CHAPTER 5

MOLECULAR EPIDEMIOLOGY, GENOMICS, AND PHYLOGENY OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS

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5.1. INTRODUCTION

Crimean-Congo hemorrhagic fever virus (CCHFV) constitutes a group of viruses of the genus *Nairovirus* (family *Bunyaviridae*). Like all members of the Bunyaviridae, the genome of CCHFV is composed of tripartite single-stranded RNA. These segments, designated small (S), medium (M), and large (L), minimally encode the nucleocapsid (N), envelope glycoproteins (Gn and Gc), and RNA-dependent RNA polymerase (RdRp), respectively [38].

Published descriptions of major epidemics, outbreaks, and the ecology of CCHFV have been reviewed extensively [18, 43, 45]. Interestingly a common theme is illustrated by the very wide distribution of the virus, which stretches over much of Asia, extending from the Xinjiang region of China to the Middle East and southern Russia, and to focal endemic areas over much of Africa and parts of southeastern Europe. Thus, CCHFV is the most widely distributed agent of severe haemorrhagic fever known.

5.2. MOLECULAR EPIDEMIOLOGY

Classic serological methods have been important in determining CCHF distribution; however, these assays do not readily differentiate between alternative strains of CCHFV. In order to characterize viral strains in more detail and facilitate a global epidemiological study, molecular methods based on partial and complete sequence data of the S segment have been used to identify certain S segment genotypes [9, 13, 36]. These genotypes show a strong relationship to the geographical area of parent virus isolation, leading to the terminology Asia 1,

O. Ergonul and C. A. Whitehouse (eds.), Crimean-Congo Hemorrhagic Fever, 45–55.

Asia 2, Europe 1, etc., which has been employed as a simple description of genotype (Fig. 5-1). Furthermore, these studies also show that similar genotypes are found in distant geographical locations (Fig. 5-2), supporting the idea that virus or infected ticks may be carried over long distances during bird migration [10]. Anthropogenic factors, such as the trade in livestock, may have also played a role in the dispersal of CCHFV. Thus, molecular epidemiological observations support a global and dynamic reservoir of CCHF virus.

Sequence information on L segments has lagged behind those of both S and M segments primarily due to the technical difficulties in working with these very long molecules. Nevertheless, several data from strains is available and while the number of alternative strains is on a different scale to those of S segments, there is evidence that the S and L segments from the same strains have similar evolutionary history (Fig. 5-3). For M segments however, the situation is different and it enables an insight into the ways CCHFV have evolved.

5.3. GENETIC VARIATION AND EVOLUTION

The driving force for evolution is provided by genetic change and variation in genomes. These lead to phenotypes which are molded by selective forces, thus genomes gradually change with their changing environments. RNA viruses, with their large population sizes, swift, and mutation-prone replication rates are generally considered capable of rapid evolution [16]. Additional evolutionary processes of (i) recombination, and for viruses with segmented genomes (ii) reassortment, also offer potentially important routes of generating genetic diversity. The genomes of arthropod-borne RNA viruses however, need to function and maintain high fitness in both arthropod and vertebrate host cells. This maintenance on two fronts is frequently thought to constrain the evolutionary processes acting on arbovirus genomes [44]. Thus, low levels of genetic diversity are frequently observed for arboviruses. The genome of CCHFV is interesting since, as well as showing features of high genetic stability [13], it also shows features of high flexibility [8]. CCHFV is often described as an emerging virus [22, 47]. Studies of its genetic fine structure aimed at developing a better understanding of the ways it can change and evolve are helping to illuminate its nature as an emerging pathogen. Complete genome entries of several CCHFV are now available in GenBank, and analysis of these sequences are enabling evolutionary hypothesis to be inferred and tested.

5.3.1. Recombination

Genetic homologous recombination – the formation of chimeric RNA molecules from sequences previously separated on different molecules – is an important means of variation open to RNA genomes. Indeed, it is clear that homologous recombination has been an important process that has shaped the evolution of RNA viruses per se [46]. However, the contribution of its effects

Fig. 5-1. Variation within CCHFV S segments and geographical correlation of genotypes. Maximum likelihood phylogenetic tree of CCHFV S RNA segments made from nucleotide alignments constructed from nucleotides 322–562 (Baghdad) enabling the incorporation of a maximum number of strains. Seven distinct lineages of S segment are extant.

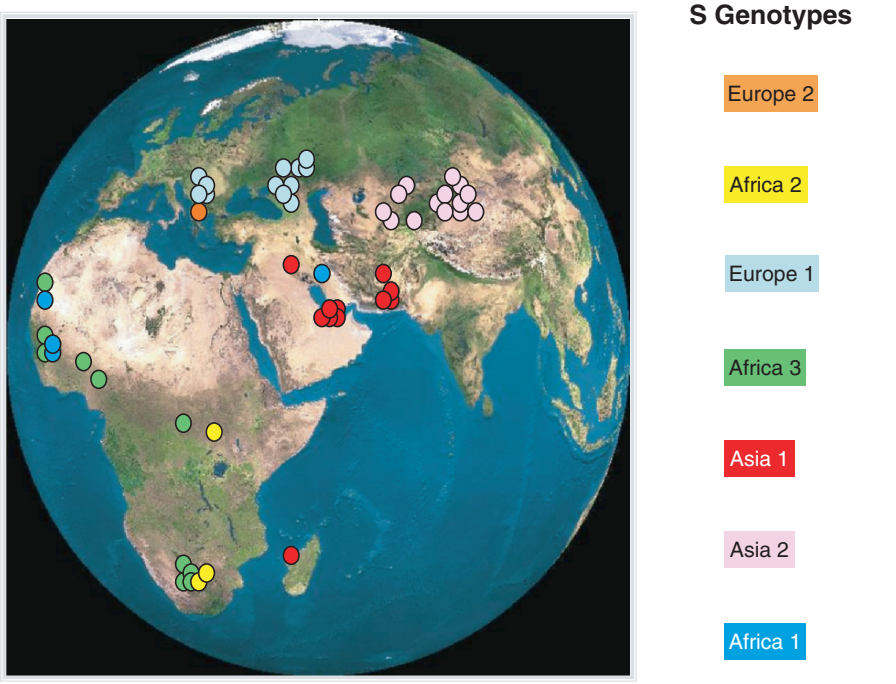

Fig. 5-2. Geographical correlation of genotypes. When superimposed onto the globe, the phylogenetic grouping of S RNA subtypes illustrates that the pattern of genetic diversity observed is largely related to the geographical distribution of the viruses. On some occasions, however, similar subtypes are sometimes found in distant geographical locations. It is possible that trade in livestock and perhaps long-distance carriage of virus or infected ticks during bird migration may have brought about links between such locations. (*See Color Plates*)

and the rate at which it occurs vary for different virus families. For example, it is known to be frequent in retroviruses [19], less common but periodic for positive-strand RNA viruses [24], but relatively infrequent in negative-strand RNA viruses [4, 32]. Yet, cases of recombination in the latter group do occur and evidence of it in the Bunyaviridae [39] and Arenaviridae [1] is well documented. Such reports have encouraged the search for recombination in CCHF viruses. Noteworthy evidence, including the demonstration of phylogenetic incongruence, often regarded as the best support for recombination [34], has been illustrated for the CCHF S segment [26]. Similar evidence for recombination in either of the M or L segments was not detected. A very recent study [8] also supported this latter observation in the majority of M and L segments. In addition, however, an analysis employing similarity plots, bootscanning and the informative sites tests, highlighted the possibility of recombination events within L segments of the Asian groups [8]. Interestingly, the cases of recombination are phylogenetically ancient and there is evidence that the sequences in question have diverged considerably after recombination. This suggests that

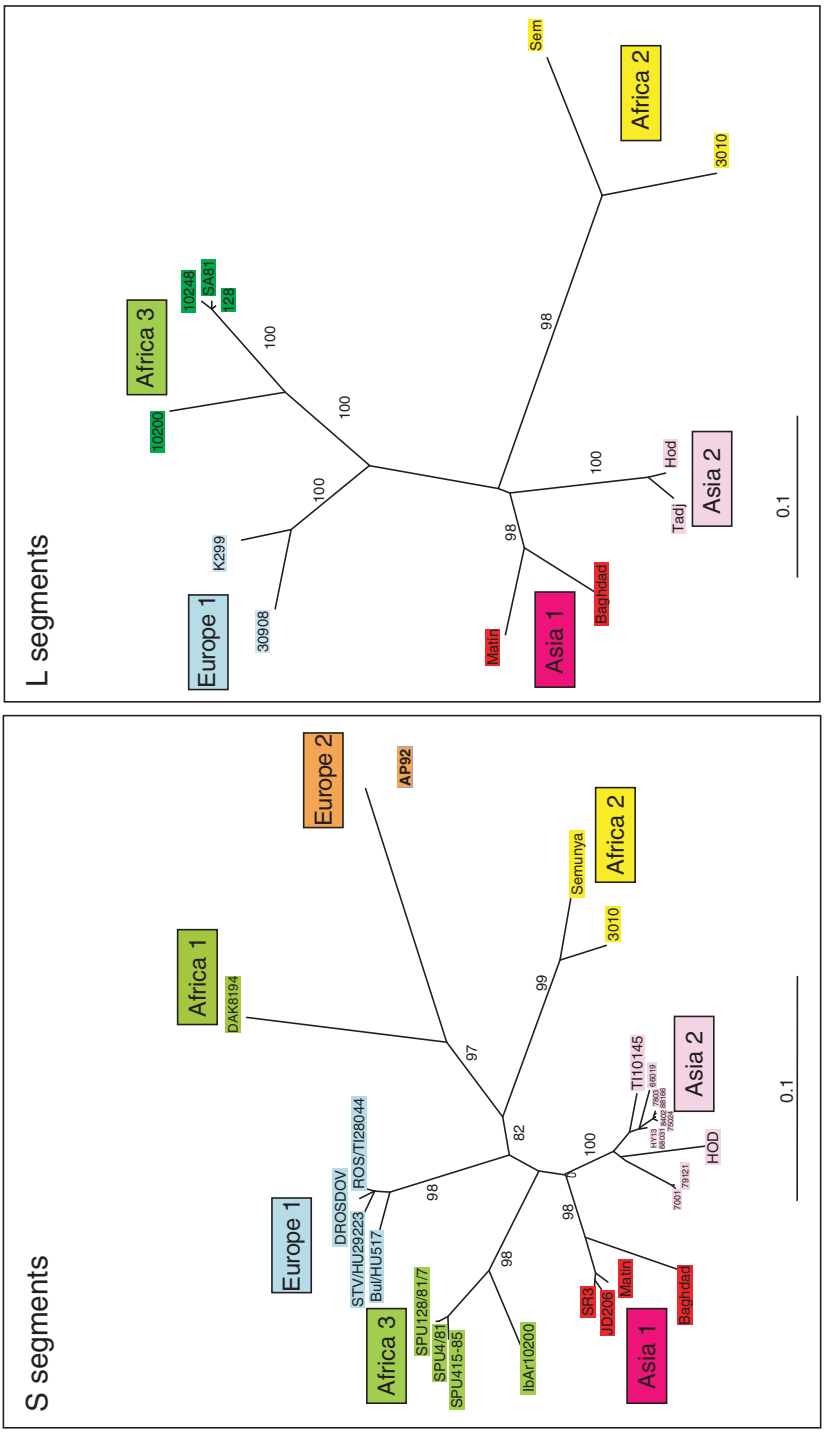

Fig. 5-3. Unrooted maximum-likelihood trees of full-length CCHFV S and L segment sequences showing phylogenetic relationships and correlation to geographic location. For the L segment, there are five different lineages or genotypes that, like S segments, have grouped according to their geographical location of isolation. From these data, it appears likely that the L segments also conform to the same grouping pattern as observed for S segments, although there are fewer L segment sequences. Additional sequence data provided by very recent work has enabled more comprehensive analysis and shows some exceptions to this idea. Nevertheless, while the more recent tree topologies of L and S segments are not analogous, they remain very similar.

recombination in CCHFV is a rare event and while it is difficult to estimate precise recombination rates, it is apparent that such rates are lower than those of point mutation. Nevertheless, an important consideration borne out of such work is that inferences about recombination events should only be entertained when molecular analysis have been constructed from complete segment sequence data. Additional consideration should also be given to the quality of published sequence data. A noteworthy example is provided by strains; (i) STV/HU29223 from European Russia (Stavropol) and (ii) Uzbek/TI10145 from Uzbekistan, which present some the of best evidence of genetic recombination in CCHFV as observed by phylogenetic incongruence [12]. However, this conclusion should be treated with caution as there is also evidence that the observed recombination may be an artifact [29].

5.3.2. Reassortment

RNA viruses with segmented genomes have the capacity to reassort their genomic segments into new genetically distinct viruses if the target cells are subject to dual infection. Indeed, this ability is believed to play a key role in the evolution, pathogenesis, and epidemiology of important pathogens such as influenza viruses, rotaviruses, and arthropod-borne orbiviruses [20, 25, 30]. Within the Bunyaviridae family as a whole, reassortment has been demonstrated for members of the genera *Orthobunyavirus* [2, 33, 42], *Phlebovirus* [40], *Hantavirus* [15, 23, 37], and *Tospovirus* [35], accordingly it is not surprising that segment reassortment in the *Nairovirus* genus has also been demonstrated [8, 14]. Here, evidence of reassortment in CCHFV is illustrated by a phylogenetic analysis of each strain or segment (Fig. 5-4). The phylogenetic groupings of S and L segments are consistent and show a correlation with the geography of parent strain isolation; however, the phylogenetic groupings of M segments are different. Distinct groups that were formed in S and L segments by Asia 1 and Asia 2 genotypes, for example, are not matched in the M segment phylogeny (Fig. 5-3). Although full-length sequence data is limited it is possible to ascertain that reassortment has taken place in the biogenesis of certain strains of CCHFV. For currently available data, the best evidence of reassortment is provided by the Matin strain isolated from Pakistan. If we consider groups for which there is full-length sequence data available on each segment (so that recombination events can be ruled out), then there appear to be strains with five types of S and L segment (Europe 1, Africa 2, Africa 3, Asia 1 [Middle East], and Asia 2 [Far East]) and five types of M segment (designated M1, M2, M3, M4, and M5). Even from the limited number of full-length sequences and the geographical location of virus isolations, it is possible to conceive that viruses of the Europe 1 lineage are composed of [S-Europe 1/L-Europe 1/M-4]; viruses of the Africa 2 lineage are [S-Africa 2/L-Africa 2/M-5]; viruses of the Africa 3 lineage are [S-Africa 3/L-Africa 3/M-2]; the majority of circulating viruses in the Middle East are composed of [S-Asia 1/L-Asia 1/M-2]; while in the Far East

viruses contain the combinations [S-Asia 2/L-Asia 2/M-2], [S-Asia 2/L-Asia 2/M-1], and [S-Asia 2/L Asia 2/M-3]. From the available information it is possible to infer that strain Matin [S-Asia 1/L-Asia 1/M-1] is the result of reassortment between a Middle Eastern virus [S-Asia 1/L-Asia 1/M-2], and a Far-Eastern virus [S-Asia 2/L Asia 2/M-1]. It is likely that other strains have also arisen by segment reassortment. Indeed, very recent work has provided more complete sequence data from a broader range of strains [8] exposing many more examples of segment reassortment. It is clear that the majority of these events involve M segment reassortment, however, L segment reassortment viruses are also observed, albeit at a lower frequency. The reassortment events involving strains from widely separated geographical locations, illustrates that coreplication enabled by the movement and mixing of viruses is quite common. It follows that there may be a global reservoir of CCHFV, with local subreservoirs supporting high levels of virus circulation and permitting frequent coinfection (in which migratory birds play a significant role in virus dispersion).

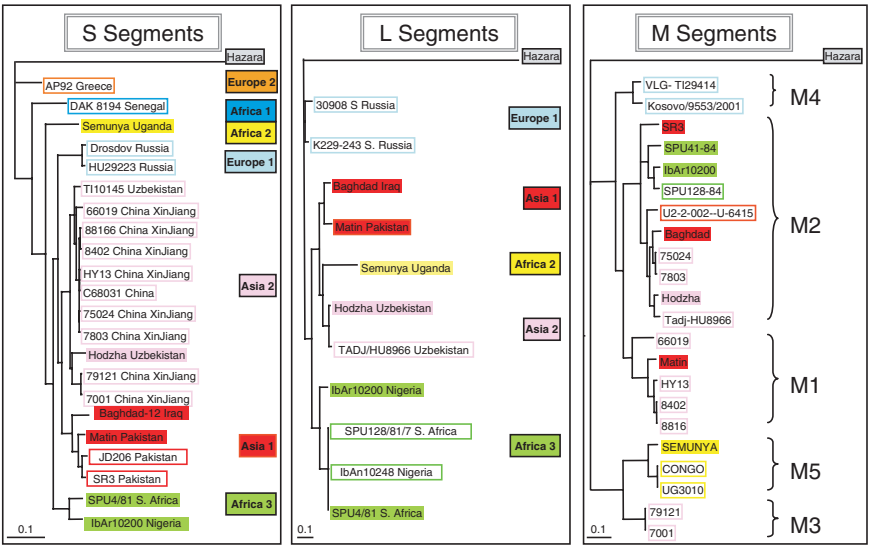

Fig. 5-4. Phylogenetic trees based on complete sequence show evidence of segment reassortment. Maximum likelihood phylogenetic groupings based on full-length sequence data and rooted against corresponding segments from Hazara virus as out groups. Filled boxes represent strains which are represented across all trees, colors correspond to grouping pattern described earlier. Comparison of trees shows that distinct geographical patterns formed by S and L segments are not maintained by M segments of the same strains. The best example of reassortment here is provided by the Matin strain. This was the first evidence of reassortment in CCHF viruses; more recent work has built on this notion and provided additional verification of RNA segment reassortment.

5.4. CONCLUSIONS

There is evidence that both recombination and reassortment are able to play roles in the evolution of CCHFV, in addition to general genetic drift. Obviously such genetic exchange requires coreplication of two or more strains within the same cell. The most likely coinfection environment where segment reassortment occurs is within ticks, where lasting virus infections persists for extended periods and superinfection with a second strain, during the strict requirement for blood meals, is very likely [28]. Given the currently available data on the low rate of recombination in CCHFV, and particularly the fact that the rate of recombination seems lower than general genetic drift, it appears that reassortment plays the most contributory role to the variability and flexibility of the CCHFV genome. Indeed, the low rate of recombination in negative-strand RNA viruses generally has led to suggestions that genome segmentation and reassortment have evolved to increase their fitness for survival [7, 31]. Specifically, while the high mutation rates of RNA viruses provide the raw material for evolutionary processes [21], mutations also introduce fitness compromising deleterious effects [6]. Genetic exchange through recombination or reassortment are recognized as adaptive methods of purging such effects [5, 27], thus in the practical absence of recombination, reassortment is able to take up the reins. In addition, reassortment enables alternative virus genotypes to be selected from a pool of functional segments.

The current evidence of reassortment in CCHFV [3, 8, 14] points principally to the exchange of M segments between viruses in mixed infections. In addition, the majority of data on L and S sequences show that in many cases these segments have evolved together as partners. Thus, in mixed virus infections where reassortment is a possibility, partner L and S segments have a propensity to end up in the same virus particle (due to the ostensibly strong interrelationships between the nuclear protein and RdRp) in order to constitute a viable new virus [3]. Some exceptions to this idea have been exposed by the availability of more sequences [8], and while it is clear that L and S segments trees are not analogous, they remain highly similar. M segments on the other hand seem to be more autonomous and could result in new virus phenotypes. Thus, as CCHFV are dispersed and introduced into new areas in which they are already endemic, the emergence of new CCHFV would principally be the result of M segment reassortment. Glycoprotein spikes encoded by M segments are well known for their ability to influence host range and cellular tropism [11, 41], furthermore, they are often associated with altered pathogenicity. These mechanisms, together with the likely contact and infection of new hosts, provide a foundation for the appearance of new CCHF disease and the emergence of new viruses [17].

These genomic studies highlight the importance of molecular surveillance to monitor and track the natural fluxes of virus and CCHF disease. A number of key questions can be asked in this context: For example, are certain viral genotypes more associated with severe disease? If so, are certain combinations of segments (or mutations) involved in the production of virulent strains? If there

is a strain basis to disease, is viral genetic diversity increasing so that new strains with novel biological properties (such increased virulence or transmission potential) might appear? A practical conclusion of the evolutionary opportunities open to this virus is that CCHF diagnostic approaches and potential vaccine research strategies should be tested against isolates from all parts of the world, regardless of the intended location of use.

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