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Understanding the molecular epidemiology of foot-and-mouth-disease virus

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ABSTRACT

The use of molecular epidemiology is an important tool in understanding and consequently controlling FMDV. In this review I will present basic information about the disease, needed to perform molecular epidemiology.

I will give a short introduction to the history and impact of foot-and-mouth disease, clinical picture, infection route, subclinical and persistent infections, general aspects of the transmission of FMDV, serotype-specific epidemiological characteristics, field epidemiology of FMDV, evolution and molecular epidemiology of FMDV. This is followed by two chapters describing the molecular epidemiology of foot-and-mouth disease in global surveillance and molecular epidemiology of foot-and-mouth disease in outbreak investigation.

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1. Introduction

The combination of molecular biology, epidemiology and population genetics in the science of molecular epidemiology is a powerful tool to develop control strategies for infectious disease.
This is especially significant for viral diseases, due to the rapid changes in the viral genome, regardless of it consists of DNA or RNA, which allows the drawing of conclusions about the origin of the virus lineage and/or the influence of immunologic and environmental pressures, like the introduction of vaccination campaigns or climate changes. The latter implies, in the authors opinion, that each researcher working with the molecular epidemiology of infectious disease should have knowledge about the actual situation in the field, which will certainly help to avoid the proposal of wrong hypotheses. It is equally important to be aware that most of the techniques used, e.g. phylogenetic analysis or selection pressure analysis, are by themselves based on hypotheses and models, with their intrinsic assumptions, biases and fuzziness. Furthermore, there should be a general awareness about the illusion that we, particularly with the derived nucleotide sequence information, have knowledge about the viral genetics. Beside the quasispecies structure of the RNA virus populations, there are manifold possibilities of virus–host interactions we still do not understand.

This review gives the minimum background information needed for the understanding of the molecular epidemiology of foot-and-mouth disease virus and presents the current developments within this field.

2. History and impact of foot-and-mouth disease

The first indication of foot-and-mouth disease (FMD) in the literature can be found in a book from the Italian physician Hieronymi Fracastorii (Wright, 1930), where he describes a cattle disease similar to the signs of FMD. Since then, several indications of FMD can be found in the literature (Thalmann and Nockler, 2001). From the second half of the 19th century, on the account of the increase of animal trade due to the development of new transport routes (O’Rourke and Williamson, 2002), the disease became more important and started to cause severe economic losses. In Germany, in 1897, a commission had been set up by the Prussian Ministry of Culture, following a request from the “Partei der Landwirte” of the Reichstag to set up measures of FMD control (Rott and Siddell, 1998). One year later, Friedrich Loeffler and Paul Frosch wrote in their report that the causative agent of FMD was neither a known bacterium nor a toxin but an “ultravisible, ultrafilterable substance” (Loeffler and Frosch, 1897), and described thereby the first animal virus, foot-and-mouth disease virus (FMDV). In 1910, the first FMD research institute was built on the island of Riems in Germany, followed by other research institutes in Europe, e.g. in Pirbright, United Kingdom in 1925 and on Lindholm island, Denmark in 1926. The major milestones in these early years of FMD research were the introduction of guinea pigs as research animals (Waldmann and Pape, 1920) and the first steps towards the development of an FMD vaccine in 1937 (Waldmann et al., 1937).

After the Second World War, FMD vaccines became available in all European countries and the disease was controlled by systematic vaccination until the end of 1991 when the European Union adopted a non-vaccination policy (Directive 85/511/CEE du conseil du 18 novembre 1985 établissant des mesures communautaires de lutte contre la fièvre aphteuse). Since then, several minor outbreaks have been detected in Europe, e.g. in Denmark in 1982/1983 (Christensen et al., 2005) and in Italy in 1993 (Nunez et al., 2006). For the latter it has been shown that the causative virus was closely related to viruses that circulated previously in the Middle East (Nunez et al., 2006). In 2001 a large FMD outbreak started in the United Kingdom (Alexandersen et al., 2003a) and spread to France, Ireland and The Netherlands. The losses to agriculture in the United Kingdom alone were about £3.1 billion (Thompson et al., 2002). The causative FMDV here showed phylogenetic similarity to the virus from the outbreak in Italy in 1993 and was closely related to viruses that circulated previously in Asia and Middle East (Knowles et al., 2005).

3. The disease

3.1. The virus

Foot-and-mouth disease is an acute, highly contagious viral disease, which has a great potential for causing severe economic losses in susceptible cloven-hoofed animals (Axelhansden and Mowat, 2005).

The causative agent of FMD is the foot-and-mouth disease virus (FMDV), a small positive sense ssRNA virus (approx. 8.3 kb) which belongs to the Aphthovirus genus of the family Picornaviridae (Belsham, 1993). FMDV replicates through negative strand RNA which was recently used in quantitative strand specific real time RT-PCR as a tool to determine strain virulence and replication patterns (Horsington and Zhang, 2007). The high genetic and phenotypic variability of FMDV is reflected in the existence of seven serotypes: O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3 and further numerous variants and lineages, described as topotypes (Knowles and Samuel, 2003). The antigenic diversity within the serotypes is crucial to consider when selecting vaccine strains, as cross-reactivity may be variable (Kitching, 2005). The virus capsid is composed of 60 copies of each of the four structural polypeptides designated VP1 to VP4. A region in the G-H loop (around residues 140–160) of the VP1 protein exposed on the surface of the viral capsid was determined to be the main antigenic site recognized by neutralizing antibodies (Bittle et al., 1982). The natural FMDV infection is initiated when the RGD motif within the G-H loop binds to certain cellular integrin receptors, which are expressed in the epithelial cells targeted during the acute phase of infection (Alexandersen et al., 2003b; Monaghan et al., 2005). In the process of cell culture adaptation, FMDV acquire the ability to use other receptors like glycosaminoglycans (GAGs), which do not require the RGD motif. The host specificity to natural FMDV infection is thought to depend on FMDV binding to specific integrins although there are some other, yet undiscovered, mechanisms which determine FMDV susceptibility.

3.2. Clinical picture

Animals that can be affected include cattle, swine, sheep, goats, buffaloes and wild ruminants (Alexandersen and Mowat, 2005). FMD in Bovinae is characterized by fever (often above 40 °C), excessive salivation, lameness, depression and decreased milk production. The mucosa of the lips, dorsum of the tongue, and the dental plate are most severely involved. The mortality rate is low and mostly young animals are affected due to myocardial necrosis (Alexandersen et al., 2003b; Gulbahar et al., 2007; Kitching, 2002).

FMD in pigs primarily affects the feet. It is dominated by rather painful formation of vesicles in the epidermis of the feet (coronary band, interdigital clefts, bulbs), associated with severe lameness. Complications are seen, such as detachment of the hoof and secondary infection of disrupted aphthae (fluid-filled blisters), which may cause purulent arthritis of the pedal joint (Kitching and Alexandersen, 2002).

Clinical signs of FMD in sheep and goats are less severe; often only lameness through aphthae and inflammation at the cloves (Alexandersen and Mowat, 2005).
3.3. Infection route

The mechanism of spread of FMDV is primarily in the form of either aerosolized droplets, saliva, or through indirect contact by personnel or contaminated surfaces and then subsequently back to the respiratory tract (Alexandersen and Mowat, 2005). However, infection can also occur through lesion of the skin or mucous membranes, but this is a very inefficient entering route, unless abrasions or cuts are present (Alexandersen et al., 2003a; Donaldson et al., 1987). The site of penetration is influenced by droplet size, since only the smallest inhaled droplets reaching the alveoli of the lungs. Initial infection occurs predominantly at the epithelial surface of the soft palate and adjacent nasopharynx, with subsequent spread of virus through regional lymph nodes (Alexandersen et al., 2003a; Donaldson et al., 1987). The virus can be detected in the oral cavity by real time RT-PCR 1-3 days before the onset of the viraemia (Alexandersen et al., 2003b).

3.4. Subclinical and persistent infections

Inapparent infections of FMDV can be divided into two types. First, those animals that become infected and spread the virus without showing clinical signs and second, those animals in which the virus persists after recovering from FMD with clinical signs (Sutmoller and Casas, 2002).

FMDV persistently infected animals are defined as those being virus positive for a minimum of 28 days (Sutmoller et al., 1968) and usually are referred to as carriers. Persistently infected animals show only low-level excretion of FMDV from the pharynx of ruminants, for periods that are species and virus lineage associated (Salt, 1993). The maximum duration of the persistent infection ranges from 4 months in goats to 5 years in African Buffaloes (Syncerus caffer) (Alexandersen et al., 2002b). FMDV persistence in pigs could not be demonstrated and there is no information about persistence in Asian Buffaloes (Bubalus bubalis) (Alexandersen et al., 2002b).

Transmission of FMDV from carrier animals to susceptible hosts, under field conditions, has so far only been shown for African Buffaloes to cattle and impala (Aepyceros melampus) (Bastos et al., 2000; Dawe et al., 1994). However, under experimental conditions, saliva obtained from carrier animals injected into cattle and pigs has caused infection, although there is no information as to whether this also can happen under field conditions (Alexandersen et al., 2002b), or with closely related species as the Asian Buffaloes.

Unlike persistently infected animals, subclinically infected animals, i.e. those animals not developing obvious clinical signs, may be highly contagious (Gibbens et al., 2001; Holzhauer et al., 2001). Beside the immune status of the host, also the involved FMDV lineage plays a role in the subclinical course of the disease (Bouma and Dekker, 2002). If an outbreak is caused by vaccine strains, which shows reduced virulence (Sa-Carvalho et al., 1997), subclinical infections can also be found. For example, during the FMD outbreak in 2007 in the United Kingdom, which was caused by a vaccine strain (Enserink, 2007), only few of the FMDV infected animals showed clinical signs.

Subclinical infection can also be produced within vaccinated ruminant herds, with a high FMDV challenge in those animals with high immunity or a low challenge in animals with low vaccine titre (Hutber et al., 1999; Yadin et al., 2007). The possibility of those animals becoming carriers has, to the author’s knowledge, not been examined. Nevertheless, it is likely that such animals may play an important role in the maintenance of FMD in endemic countries (Kitching, 2005).

4. Epidemiology of FMD

Even though there are some common features in the spread of FMD, each of the seven serotypes, or even their variations (topotypes), has a different way of transmission, clinical appearance and species tropism (Kitching, 2005).

4.1. General aspects of the transmission of FMDV

Large amounts of the virus are found in all body secretions and excretions over a relatively long time and can be carried in contaminated feed (Donaldson, 1997; Wijnker et al., 2007), on the tires of vehicles, and on the shoes and clothes of people (Alexandersen et al., 2003a; Grubman and Baxt, 2004).

The transmission via aerosols can also occur, depending on weather conditions and the characteristics of virus survival and dissemination (Alexandersen et al., 2002a; Alexandersen and Donaldson, 2002; Donaldson, 1972; Donaldson et al., 1987; Sellers and Gloster, 2008). The host plays a major role for this transmission route; cattle, sheep and goats may exhale up to 5.2 log10 TCID50 of virus per day, but pigs which produce up to 8.6 log10 TCID50 per day represent a significantly greater source of airborne FMDV infections than infected ruminants (Alexandersen et al., 2002a, c; Alexandersen and Donaldson, 2002).

However, the most common route of introduction of FMD into a country free of FMD has been the illegal use of contaminated swill feed (Hartnett et al., 2007). The reduction in pH of meat at post mortem due to accumulation of lactic acid is usually sufficient to kill FMDV, but there is no change of pH in the glands and bone marrow, and the virus will persist between −20 °C and 4 °C (Kitching, 2005).

4.2. Serotype-specific epidemiological characteristics

4.2.1. Serotype O

The pandemic strain of FMDV serotype O is a major threat to Europe. It is the most prevalent serotype worldwide, although there is no precise genetically explanation for this higher prevalence (Mason et al., 2003). Some lineages of the type O are restricted in their host range to suidea (Cheng et al., 2006), whereas others, like the Panasia strain, are not restricted to a specific host (Knowles et al., 2005).

4.2.2. Serotype A

There is a great antigenic diversity between the members of this serotype, and there is also often no cross-protection between them (Bronsvoort et al., 2004; Islam et al., 2001; Klein et al., 2006; Konig et al., 2007; Matton et al., 2004; Muthuchelvan et al., 2001; Tosh et al., 2002a). There is also evidence that recombination in serotype A occurs much more than in the other serotypes (Carrillo et al., 2005; Jackson et al., 2007; Simmonds, 2006). However, it is still unclear why so many new lineages of serotype A regularly appear and disappear (Kitching, 2005).

4.2.3. Serotype Asia 1

The members of this serotype seem to be the less virulent and are usually restricted to Asia (Biswas et al., 2006; Chang et al., 2007; Mohapatra et al., 2002, 2004; Sanyal et al., 2003; Sanyal et al., 2004a,b). However, in recent years an increase of Asia 1 caused outbreaks in Asia has been reported, as well as further spread of viruses of this type to Turkey and Greece in 2000 (Valarcher et al., 2005).

4.2.4. Serotypes SAT 1, 2, 3

The SAT (Southern African Territories) serotypes are usually found in Africa, but there have also been sporadic outbreaks in Saudi Arabia and Kuwait in 2000 (Aidaros, 2002). There is a higher
sequence variation within each of the three SAT types than in serotype O (Bastos et al., 2001, 2003a).

4.2.5. Serotype C

During the last 20 years there have been no major outbreaks reported of this serotype (only sporadically in South America, East Africa and Pakistan between 2000 and 2006 (OIE, 2008)), and there is no explanation for the apparent disappearance. However, vaccines containing serotype C are still in use in some countries, comprising a risk of vaccine induced serotype C outbreaks (Kitching, 2005).

4.3. Field epidemiology of FMDV

Generally, field epidemiology is the practice of epidemiology in response to problems of a magnitude significant enough to require a rapid or immediate action (Goodman and Buehler, 2002), i.e. it is required to consider when the obtained data are sufficient to make practical recommendations, rather than to ask what additional, more academic, questions might be answered by the data.

Rapid field epidemiological action in case of FMD outbreaks is required to trace potential sources of infection to limit the spread of the disease. For example, during the 2001 outbreak of FMD in the United Kingdom (Gibbens et al., 2001; Gibbens and Wilesmith, 2002), all animal, vehicle and personnel movements through animal markets were traced and investigations of infected farms performed, in order to identify potential contact farms and to subsequently apply the appropriate control measures.

Disease surveillance, on the other hand, can be considered as immediate field epidemiological action. According to the Office International des Épizooties (OIE), surveillance means “the investigation of a given population or subpopulation to detect the presence of a pathogenic agent or disease; the frequency and type of surveillance will be determined by the epidemiology of the pathogenic agent or disease, and the desired outputs” (Thiermann, 2008).

FMD surveillance usually has the following objectives: (a) confirmation of absence of disease (DEFRA, 2008) (b) development and/or evaluation of FMD control programs in endemic countries (Chung et al., 2002) or (c) to detect the occurrence of new FMDV lineages (del Rio Vilas et al., 2006).

Sources of data for FMD surveillance can be either purely laboratory results of biological samples collected or participatory information obtained from the farmers or a combination of both. Key information, beside laboratory data like PCR results, sequence analysis and serology, are in the authors opinion:

1. clinical signs
2. vaccination status and date
3. vaccine used
4. species and age of the animal
5. beginning of outbreak (month/year)
6. sampling date and place
7. outbreak history
8. likely source of outbreak
9. morbidity rate
10. environmental information

Though it should be a strived aim to obtain all information, it has to be accepted that this is sometimes, due to various reasons, not possible. This is especially true for rural areas and less developed countries.

4.4. Evolution and molecular epidemiology of FMDV

FMDV shares its antigenic variability with many other picornaviruses. However, the extent of this variation is not equal in all picornaviruses, e.g. there are three polio serotypes and more than hundred rhinovirus serotypes (Domingo et al., 1996; Duarte et al., 1994).

There are three major forces driving the molecular evolution of all RNA viruses:

1. Spontaneous mutation: RNA virus encoded RNA-dependent-RNA-polymerase lacks the 3′−5′ exonuclease activity (proof-reading activity), leading to a very high mutation rate (approx. one mutation per genome per replication (Drake, 1993). These viruses have adapted their genome size to the so called error threshold (maximum mutation rate, tolerable for a genome size) to avoid fitness loss (Biebricher and Eigen, 2005, 2006; Bull et al., 2005; Escarmis et al., 2006; Gonzalez-Lopez et al., 2005; Sole et al., 2006).

2. The phenomenon of spontaneous mutation leads to a statistical effect called genetic drift. For an extremely large, highly diverse population, the ratio of highly fit to less fit genotypes may be very small. During a transmission event, only a few particles might be transmitted. Those genotypes do not represent the full genetic or fitness distribution of the original population and therefore often do not include the highly fit genotypes, because of their comparatively small numbers. The outcome is a new population established in a similar environment but with members of a small, less fit (on average) class of viruses (Knipe et al., 2006). FMDV is thought to evolve mainly through genetic drift, due to the high error-prone nature of its RNA polymerase (Domingo et al., 2005a-c). The FMDV RNA transcription error frequencies are estimated to vary between 10−3 and 10−5 nucleotide misincorporations per site per replication cycle (Haydon et al., 2004).

3. Recombination: Recombination describes a process in which the fragments of nucleic acid sequences from two genotypically different parental viruses are exchanged so that the progeny contain sequences derived from both parental viruses. Inter alia picornaviruses, coronaviruses and togaviruses display efficient recombination, which probably occurs during replication via “copy choice” (Enami et al., 1989; Jarvis and Kirkegaard, 1992; Kirkegaard and Baltimore, 1986; Kuge et al., 1989; Meier et al., 1984). Recombination seems to play an important role in FMDV evolution and occurs mainly in the gene regions coding for the non-structural proteins (Carrillo et al., 2005; Domingo et al., 2003; Jackson et al., 2007; Klein et al., 2007; Lee et al., 2009), but also sometimes in the gene regions coding for the structural proteins (Haydon et al., 2004; Haydon and Woolhouse, 1998; Tosh et al., 2002b). Changes in the non-structural proteins may potentially modify the virulence of the virus (Klein et al., 2007). The evolution of the structural proteins, especially VP1, which plays a major role in virus entry, seems to be mainly shaped by genetic drift (Domingo et al., 2005b,c; Domingo, 2006).

4. Migration: A population acquires new genetic information primarily through the immigration of individuals from surrounding populations (gene flow or genetically effective migration). This also applies within a single infected host, where different virus variants sometimes can be isolated from different tissues, reflecting the adaptation of quasispecies (Domingo et al., 2005a,b,c, 2003). This means that single host populations will compete with new, different virus genotypes for the limited number of susceptible host cells; which may lead to an increase in virulence. The virus strain with the higher basic reproductive rate will overtake the less-virulent, but finally end in a stage of equilibrium (Alexopoloulou and Dourakis, 2003; Mao et al., 2001; Miralles et al., 1999; Sítia et al., 2001). Evolution experiments in vitro (Domingo et al., 2002; Domingo et al., 2005a,b,c; Martinez et al., 1992, 1991, 1997; Villaverde et al., 1991) demonstrate FMDV’s capability of survival, despite...
accumulation of mutations upon repeated bottleneck events (where population size is greatly reduced by a catastrophe). Therefore, a range of mutation rate must have evolved to ensure a continuous heterogeneity to find population subsets that can invade cells after bottleneck events and staying below the error-threshold. This can be exemplified by the RGDLXXL motif in the highly variable GH loop of VP1. This motif is responsible for receptor recognition (ovb6) and is invariant in most field isolates, even after bottleneck events like the introduction of vaccination (Monaghan et al., 2005).

A consequence of the high evolutionary rate of FMDV is that strains of virus with a common origin quickly diverge in sequence as they replicate and spread into new areas. Therefore, the greater the number of differences, the greater the distance between the strains in space and time. Conversely, strains with homologous sequences must have had a common, more recent, origin (Bastos et al., 2003b; Bronsvoort et al., 2004; Cottam et al., 2006; Klein et al., 2006; Knowles and Samuel, 2003; Konig et al., 2007; Malirat et al., 2007; Martin et al., 2006; Sahle et al., 2004; Sangare et al., 2004).

This usage of molecular biological methods for the study of the distribution and determinants of infectious disease is the subject of molecular epidemiology (Thompson, 2000).

It is however, important to recognize, that for the study of the molecular epidemiology of a certain virus, like FMDV, the knowledge of the molecular evolution is equally important, thus knowing the (genetic) factors which have shaped the evolution of the pathogens will improve the development of vaccines, as well as control strategies (Charonsirisuthigul et al., 2007; de Freitas et al., 2007; Holmes, 2007; Holmes and Drummond, 2007).

Unlike taxonomy, which describes how closely related organisms are based on evolutionary grouping by ancestral traits, molecular epidemiology correlates phylogenetic information with biological variables and classical epidemiological information. Furthermore, it develops hypotheses about evolutionary changes considering different selection pressures, inter alia caused by the host’s immune system and new environmental challenges.

Despite its potential, it is important to stress the limits of molecular epidemiology:

- Phylogenies are based on simplified models.
- The available sequences are most likely biased, and very often do not represent a complete spatial and temporal random sample.
- The majority of sequence analysis for RNA viruses is biased, because they are, despite the quasispecies structure of RNA viruses, based on a single consensus sequence, and thus not mirroring all intrahost viral populations.

In addition, two types of probability error must be considered in molecular epidemiology (Thompson, 2000):

- Type 1 probability error: a subtyping assignment claims epidemiologic relationship of strains when there is none.
- Type 2 probability error: a subtyping assignment concludes that there is no epidemiological relationship when in actuality there is.

Generally, phylogenetic analysis is a statistical inference of relationship, using a probability model of nucleotide and amino-acid substitution (evolutionary model). Assuming the wrong evolutionary model can affect the outcome of a phylogenetic analysis, e.g. by incorrectly estimating tree topology (Bruno and Halpern, 1999; Penny et al., 1994), influencing branch length estimation (Posada, 2001; Posada and Crandall, 2001), and biasing statistical support values (Buckley and Cunningham, 2002). The correct evolutionary model becomes a powerful tool when, despite its simplified assumptions, it can fit the data and make accurate predictions (minimizing probability error 1 and 2). The correct model can help to understand the linkage among the spatial, temporal, and genetic processes taking place during virus transmission in a changed environment, thus evolutionary events occur early during the initial expansion and advantageous changes become fixed in the genetic population structure.

By using hierarchical likelihood ratio tests (Goldman, 1993; Posada and Crandall, 2001; Rzhetsky and Nei, 1995) or Akaike information criterion (AIC) tests (Saffron et al., 2006; Seghouane and Amari, 2007), it is possible to identify the most appropriate model for the sequence analysis, through a hierarchical set of evolutionary models (Arndt, 2007; Buckley and Cunningham, 2002; Fan et al., 2007; Lemey et al., 2005a,b; Lemey and Vandamme, 2005; Lunter and Hein, 2004; Salemi et al., 1999; Van Brussel et al., 1999; Vandamme et al., 2000, 1998; Vercauteren and Vandamme, 2006; Yang, 2002).

4.5. Molecular epidemiology of foot-and-mouth disease in global surveillance

FMD endemic areas in the world are high-risk zones for introducing FMD to countries free of the disease and for the origin of new FMDV lineages. Therefore, a continuing surveillance of FMDV is needed for the early recognition and understanding of emerging risks or changes in the global FMD situation. The detection and characterization of new FMDV lineages and control of possible conformation changes of circulating immuno-relevant epitopes, is of special interest, as it may indicate that a change in the current used vaccine strains is needed.

One recent example of a newly occurred FMDV lineage is A/IRN/2005.

During 2005, a new FMDV A subtype, A/IRN/2005, spread throughout Iran and moved westwards into Saudi Arabia, Turkey and in 2007 reached Jordan (ProMED, 2007). In 2006 this subtype was also detected in Pakistan (Klein et al., 2007). This particular FMDV subtype has proven to be highly virulent and has caused severe disease in all ages of cattle (EUFMD, 2007).

Serum neutralization assays demonstrated a closer relationship to A22 than to other serotype A subtypes (Paton, 2006) and the World Reference Laboratory as well as the FAO European Commission for the control of FMD recommend, in the absence of an homologous vaccine strain, the use of the widely available A22 Iraq strain as vaccine (Paton et al., 2006). It has been hypothesized that the A/IRN/2005 sublineage has undergone two different paths of evolution for the structural and non-structural genome regions (Klein et al., 2007).

The structural genome regions may have had their evolutionary starting point in the A22 sublineage. It can be assumed that, due to the quasispecies structure of FMDV populations and the error-prone replication process, advantageous mutations in a changed environment have been fixed and have lead to the occurrence of the new A/IRN/2005 sublineage.

Together with this mechanism, recombination within the non-structural genome regions, potentially modifying the virulence of the virus, may be involved in the success of this new sublineage. The possible origin of this recombinant virus may be a co-infection with Asia1 and a serotype A precursor of the A/IRN/2005 sublineage within Asian Buffaloes.

Another example of a new FMDV lineage is the currently described PanAsia II lineage described so far in Bhutan, Nepal, Malaysia and Pakistan, which seems to evolve away from the "old"
PanAsia lineage (Klein et al., 2008), leading possible to a decrease in the used O1/Manisa vaccine efficiency.

A special situation is the Office International des Epizooties (OIE) status of “FMD-free countries where vaccination is practiced” in. In these countries FMDV is not eradicated and still circulates in the susceptible population. This leads to a permanent bottleneck situation for the virus, and the emergence of vaccine escape mutants is likely, as seen for the serotype A outbreaks in South America during the last years (Konig et al., 2007; Mattion et al., 2004; Perez et al., 2008). However, molecular epidemiology may also be helpful to detect low vaccine coverage (Klein et al., 2006; Malirat et al., 2007).

4.6. Molecular epidemiology of foot-and-mouth disease in outbreak investigation

In an ongoing outbreak situation molecular epidemiology is a useful tool to track virus and to consequently stop the transmission. But also the post-outbreak investigation is important with regard to the claim for damages.

The best examples for this particular field are given by work done on the United Kingdom outbreaks in 2001 and 2007. In 2001, Great Britain experienced an epidemic of FMDV caused by the Pan Asia O strain of the virus (Alexandersen et al., 2003a; Cottam et al., 2006). The disease spread rapidly and widely throughout the country and further to the Republic of Ireland, France and the Netherlands. Due to the size of the outbreak it was not possible to track the transmission route and many cases were simply attributed to “local spread.” However, many tissue samples have been collected during the seven-month lasting outbreak and it was possible to study the microevolution of the virus and thereby recover particular transmission pathways of the acting FMDV lineage (Cottam et al., 2006).

On August 3, 2007 a new outbreak of FMD was confirmed in Surrey, United Kingdom. The causing FMDV lineage was identified as OFS 1860/UK/67, a virus isolated not in circulation in animals since 1967. However, this lineage was used at the nearby Pirbright laboratory site, which houses separate units of the Institute for Animal Health and Merial Animal Health Ltd and it was soon clear that this was the origin of the virus. Thus, the question arose from which unit the virus escaped. Full length genome sequencing of the virus isolates obtained from infected animals have shown typical amino acid adaptations for FMDV propagated for several passages in cell culture systems, as used in vaccine production, and Merial Animal Health Ltd. was identified as the source of the outbreak (Cottam et al., 2008).

5. Conclusion

Molecular epidemiology of FMD is a powerful tool if applied correctly with regard to the statistical methods and when supplied with classical epidemiological information. It is important to stress, that taxonomical relationship deduction alone is not enough to understand the dynamics of FMDV. Furthermore, it should be a strived aim to develop bioinformatics tools, which are able to handle the complex virus–host interactions during infection, as for example seen in the action of microRNA’s during viral infection.

Although active surveillance of FMD has decreased dramatically the incidence of the disease in Europe, the fear of new outbreaks still exists and, as the example of last outbreak in UK in 2007 demonstrates, it is not unreasonable (Cottam et al., 2008). Moreover, FMD remains endemic in developing countries of Asia, South America, and Africa, where new FMDV strains are most likely to evolve and where the only solution for the effective eradication/reduction is the application of measures based on the molecular and genetic characterization of the virus (Bronsvoort et al., 2004; Klein et al., 2007, 2006, 2008). Introduction of new fast methods such as real time RT-PCR into FMD diagnostics together with viral sequence analysis allows the study of viral biodiversity and evolution and thereby to track transmission events and sources, as well as to assure vaccine coverage of corresponding field FMDV lineages.

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