutaneously. Twenty-one days later, the birds were reinoculated intravenously with 1 mL of the virus suspension and were bled on the 28th day. The sera collected from each bird in each group were pooled and stored at -20°C in small aliquots until needed.

SERUM NEUTRALIZATION TEST

The antisera were inactivated at 56°C for 30 minutes prior to their use in the *in ovo* serum neutralization test using varying virus constant serum method as described by Hofstad (6).

EPIDEMIOLOGICAL SURVEY

The history of each flock from which a virus was isolated was examined to establish clinical signs of disease, age of birds, location and vaccination status.

RESULTS

SPECIFICITY OF THE HOMOLOGOUS ANTISERA

As shown in Table II, all antisera produced to the reference strains were specific except for antiserum to strain SE-17 which showed an apparent one way neutralization with the Gray strain.

TYPING OF THE FIELD ISOLATED

Table III shows that six isolates were of the Connecticut type, six of the Holland type, four were related to Connecticut and Florida types and one to type SE-17. Seven others did not react with any of the reference strains antisera.

EPIDEMIOLOGICAL DATA

As shown in Table IV, 19 out of 24 isolates were recovered from three to seven week old flocks. The other isolates were from young layers. Six isolates were recovered from nonvaccinated birds. Two of these isolates belonged to the Holland type, one to the Connecticut and SE-17 types and two others to undetermined types. All the other isolates were recovered from birds vaccinated 11 days to 15 weeks before the onset of the illness.

Respiratory signs predominated in 23 of the 24 outbreaks. Nephritis-nephrosis was the predominant lesion in two outbreaks and a severe dropping in egg production was observed in one outbreak. Detailed examination of submitted cases did not find a correlation between the different serotypes of identified viruses and onset of disease, seasons and transportation from an area to another. Moreover, the epidemiological data did not allow follow up on the emergence of spreading of unknown types in our previous publication (9).

DISCUSSION

The analysis of the cross reactions observed in Table II and III demonstrates the specificity of the homologous sera produced against the reference strains. The finding that four isolates reacted strongly with the Connecticut and Florida antisera confirms the results of Hopkins (4) who stated that the Florida strain 18288 was an antigenic variant of the Connecticut serotype. Consequently, it is concluded that ten out of 24 isolates were of the Connecticut type.

Seven isolates did not react with antisera to the 13 reference strains. However, these should not be necessarily considered as new serological types. Due to the fact that only 13 types out of a possibility of 20 have been used in this work, a more complete study, by

TABLE II. Cross Reactions Between Reference Sera Obtained by Serum Neutralization Test on Chick Embryo

Virus strain \ Antisera	Ark 99	Aust T	Clark	Conn	Flo	Gray	Holl	Holte	Ia 97	Ia 609	JMK	Mass	Se-17
Arkansas 99	>6.2[a]	1.7	1.3	1.0	0.8	1.0	1.6	1.8	0	0.8	3.0	1.6	1.8
Australian T	0.5	>5.7	0.2	0.9	0.1	0.7	1.1	1.1	0.2	0.7	0.5	0.1	1.5
Clark 333	1.2	0.3	>6.8	1.6	1.3	0.4	0.8	0	0.6	0	1.3	0	0.4
Connecticut	2.0	3.0	2.5	>6.3	2.8	3.0	2.0	2.5	2.3	2.3	2.5	2.0	2.4
Florida 18288	2.5	2.7	2.8	3.6	>7.0	1.8	2.3	2.3	1.0	2.3	1.0	3.5	2.0
Gray	3.2	1.7	2.2	3.1	2.6	>7.2	2.7	2.8	3.7	3.5	1.8	2.0	>7.3
Holland	1.7	1.3	1.8	1.0	1.5	2.3	>5.8	2.0	1.0	2.0	2.7	4.2	1.7
Holte	2.7	2.4	1.2	2.0	1.7	2.5	2.8	>6.0	3.0	2.0	2.8	2.5	1.8
Iowa 97	0.4	2.8	1.0	2.0	1.0	1.7	2.7	3.2	>6.2	0.6	3.0	1.4	0.7
Iowa 609	2.0	2.1	1.6	1.8	2.1	2.4	1.9	0	2.0	>6.6	2.4	2.6	1.4
JMK	2.0	0.6	2.7	1.6	1.3	3.5	3.0	3.0	0.6	3.3	>6.0	0.4	2.7
Massachusetts	0	1.6	2.0	1.0	1.0	2.0	4.2	2.3	0.6	1.3	3.0	>6.6	1.4
SE-17	3.1	2.1	3.0	2.0	1.5	3.5	1.2	1.7	0.7	3.3	1.2	1.3	>6.5

[a]Log 10 neutralization index

using all the reference strains reported in the literature should be undertaken to clarify this point. Considering also that the serological procedure as used in this work is a reliable method but which is very laborious and expensive, the suggested study of the untypeable isolates should be undertaken when a less cumbersome technique can be developed. Recent work on the characterization of IBV polypeptides (3, 8, 10) could certainly simplify the taxonomy of the infectious bronchitis viruses as suggested by Darbyshire (4).

Ten out of the 24 outbreaks investigated were related to Connecticut and the others to Holland, SE-17 and some unrelated serotypes.

The epidemiological analysis of these outbreaks does not find a correlation between the isolated

TABLE III. Cross Neutralization Between Field Virus Isolates and Reference Antisera

Antisera / Virus isolates	Ark 99	Aust T	Clark	Conn	Flo	Gray	Holl	Holte	Ia 97	Ia 609	JMK	Mass	Se-17
76-318	0.3[a]	1.0	0.5	>5.8	0.5	1.3	1.2	0.3	0.2	0.5	0.5	1.2	0.5
77-178	0.1	0.6	1.0	>6.5	2.4	0.1	0.5	0.4	0	0	0.6	0	0.1
77-426	0.2	0.5	2.0	1.5	0.7	1.4	1.0	1.0	0.5	0	1.7	1.4	0.2
77-477	2.7	0	2.4	2.2	1.9	2.5	1.1	2.4	1.3	2.5	0.4	1.3	1.1
77-536	1.8	1.8	2.1	2.6	2.4	1.3	>6.2	0.7	0.7	0.7	1.0	0.8	1.3
77-600	1.3	1.0	3.3	>5.5	>5.5	2.3	1.9	2.0	0.6	0	1.4	0.3	1.8
77-966	1.3	0.5	1.8	0.5	1.8	1.3	>5.3	1.0	0	1.8	1.6	0.5	1.1
78-21	1.4	1.9	1.7	>6.6	3.0	0.8	2.1	1.1	0.1	0.6	1.8	0	2.1
78-212	0.2	0.5	1.6	>5.5	2.8	0.9	0.8	1.2	0.5	1.1	1.8	0.8	1.3
78-284	0.7	3.2	1.5	>6.6	>6.6	1.7	2.8	1.2	0.7	2.7	2.3	0	2.0
78-483	2.5	1.7	3.0	>6.5	>5.1	2.9	3.0	1.2	0.5	0.2	1.8	0.2	2.9
78-486	1.9	2.3	1.5	>6.3	3.5	1.5	2.0	1.9	1.7	0.3	2.8	0.7	0.9
78-598	0	0.5	1.5	0.9	0	0	0	0.9	0	0	0	0.8	0.7
78-629	1.3	1.4	1.8	0.8	0.8	2.1	>6.6	1.4	1.1	1.2	3.2	1.0	1.8
78-638	0	0	1.5	0	0.2	0	4.5	1.0	0	1.3	3.5	0	0.3
78-656	1.3	0.1	3.0	2.1	2.6	1.4	>6.6	1.8	0.3	2.1	1.1	1.1	1.4
78-824	0	0	0.4	0.1	0.9	0	1.6	0	0	0	0	1.5	0
78-1095	0.3	0.5	1.3	2.5	0.8	0	0.5	0	0	0.7	1.0	0.3	0.7
78-1221	2.0	2.0	2.5	3.0	2.0	4.1	1.5	1.2	1.5	2.5	2.5	2.3	>6.3
78-1297	1.7	2.5	2.9	2.6	3.0	2.3	3.3	2.3	2.3	3.0	2.0	1.3	2.5
79-83	0.2	0.7	0.8	5.0	1.3	1.0	2.7	0	0.9	0.5	2.0	2.0	0.9
79-158	0.6	1.1	1.6	2.3	1.8	1.0	2.3	1.1	0	1.3	0	1.8	0.3
79-241	1.7	2.5	1.5	>5.5	4.2	1.0	0.9	0.2	0	0.5	1.0	0.2	1.0
79-323	0.6	1.1	0.7	2.6	1.3	2.1	>6.3	0.3	1.7	1.3	1.3	0.2	1.3

[a]Log 10 neutralization index

TABLE IV. Description of the History of the Isolates and the Serotypes Obtained in this Study

Reference Number	County of Origin	Age of Birds in Weeks	Time of Vaccination in Weeks	Associated Disease	Serotype
76-318	Terrebonne	6½	1	Respiratory	Connecticut
77-178	Shefford	6½	No	Respiratory	Connecticut
77-426	Terrebonne	4½	No	Respiratory	Unknown
77-477	Terrebonne	4	1¼	Respiratory	Unknown
77-536	Joliette	5	1¼	Respiratory	Holland
77-600	Charlevoix	6	Unknown	Respiratory	Conn. + Florida
77-966	Lotbinière	5	No	Respiratory	Holland
78-21	Joliette	3	1¼	Respiratory	Connecticut
78-212	Berthier	?	Unknown	Respiratory	Connecticut
78-284	Napierville	6	1¼	Respiratory	Conn. + Florida
78-483	Joliette	3	1¼	Respiratory	Conn. + Florida
78-486	Rimouski	4	1	Respiratory	Connecticut
78-598	Joliette	24	19	Respiratory	Unknown
78-629	Drummond	27	12	Resp. + ↓ egg product	Holland
78-638	Iberville	19	Yes, unknown	Respiratory	Holland
78-656	Terrebonne	4	No	Respiratory	Holland
78-824	Joliette	7	No	Respiratory	Unknown
78-1095	Beauce Nord	6	Unknown	Respiratory	Unknown
78-1221	Drummond	6	No	Respiratory	SE-17
78-1297	Joliette	5½	4	Respiratory	Unknown
79-83	Berthier	3	Unknown	Respiratory	Connecticut
79-158	Johnson	4	1¼	Nephritis-nephrosis	Unknown
79-241	Joliette	4	2	Respiratory + Nephritis-nephrosis	Conn. + Florida
79-323	Iberville	20	Yes, unknown	Respiratory	Holland

viruses and all the parameters considered in materials and methods. Consequently, no particular explanation is given for the appearance of new virus serotypes.

In our former survey (9) most isolates originated from nonvaccinated flocks and were mainly of the Massachusetts serotypes. It is quite possible that better management, especially the continued vaccination of flocks has contributed to establish a resistance to this type and possibly permitted the emergence of new serotypes.

Most of the isolates originated from flocks immunized with a vaccine containing the Connecticut and Massachusetts serotypes. The last one was not recovered from the diseased flocks. However, nine of the ten Connecticut isolates originated from vaccinated flocks. Although it could be suspected that these isolates were originating from the vaccine previously used, the clinical symptoms observed during these outbreaks tends to eliminate this possibility. Furthermore, one isolate was recovered from a nonvaccinated flock suggesting that the virulent Connecticut serotype was present in Quebec at this time. Also, most Connecticut strains were isolated at the time when the theoretical presence of the virus in vaccinated animals had vanished according to the work of Alexander and Gough (1, 2).

All other serotypes isolated including the Holland strain, were not contained in any of the vaccines used in the field and should be considered as partly responsible for the outbreaks. At this point, it is concluded that strains used for immunization of birds do not confer protection to all serotypes found in Quebec and that more polyvalent vaccine should be used to give better protection against different field virus strains.

In conclusion, it is suggested that flocks should also be vaccinated with the Holland strain (5) which possesses a larger antigenic spectrum and offers better protection than the previously used serotype (11, 12).

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