OLIGONUCLEOTIDE FINGERPRINTING OF THE RNA OF DIFFERENT STRAINS OF INFECTIOUS BRONCHITIS VIRUS

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INTRODUCTION

Infectious bronchitis virus /IBV/, the prototype of the family coronaviridae[1] causes pathological conditions of the respiratory tract, the reproductive tract and the kidneys of chickens[2]. The disease was first described in the United States[3], but by the early sixties it had been identified all over the world. Early work showed that more than one serotype of IBV existed[4]. Since then a large number of strains have been isolated and attempts have been made to classify these strains using a serological approach. On the basis of immunofluorescence data, and virus neutralization tests carried out mostly with reference strains, at least eight serotypes[5] were established. However, in a study where large numbers of field strains were examined and compared to reference strains, it became apparent that it was not feasible to classify IBV isolates using a serological approach[6]. In an attempt to circumvent this problem strains were examined by cluster analysis using Euclidean distance as the measure of similarity and two main groups were established[7]. It was also found that twelve strains, fell into one or other of two categories of protein pattern differing in the mobility of a small glycoprotein on polyacrylamide gels[8,9]. It is of interest that reference strains placed in different groups by the cluster analysis also showed different protein patterns[8].

Under circumstances when live vaccine strains are used against a disease or when the relatively rapid emergence of new field strains

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Table 1. Description of IBV strains used for fingerprinting.

Serotype and strain designation	Symbol	Isolation Place	Isolation Year	Protein pattern[8]	Reference
1. Massachusetts					
Massachusetts 41	Mass 41	USA,Ma.	1941	M*	11
Connaught	Mass-C	USA	1954	M	12
Beaudette No.66579	Bea	USA	1937	M	13,14
927	927	England	1965-70	M	6
Houghton, HVI 140	HVI 140	England	1965-70	M	15
H120	H120	Holland	1956	M	17
2. Georgia					
SE 17	SE 17	USA,Ga.	1969	M	18
3. Connecticut					
Connecticut	Conn	USA,Co.	1951	C*	19
LED	LED	Hungary	1968	C	20
4. Delaware					
Holte	Holte	USA,Wisc.	1954	C	21
5. Iowa 97					
Iowa 97	Iowa 97	USA,Io.	1947	C	22
6. Australia T					
Australia T	Au T	Australia	1963	C	23
7. New Zealand A					
7533	NZ-A	New Zealand	1976	C	24

*Characterized by gp30 /M/ and gp28 /C/.

occurs /as in the case of infectious bronchitis/ a reliable method for strain identification is essential. The increasing understanding of the molecular biology of coronaviruses during the past few years prompted us to compare the RNAs of different IBV strains by oligonucleotide fingerprinting, which has been found to be useful in establishing the genetic basis of a serological classification of rhabdoviruses, picornaviruses, polioviruses, bunyaviruses, alfaviruses and retroviruses[10]. Previous studies have shown that viruses which cannot be antigenically differentiated usually give comparable but distinguishable oligonucleotide fingerprints. When few differences are seen the viruses are called variants of one another. In other cases where many differences occur between the fingerprints of

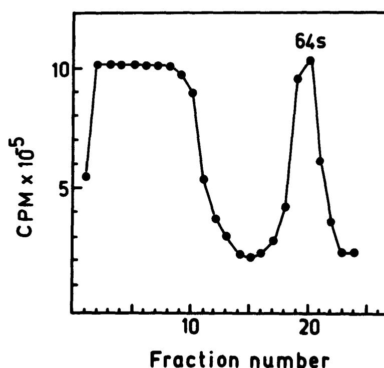

Fig. 1. 64s IBV RNA fractionated on sucrose gradient.

two isolates classified by other methods as the same virus type, the viruses are called varieties of the same type.

In this study the RNAs of strains of IBV belonging to the same serotype, and strains representing different serotypes have been compared by oligonucleotide fingerprinting.

MATERIALS AND METHODS

The identification code, year of isolation and geographical origin of the IBV strains used in this study are summarized in Table 1. The exact number of passages of the strains since their isolation is unknown but is probably in the order of ten to twenty. Strain Bea

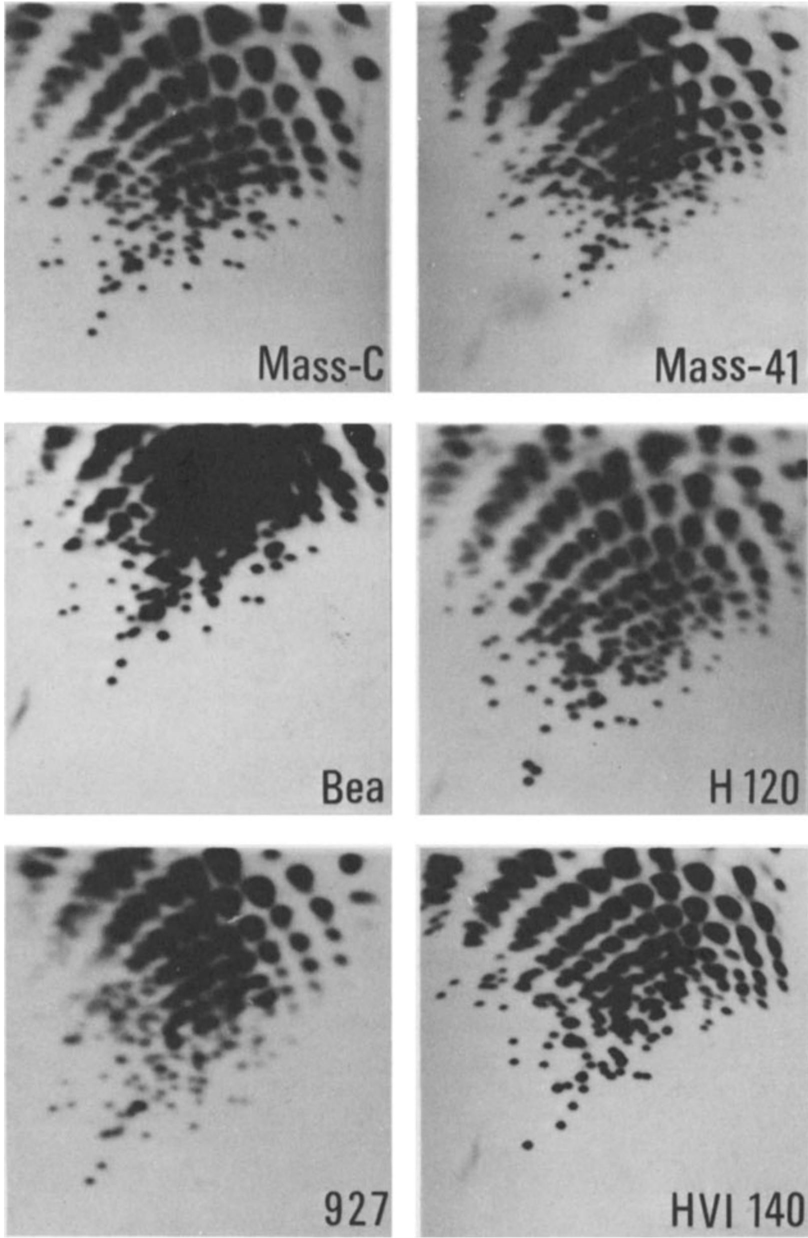

Fig. 2. Oligonucleotide fingerprints of RNA from six strains of IBV belonging to the Massachusetts serotype.

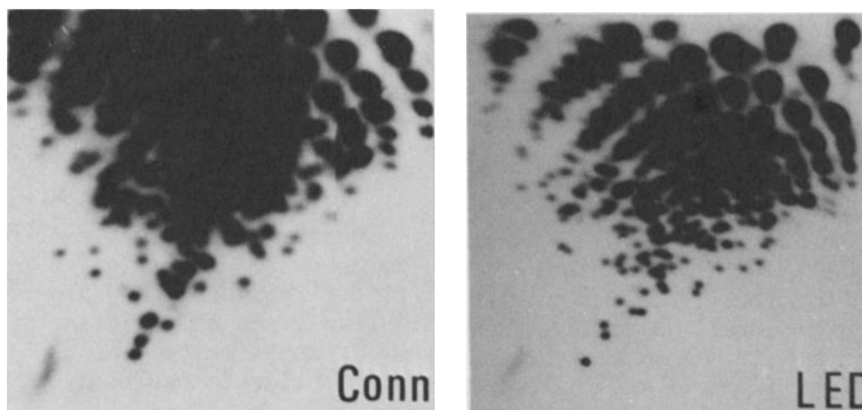

Fig. 3. Oligonucleotide fingerprints of RNA from two strains of IBV belonging to the Connecticut serotype.

is a highly passed strain, whose passage number is close to 300, for strain H120 the passage number is above 120 and for strain LED it is between 60 and 70.

IBV was grown in CEK cells as previously described[25].

^{32}P-labelled RNA was extracted from purified virus and fractionated on sucrose density gradients as described in detail earlier[25]. A typical profile of IBV RNA used for further analysis is shown on Fig. 1.

Labelled RNA precipitated from ethanol was digested with RNase T_1, and oligonucleotides were resolved on 2-dimensional gels, before visualization by autoradiography[26].

RESULTS

RNA was isolated from six strains of the Massachusetts serotype and fingerprinted /Fig. 2/. Three of the patterns obtained were obviously related to each other, showing only a few differences in their characteristic oligonucleotides. These were from strains Mass-C, Bea and 927, which are therefore variants of the same parental variety. The fingerprints of the other three strains are dissimilar to the extent that no common large oligonucleotides could be recognized. Thus Mass 41, HVI 140 and H120 represent other varieties of the Massachusetts serotype, and a total of 4 distinct varieties were identified in the 6 strains examined. These results show that oligo-

nucleotide fingerprinting is much more incisive for strain differentiation than any methodology previously applied to IBV.

The relationships between viruses in the Connecticut serotype was investigated in a similar way. The reference strain Conn and a more recent isolate LED were compared and may have a few common spots, but their overall difference is conspicuous /Fig. 3/. Thus these represent different varieties within the Connecticut serotype.

The difference between the two major serotypes, Massachusetts and Connecticut was more rigorously investiagaed by co-electrophoresis of T_1 digests of their RNAs /not shown/. The majority /more than 70 per cent/ of the characteristic oligonucleotides did not co-electrophorese, suggesting that the viruses are only distantly related with little sequence homology. This is reflected in the differences that have been observed in the polypeptide composition of these viruses[8,9].

Representative viruses from five other serotypes were analysed by oligonucleotide fingerprinting in order to ascertain whether any have a significant degree of sequence homology. The fingerprints of three isolates from the Northern Hemisphere SE 17, Holte and Iowa 97 are shown in Fig. 4. There is no apparent relationship between these viral RNAs.

It was of interest to examine two strains from the Shouthern Hemisphere /NZ-A and Au T/ to determine whether it was possible to shed any light on the origin of these strains, since they were isolated later than Iowa 97 and Holte /see Table 1/. The fingerprints of these RNAs are shown in Fig. 5. As far as it is possible to tell NZ-A and Au T are unrelated to each other and to Iowa 97, Holte and

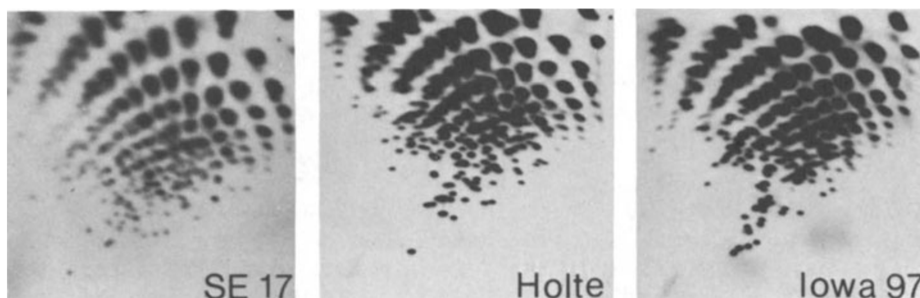

Fig. 4. Oligonucleotide fingerprints characteristic of the Georgia /SE 17/, Delaware /Holte/ and Iowa 97 serotypes of IBV, all of which are other Northern Hemisphere isolates.

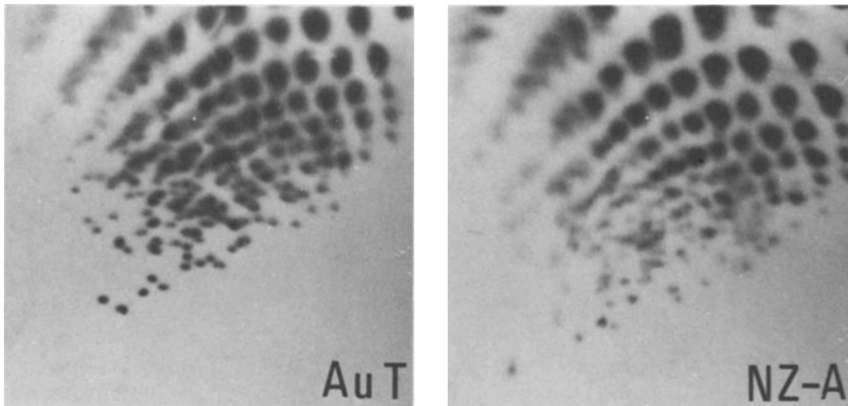

Fig. 5. Oligonucleotide fingerprints characteristic of Australia T /Au T/ and New Zealand A /NZ-A/ serotypes isolated in the Southern Hemisphere.

SE 17, suggesting that NZ-A was not derived from the Australian strain although New Zealand allowed the import of chickens from Australia before the NZ-A virus was isolated.

In addition, none of these five strains showed any obvious similarity with the two other major serotypes, Massachusetts and Connecticut.

DISCUSSION

We present here the T_1 oligonucleotide fingerprints of RNA prepared from 13 virus isolates. All these isolates were originally classified as IBV using pathological criteria. We have confirmed that all the viruses studied in this work have the virion density, genome size and protein profile expected for IBV /data not shown/. Nevertheless at least 11 different fingerprints were recognized. These were given by Mass 41, Mass-C, HVI 140 and H120 from the Massachusetts serotype, Conn and LED from the Connecticut serotype and five representatives of other serotypes, SE 17 from the Georgia serotype, Holte from the Delaware serotype, Iowa 97 from the Iowa 97 serotype, Au T from the Australia T serotype and NZ-A from the New Zealand A serotype. Thus different serotypes gave distinct fingerprints, but so did varieties within a serotype. Whether variants with similar fingerprints, such as Mass-C, Bea and 927 are antigenically closer to each other than to other varieties of the same serotype with different fingerprints is not yet established.

Further generalizations about the genetic basis of antigenic relationships among IBV isolates would be premature, and must await the introduction of more precise methods capable of recognizing subtle antigenic differences between strains.

One problem raised by this study is the question of which isolate should be considered the prototype virus of the Massachusetts serotype. Three of the viruses from the Massachusetts serotype characterized in this study, have virtually identical fingerprints. These are Mass-C, Bea and 927. These fingerprints differ substantially from the fingerprint of another virus which is currently regarded as the prototype virus of the Massachusetts serotype /Mass 41/. The differences are unlikely to have arisen during laboratory passage because the fingerprint of Bea produced in this work is identical to that previously published[25]. Thus the limited number of passage between these viruses /fewer than ten/ has not allowed the accumulation of sufficient mutations to change the pattern of characteristic oligonucleotides. This agree with results from other viruses[10]. Stern and Kenndey[27] have recently published a fingerprint of strain Beaudette /IBV 42/ obtained from the American Type Culture Collection which differs only slightly from the fingerprints we have obtained from Mass-C, Bea and 927. IBV 42 and strain Bea /No. 66579/ used by us, have been maintained as separate passage lines. Thus these four viruses are all variants of the same parental virus.

IBV was first recognized as a disease in the U.S.A. in 1931[3], but by the early sixties outbreaks had occurred all over the world. The viruses examined in this study were isolated over a period of years from widely separated geographical locations.

A great diversity among the fingerprints of these viruses all identified as IBV by other criteria indicates that considerable variation of the genome is possible. Fingerprint analysis provides information concerning theories of origin and spread of IBV. For example the NZ-A virus could be postulated to be derived from the Au T virus. This would seem reasonable since Au T was present in Australian chickens during the period in which these were imported into New Zealand. However fingerprint analysis of the virus subsequently isolated in New Zealand shows that it is not related to Au T. Therefore either the New Zealand virus has diverged very rapidly from Au T or it is derived from another source. This source, however, could not be any of the other viruses studied here because their fingerpints were also different.

Vaccines have been used prophylactically in attempts to control the disease caused by IBV. Some of these vaccines have been composed of live attenuated virus. When live vaccines are used there is an obvious danger that if the virus escapes, and if virulence is recovered, then the vaccine itself can actually be the cause of disease outbreaks. Two of the viruses used in this study /H120 and

Mass-C/ have been used as live vaccines. Mass-C was used as a vaccine in North America in the late fifties and early sixties. Subsequently /1965-70/ 927, whose fingerprint analysis showed to be very closely related to Mass-C, was isolated in England. Thus it is possible that 927 could be derived from escaped Mass-C virus, although at that time no live vaccine was licensed for use in England. Alternatively this variety of the Massachusetts serotype /Mass-C, Bea and 927/ has a wide temporal and geographical spread. In order to resolve this question, and others about the origin and spread of IBV varieties, further studies will be required following the evolution of IBV in the field.

SUMMARY

11 distinct oligonucleotide fingerprints were obtained in studies of the RNA of 13 isolates of IBV. Different serotypes had distinct fingerprints, but so did varieties within a serotype; allowing a greater degree of strain differentiation than was previously possible. Some conclusions can be drawn from the fingerprints concerning theories of origin and spread of IBV.

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REFERENCES

1. D. A. J. Tyrell, D. J. Alexander, J. D. Almeida, C. H. Cunningham, B. C. Easterday, D. J. Garwes, J. C. Hierholzer, A. Kapikian, M. R. MacNaughton, and K. McIntosh, Coronaviridae: second report, Intervirol. 10:321 /1978/.
2. C. H. Cunningham, Avian infectious bronchitis, Adv. Vet. Sci. Comp. Med. 14:105 /1970/.
3. A. F. Schalk, and M. C. Hawn, An apparently new respiratory disease of baby chicks, J. Amer. Vet. Med. Ass. 78:413 /1931/.
4. M. S. Hofstad, Antigenic differences among isolates of avian infectious bronchitis virus, Amer. J. Vet. Res. 19:740 /1958/.
5. S. R. Hopkins, Serological comparisons of strains of infectious bronchitis virus using plaque-purified isolants, Avian Dis. 18:231 /1974/.
6. P. S. Dawson, and R. E. Gough, Antigenic variation in strains of avian infectious bronchitis virus, Arch. g. Virusforsch. 34:32 /1971/.

7. J. G. Darbyshire, J. G. Rowell, J. K. A. Cook, and R. W. Peters, Taxonomic studies on strains of avian infectious bronchitis virus using neutralization tests in tracheal organ culture, Arch. Virol. 61:227 /1979/.
8. E. Nagy, and B. Lomniczi, Polypeptide patterns of infectious bronchitis virus serotype fall into two categories, Arch. Virol. 61:341 /1979/.
9. M. S. Collins, and D. J. Alexander, Avian infectious bronchitis virus structural polypeptides: effect of different conditions of disruption and comparison of different strains and isolates. Arch. Virol. 63:239 /1980/.
10. J. P. Clewley, and D. H. L. Bishop, Oligonucleotide fingerprinting of viral genomes, in: " Newer Techniques in Practical Virology," C. R. Harvard, ed., Alan R. Liss Inc., New York /in press/.
11. H. Van Roekel, K. L. Balls, M. K. Clarke, O. M. Olesiuk, and F. G. Sperling, Infectious bronchitis. Mass. Agric. Exp. Stn. Bull. No. 460. /1950/.
12. S. B. Hitchner, and P. G. White, Groth-curve studies of chick embryo-propagated IBV. Poultry Sci. 34:590 /1955/.
13. F. R. Beaudette, and C. B. Hudson, Cultivation of the virus of infectious bronchitis. J. Amer. Vet. Med. Ass. 90:51 /1937/.
14. H. G. Purchase, C. H. Cunningham, and B. R. Burmaster, Identification and epizootiology of infectious bronchitis in a closed flock, Avian Dis. 10:111 /1966/.
15. J. W. Harkness, and C. D. Bracewell, Morphological variation among avian infectious bronchitis virus strains, Res. Vet. Sci. 16:128 /1974/.
16. J. Hoekstra, and B. Rispens, Infectieuze bronchitis bij pluimvee. I. Laboratorium-experimenten met een sterkwerkende entstam, Tijdschr. Diergeneesk. 85:398 /1960/.
17. G. Bijlenga, Het infectieuze bronchitis virus bij kiuken in Nederland, aangetoond mit behulp von ei-enting, dierexperimenteel onderzoek en serumneutralisatie-proveven, Tijdschr. Diergeneesk. 81:43 /1956/.
18. S. R. Hopkins, Serological and immunological properties of a recent isolate of infectious bronchitis virus, Avian Dis. 13: 356 /1969/.
19. E. L. Jungherr, T. W. Chomiak, and R. E. Luginbuhl, Immunologic differences in strains of infectious bronchitis virus, Proc. 60th Ann. Meeting U.S. Livestock San. Ass. Chicago. 203 /1956/.
20. B. Lomniczi, and L. Stipkovits, Isolation of the virus of infectious bronchitis, Magy. Áo. Lapja 23:600 /1968/.
21. R. W., Winterfield and S.B. Hitchner, Etiology of an infectious nephritis-nephrosis syndrome of chickens, Amer. J. Vet. Res. 23:1273 /1962/.
22. M. S. Hofstad, and H. W. Yoder, Jr., Avian infectious bronchitis – Virus distribution in tissues of chickens. Avian Dis. 10:230 /1966/.

23. R. B. Cummings, Infectious avian nephrosis /uraemia/ in Australia, Austral. Vet. J. 39:145 /1963/.
24. J. E. Lohr, Serological differences between strains of infectious bronchitis virus from New Zealand, Australia and the United States, Avian Dis. 20:478 /1976/.
25. B. Lomniczi, and I. Kennedy, Genome of infectious bronchitis virus. J. Virol. 24:99 /1977/.
26. J. P. Clewley, J. Gentsch, and D. H. L. Bishop, Three unique species of snowshoe hare and LaCrosse bunyviruses. J. Virol. 22:459 /1977/.
27. D. F. Stern, and S. I. T. Kennedy, Coronavirus multiplication strategy. I. Identification and characterization of virus-specified RNA. J. Virol. 34:665 /1980/.