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Sequence analysis of strains of avian infectious bronchitis coronavirus isolated during the 1960s in the U.K.

Brief Report

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Summary. Sequencing of parts of the spike, small membrane, and integral membrane protein genes of English isolates of avian infectious bronchitis virus (IBV) isolated in the 1960s revealed that they were not the direct ancestors of those isolated in the 1980s.

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In the late 1970s and early 1980s in the Netherlands and the U.K. pathogenic strains of IBV were isolated which were unrelated by virus neutralization (VN) tests to those of the Massachusetts and other serotypes which had been detected previously in the U.S.A. [7, 10, 12]. The question arose as to whether the 1970/80s European isolates were closely related to strains present in earlier years or represented a distinct group(s) of strains. Fortunately Dawson and Gough [11] had isolated IBV strains from many regions of England between the years 1965 and 1967. Since some IBV strains appear to be only distantly related on the basis of serum VN tests while actually having very similar ($\geq$ 97% amino acid identity) S 1 proteins [6] it was decided to compare the 1960s isolates by nucleotide sequencing. The VN antibodies that have been the basis for comparison of IBV isolates are induced largely by the N-terminal S 1 subunit of the S protein [3, 14, 16] and S 1 genes of several of the 1970/80s isolates have been sequenced [1, 6, 13, 15, 17, 20] as have sM (small membrane; previously referred to as 3 c) and M genes [2, 19].

The following isolates, isolated in England between 1965 and 1967 and

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propagated in embryonated domestic fowl eggs [11], were kindly provided by R. Gough, Central Veterinary Laboratory, Weybridge, U.K.; the second number shows the year of isolation: 48/65, 183/65, 265/66, 860/66, 225/67, 227/67, 551/67, 690/67, 918/67. Strain Allen had been isolated in 1947. Isolate 101/86 was provided by J. K. A. Cook of this institute. In our laboratory each isolate was passaged up to three times in eggs to obtain a working stock and once more to produce virus RNA. RNA was extracted [19] and used for dideoxy sequencing [2], modified as described previously [6], using the following negative-sense oligonucleotide (numbers in parenthesis correspond to the sequence of IBV-M 41 S sequence, excluding the 54 nucleotide amino-terminal signal sequence [1]:

5′ GGTGCTGTCATAGC 3′ (190–203); TTTTCATGGCAGAGATG (348–364);

GCCAGGGCTTTAACT (561–575); GGATAAAAGCCATCTGA (667–683);

CCTTCCTGCATACGACC (915–931); GCCACCGCTCTTAGTA (1155–1170);

TTAGTAATAAAACCTTG (1276–1292); ACTACAAACTGCTG (1438–1451);

TTTGGTACTATTGTGG (1641–1656).

The sequence of the S 1 part of the S gene of isolate UK/918/67 has been submitted to the EMBL Nucleotide Sequence Database, accession number X 64737.

The end of the sM gene and the beginning of the M gene overlap and we have previously examined this region for many strains isolated in the 1980s in the U.K. and the Netherlands and for a large number of Massachusetts serotype strains isolated in the U.S.A. and Europe [2]. Therefore we examined this part of the genome in respect of 11 of the pre-1970 U.K. isolates and compared the sequences with the consensus sequence of 12 Massachusetts serotype strains (Fig. 1). Two of the 11 isolates (Allen/47 and 265/66) differed by only one nucleotide from the consensus sequence of the Massachusetts serotype, a similarity subsequently confirmed by sequencing of the S 1 gene. The remaining nine isolates differed substantially from Massachusetts serotype strains and eight of them formed a recognisable group on the basis of a six nucleotide deletion, effectively making the M protein two amino acids shorter at the extreme amino-terminus compared with Massachusetts strains. The remaining isolate (604/67) had a nine base deletion but was otherwise similar to the other eight strains. This particular six/nine nucleotide deletion was one that we had not encountered previously except for one 1980s isolate, UK/101/86 (Fig. 1). The sequence of three other 1980s strains, representative of three different deletion types, is shown for comparison in Fig. 1.

The sequence of S 1 of strain 918/67 was determined in its entirety and comprised 1577 nucleotides (plus a leader sequence of 54 bases) encoding a polypeptide of 526 amino acids, making this the longest IBV S 1 sequenced to-

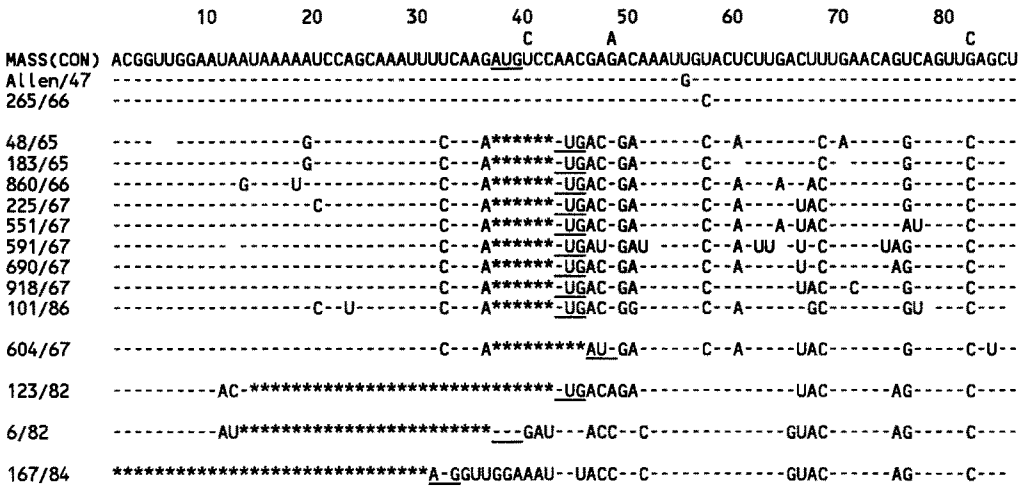

Fig. 1. Nucleotide sequences at the end of the sM gene and beginning of the M gene of IBV strains isolated in the U.K. before 1970, compared with a consensus sequence of 12 Massachusetts (*MASS*) serotype strains. The nucleotides above the Massachusetts consensus sequence show where some Massachusetts differ from the consensus sequence. The sequence of three strains isolated in the 1980s are also shown. The AUG translation start codon for the M protein is underlined. Nucleotides absent in comparison with Massachusetts strains are shown by asterisks (*). Gaps in the sequence indicate that the nucleotides could not be unequivocally identified

Table 1. Number (and %) of amino acid differences between each quarter of S1[a] of UK/918/67 and that of UK/6/82, M41 and NL/D 1466/78

Comparison with isolate	Number (%) of differences in amino acid sequences			
	1–130	131–260	261–391	392–526
6/82[b]	34 (26)	27 (13)	31 (24)	22 (17)
M 41[b]	49 (38)	22 (17)	31 (24)	16 (12)
D 1466[c]	87 (67)	71 (55)	61 (47)	59 (45)

[a] Excluding the 18 residue amino-terminal signal sequence
[b] Sequence data from [1]
[c] Sequence data from [17]

date. The deduced amino acid sequence differed from that of 6/82 and M 41 [1] and NL/D 1466/78 [17] by 19, 22, and 52%, respectively. The differences between isolate 918/67 and strains 6/82 and M 41 were greatest in the first and third quarters of S 1 (Table 1), as was the case when a group of 1980s strains was examined [6]. It is these areas which, in other strains, are known to induce VN antibodies [4, 14, 16]. Some of the oligonucleotides did not generate sequence data with the other isolates so sequence has been reported for eight additional strains in up to four regions (I–IV) of S 1 comprising 826 nucleotides

(Table 2). Isolates Allen/47, 265/66 and 227/67 resembled M 41 very closely (Table 2), in keeping with the sM/M sequence determined for Allen/47 and 265/66 (Fig. 1). The four other 1960s strains for which S 1 data was obtained resembled 918/67 more than M 41. However, variation among these 1960s isolates was 3- to 5-fold greater in regions I–IV than in the corresponding regions of a group of 12 Massachusetts serotype strains [4, 5] and 3- to 4-fold greater in regions I–III and 6- to 10-fold more in region IV than in a group of seven 1980s strains [6]. Thus, even when the M 41-like strains were excluded, the remaining 1960s isolates formed a rather heterogeneous group.

One of the most variable S 1 regions (M 41 nucleotide sequence 270 to 394, excluding the signal sequence) contained an additional 15 contiguous bases for some of the non-Massachusetts-like 1960s strains (demonstrated for 225/67, 690/67, and 918/67), which largely accounted for the greater length of S 1 of these strains when compared with published sequences (data not shown). These bases were located between nucleotides 369 and 370 (amino acids 123 and 124) in the M 41 sequence. Isolate 48/65 had nine rather than 15 additional bases at this position. These amino acids were within a region (site E) which, for many 1980s strains, induced VN antibodies [14].

The data indicates that it is unlikely that the IBV strains isolated in the late 1970s and 1980s in the U.K. had evolved directly from those present during

Table 2. Nucleotide variation in four regions of the S1 gene of UK IBV isolates and M 41

Isolate	Differences (%) from UK/918/67 and M 41							
	Region I[a]		Region II[b]		Region III[c]		Region IV[d]	
	918/67	M 41	918/67	M 41	918/67	M 41	918/67	M 41
48/65	15	26	19	24	–	–	7	11
225/67	–[e]	–	18	28	14	22	–	–
551/67	19	34	–	–	–	–	7	6
690/67	–	–	14	33	14	24	12	9
918/67	0	31	0	32	0	24	0	5
101/86	–	–	27	29	27	7	6	3
Allen/47	29	4	32	3	24	1	6	3
265/66	31	8	32	3	24	2	10	5
227/67	31	12	32	3	24	2	–	–
M 41/41	31	0	32	0	24	0	5	0

[a] Bases 1–172. Base number 1 is the first nucleotide of the mature S 1, i.e., excluding the 5′-terminal 54 nucleotides which encode the signal sequence

[b] Bases 268–552

[c] Bases 709–907

[d] Bases 1409–1578

[e] Sequence not determined

the 1960s. The exceptions were those strains (265/66 and 227/67) which closely resembled strains of the Massachusetts serotype which had been isolated before and since that decade [4, 5, 9]. The more recent isolate 101/86 had a sM/M region which resembled that of most of the 1960s strains (Fig. 1) which led us to believe that it was directly related of them. However, S 1 data suggested otherwise (Table 2). Rather, it would appear that the major IBV strains present in the U.K. during the 1980s either were absent in the 1960s, were present only at a low level or were possibly located in other areas from which virus isolations had not been attempted. It is possible that the later strains had evolved directly from the earlier ones, the process having been disguised by a high mutation rate, of the order of 20% in a decade or so. However, this seems unlikely. Analysis of Massachusetts serotype strains isolated over a half century period has indicated that rapid mutation is not necessarily intrinsic to IBV [4, 6]. Some strains isolated between 1978 and 1986 in the Netherlands and the U.K. differed by only 2% or so of S 1 nucleotides, again counter-indicative of rapid mutation rates [1, 6, 13, 15, 17]. Rather it would appear that the strains dominant in the U.K. 25 years ago have declined and been replaced by others. Analysis, by VN tests, of 1980s strains indicated that a similar change in the U.K. IBV population was occurring during the 1980s [8].

As the number of IBV isolates sequenced increases it emerges that there is a wide spectrum of IBV strains. Isolates with S sequence intermediate between one group and another blur the distinction between them, defying attempts to allocate an isolate to one IBV group or another. This is compounded by the fact that isolates which closely resemble each other in one gene may be very different in another, suggestive of recombination [2, 5, 18]. Our results emphasise that, on the one hand, IBV strains with 20% differences in S 1 can coexist over a relatively small area, while on the other that the IBV population in a given area is in a state of flux.

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