Clinical variation in foot and mouth disease: sheep and goats

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Summary
Foot and mouth disease (FMD) in adult sheep and goats is frequently mild or unapparent, but can cause high mortality in young animals. The recent outbreak of FMD in the United Kingdom has highlighted the importance of sheep in the epidemiology of the disease, although there have been numerous examples in the past where small ruminants have been responsible for the introduction of FMD into previously disease-free countries. The difficulty in making a clinical diagnosis should encourage the development of more rapid screening tests to assist in future control programmes.

Keywords
Control – Diagnosis – Foot and mouth disease – Goats – Sheep.

Introduction
The outbreak of foot and mouth disease (FMD) that occurred in the United Kingdom (UK) in 2001 has provided a good example of how difficult it is to make a clinical diagnosis of the disease in sheep (3). The outbreak predominantly affected the sheep population, and the policy adopted by the UK Government of slaughtering infected animals within 24 h of suspicion of disease put intolerable pressure on field veterinarians to make a clinical diagnosis on the evidence of lesions indistinguishable from those produced by a variety of other causes. Where samples were collected, over 50% of the flocks slaughtered were not confirmed as positive for FMD virus, viral antigen or genome by the laboratory.

The susceptibility of sheep and goats to FMD can vary with the breed of animal and strain of virus. For example, the Hong Kong topotype of serotype O FMD virus has only once been isolated from a species other than the pig, and although never specifically used to infect sheep or goats, the inability of this strain to grow in experimentally inoculated cattle would suggest that it would also not naturally infect small ruminant hosts either (9). Conversely, there are strains of serotype O FMD virus circulating in the Middle East where sheep and goats form the majority of the susceptible population, which appear very well adapted to small ruminants, and there are many examples of FMD being carried into countries previously disease-free by the movement of infected sheep and goats. In 1983, FMD spread from Spain into Morocco with infected sheep. In 1989, FMD was introduced to Tunisia by infected sheep, and was originally misdiagnosed as bluetongue, because of the signs of lameness; not until the disease had spread to cattle was it recognised as FMD, by which time it had already been carried into neighbouring countries. In 1994, FMD was transported by illegally imported sheep from Turkey onto the Greek island of Lesbos, from where it was taken onto the mainland, infection was certainly present on Lesbos from May, but was not positively diagnosed until August, which led to extensive spread of the disease in eastern Greece. There are other examples of sheep carrying virus from Turkey to Greece and Bulgaria, in 1994 and 1993, respectively. Saudi Arabia banned the importation of cattle from India in the early 1990s because of the threat of rinderpest, but continued to import sheep and goats. Nucleotide sequencing of isolates of serotypes O, A and Asia 1 collected in Saudi Arabia during this period showed them to be identical to those circulating previously in India (Figs 1, 2 and 3). In 1994, an isolate later to be known as the pan-Asian topotype was imported into Saudi Arabia by infected livestock, probably from India; the disease then spread throughout the Middle East, eventually causing the 1996 outbreaks in Bulgaria and Greece. Today this strain is still present in the region, and has replaced all other strains of serotype O. A close relative of this strain, but probably imported from South-East Asia, caused the 2001 UK outbreak (20).

Serotype O FMD virus has been recovered from over 90% of the positive samples from sheep submitted to the World Reference Laboratory for FMD, Pirbright, UK. Asia 1 serotype
has also been isolated from goat samples submitted from Bangladesh and goats imported from this country were responsible for an outbreak of Asia 1 in Kuwait. Isolation of other serotypes is rare, but does not necessarily indicate that infection of small ruminants with these serotypes does not occur; for example, Kuwait reported isolating South African Territories (SAT 2) virus from sheep during the incursion of FMD into Saudi Arabia during 2000. However, even in East Africa, where outbreaks due to serotypes O, A, C, SAT 1 and 2 are common, predominantly serotype O virus was identified in clinically affected sheep and goats.

**Transmission**

As is the case with other ruminants, sheep and goats are highly susceptible to infection with FMD virus by the aerosol route, with as little as 20 TCID<sub>50</sub> (tissue culture infectious doses) being sufficient for infection. Aerosol production by infected pigs can be as high as log<sub>10</sub> 8.6 TCID<sub>50</sub> per day, theoretically sufficient to infect over 20 million sheep. Aerosol production by infected sheep, however, is considerably less, and whereas there are reports of airborne virus spreading from pigs over 250 km to infect cattle (France to England in 1981) (5), aerosol
transmission from infected sheep is unlikely to occur over distances greater than 100 metres (7, 26). Sheep are also less likely to become infected by airborne virus than cattle because of their lower respiratory volume. Sheep and goats are probably most often infected by direct contact with infected animals. The virus may infect sheep and goats through abrasions on the skin or mucous membranes, through contaminated food, as well as by the respiratory route. During the FMD outbreak that took place in the UK in 2001, disease spread was reported to occur frequently by mechanical carriage of virus between flocks by humans or vehicles.

Sheep-to-sheep spread by contact appears to be restricted, to the extent that the rate of transmission within an affected flock is lower than that observed in infected pig or cattle herds. A good example of this phenomenon is illustrated by the outbreak of FMD that took place in Greece during 1994. Serological investigations showed that in many of the affected flocks not all individuals had sero-converted to the virus, indicating that the virus had not disseminated sufficiently to infect entire flocks. In some flocks affected towards the end of the outbreak, only 20% of the sheep were sero-positive (21). There was suspicion, but little firm evidence, that the 1993 outbreak of FMD in Italy had also failed to maintain itself in affected sheep flocks. Similarly, evidence from the recent UK epidemic shows considerable variation in the level of intra-flock infection rates. On one farm visited, only 5% of 237 sheep that were blood tested were sero-positive, and 3% were virus-positive, whereas 91% of the 75 cattle present were clinically affected. On a second farm tested, 8% of 148 sheep were sero-positive, 24% virus-positive, whilst 98 of 100 cattle showed clinical signs (1). A recent study by Hughes (14) has provided supportive evidence for the observed difference between the dynamics of FMD transmission in sheep populations as compared with cattle and pigs. The study showed that, using the 1994 Greek outbreak strain, there was significant reduction in the level of infection and estimated transmission rates over time during serial passage through groups of sheep. These results infer that some, possibly most, strains of FMD virus may die out if they are restricted to sheep. Infection of cattle or pigs may be sufficient to increase the level of circulating virus and consequently the probability of transmission of infection to...
in-contact sheep, thereby re-establishing the disease. This hypothesis requires further investigation using other strains of FMD virus. The limited transmission that may occur within closed sheep populations is also often masked by the lack of clinical signs (see above).

The probability of transmission of FMD virus from infected sheep is highest during the viraemic phase and peaks at or just before the appearance of clinical signs. This period correlates well with the period of virus excretion (4) which ends at the point of sero-conversion (2). Levels of virus excretion are strain-specific (4).

**Clinical signs**

The incubation period in sheep following infection with FMD virus is usually between three and eight days (19), but can be as short as 24 h following experimental inoculation, or as long as twelve days, depending on the susceptibility of the sheep, the dose of virus and the route of infection. The duration of viraemia is between one and five days. Hughes et al. (15) were unable to detect viraemia in 8% of sheep that sero-converted in a series of transmission experiments. Clinical signs appear up to three days after the start of viraemia, approximately seven days after exposure to contact infection – giving the period between exposure to infection and the onset of viraemia as between three and seven days (13, 15). Vesicular disease may fail to develop in approximately 25% of infected sheep (12, 15), a further 20% may develop only a single observable lesion. In 79 sheep infected with the 1994 Greek strain, lesions in those animals that developed vesicular disease were visible for less than three days (15).

Lameness is usually the first indication of FMD in sheep and goats (Fig. 4). An affected animal develops fever, is reluctant to walk, and may separate itself from the rest of the flock. In the field situation, lameness due to other causes may already be present and may conceal the presence of FMD. Vesicles may develop in the interdigital cleft, on the heel bulbs and on the coronary band, but they usually rupture rapidly (Fig. 5) and their appearance may be hidden by the coexisting presence of foot rot. Hair or wool may have to be deflected upwards to render lesions on the coronary band visible, but, in sheep, lesions can easily be confused with the coronitis seen with bluetongue. Vesicles also form in the mouth, but they rupture easily and are usually only seen as shallow erosions, most commonly on the dental pad, adjacent to the incisors (Fig. 6), but also on the tongue, hard palate, lips and gums. In one study, of 57 sheep with foot lesions due to FMD, only 4 had mouth lesions and only 2 of these had mouth lesions without foot lesions (15). Vesicles may also be observed on the teats, particularly of milking sheep and goats and rarely on the vulva.

Fig. 4 Lameness is usually the first sign of foot and mouth disease in sheep

Fig. 5 Coronary band lesions due to foot and mouth disease are usually mild, and difficult to see

Fig. 6 A ruptured vesicle on the dental pad of a sheep infected with foot and mouth disease virus
and prepuce. Affected rams are unwilling to work, and lactating animals suffer a temporary loss of milk yield. Secondary infections may cause mastitis and persistent lameness and the compromised epithelium can predispose to rapid transmission of other viral infections such as sheep and goat pox and peste des petits ruminants. Uncomplicated infections with FMD virus are usually followed by rapid recovery in the adult animal.

The clinical disease in young lambs and kids is characterised by death without the appearance of vesicles, due to heart failure. Affected flocks may lose up to 90% of the lamb crop, and the image of large numbers of lambs falling down dead when stressed, as may occur when a stranger walks into the flock, is dramatic.

Pathology

Local replication of FMD virus occurs at the site of entry, in the mucosa of the respiratory tract or at a skin or mucous membrane abrasion. The virus then spreads throughout the body favouring epithelial tissue in the adult and heart muscle in the juvenile. Lytic changes in the cells of the stratum spinosum and consequent oedema give rise to the characteristic vesicles and accumulation of granulocytes, and in the developing myocardium of young animals, to a lympho-histiocytic myocarditis (6). Depending on the speed with which the virus overpowers the function of the heart, gross lesions may be apparent on post mortem as diffuse grey spots or more organised ‘tiger’ stripes, particularly in the left ventricle and interventricular septum.

In adult animals, recovery from FMD uncomplicated by secondary pathogens, is usually rapid, but the virus will persist in the tonsillar tissue for up to nine weeks in sheep and for a shorter period in goats.

Diagnosis

Clinical diagnosis of FMD in sheep and goats is difficult because of the usually transient appearance of lesions and their similarity to those caused by other common diseases of small ruminants. Laboratory confirmation of a diagnosis of FMD is therefore essential. Samples of vesicle epithelium, if available, or heart muscle from a dead lamb or kid, should be collected into 50% phosphate/glycerol, buffered to pH 7.4-7.6, and submitted together with whole and clotted blood to a laboratory equipped to handle the diagnosis and with the necessary disease-secure facilities. This will either be a designated government laboratory or the regional FMD reference laboratory. Alternatively, samples can be sent to the World Reference Laboratory for FMD at Pirbright in the UK, following the necessary procedures (23). In the laboratory, the tissue samples will be prepared as a 10% suspension for antigen detection enzyme-linked immunosorbent assay (ELISA) (23) or used directly in a polymerase chain reaction (PCR) (24) to serotype the virus. Sensitive tissue cultures, such as primary bovine thyroid or lamb kidney cells, will also be inoculated with the tissue suspension and/or the whole blood and serum, to grow the virus for further characterisation, and to amplify the antigen if insufficient quantities were present in the original sample to provide an initial diagnosis by ELISA. The antigenic characteristics of the strain will be compared with existing vaccine strains in order to identify a suitable vaccine if one is required, or to confirm the use of one that is already helping to control the outbreak (17). A segment of the viral genome (part of the 1D gene) can also be sequenced and compared in the reference laboratory database to determine the relationship of the virus to other viruses circulating in the region, which may give an indication of its origin (18).

Due to the difficulty in detecting clinical FMD in sheep and goats, the disease may be present in the flock for a considerable time prior to discovery and samples may be collected from recovering animals. These animals will no longer have live virus in their tissues, except possibly in the pharynx, but antibodies to FMD virus will be detectable using either the liquid phase blocking ELISA (23), the solid phase competition ELISA (22) or the virus neutralisation test (23). However, if vaccine has been used in the flock, these tests will not distinguish between antibodies resulting from infection and those resulting from vaccination. Animals that have been infected with replicating virus develop antibodies to the non-structural proteins of FMD virus, and these may be detected using the 3ABC ELISA or enzyme-linked immuno-electrotransfer blot (EITB), although neither test has been fully validated for use in small ruminants (23). Alternatively, the presence of infection within the flock can be investigated by collecting samples using a probang sampling cup which recovers mucous and superficial epithelial cells from the pharynx, the site of virus persistence (16). The probang sample is then tested for the presence of FMD virus as for tissue and blood samples.

A pen-side diagnostic test would have been particularly valuable during the recent UK outbreak to assist field veterinarians in their clinical diagnosis. One, similar to a test which had been developed for rinderpest diagnosis, was in the process of validation and was used with some success towards the end of the outbreak (11, 25). The test relies on FMD viral antigen being recognised by a monoclonal antibody (Mab) attached to a coloured latex bead. The antigen/Mab/ bead complex is trapped by a fixed band of additional anti-FMD virus monoclonal antibody as it migrates along a chromatographic strip, creating an easily identifiable coloured line over the fixed band of Mab as the latex beads concentrate. The result can be read in 10 minutes. However, the test requires the amount of antigen usually found in the epithelium of a ruptured vesicle, but would not be sufficiently sensitive to detect antigen in a blood sample; in many cases of FMD in sheep, there may not be sufficient epithelium available for the
test to be used. Adaptations of the PCR could also provide a more rapid diagnosis, either to detect viral genome in nasal swabs before the development of clinical disease, or for use in portable machines taken to suspect premises (8).

Once the sample is received by a competent diagnostic laboratory, and assuming the sample was properly collected and stored during transport, the available tests are extremely sensitive and specific, and for positive samples, the result can usually be available in a few hours. However, before the laboratory can report that no virus was detected, the sample must be passaged on tissue culture and then blind-passaged a further one or two times, each passage requiring 48 hours. Thus a week may elapse after receipt of a sample and before a final report is issued from the laboratory. This can and frequently does cause some frustration with the field staff, as quarantine and movement restrictions would probably have been kept in place until a result was available. However, there are many examples where preliminary negative results have been issued and restrictions prematurely removed, and then the final result has been reported positive.

Control

The control of FMD in sheep and goats follows the same principles as would apply to the disease in other susceptible farm livestock, i.e. movement restrictions, disinfection and either slaughter of affected and in-contact animals or vaccination. In countries previously free from FMD, an initial attempt is usually made to eradicate the disease by slaughter. However, most countries will retain the option to vaccinate and thus maintain membership of a vaccine bank which can provide vaccine at short notice. In countries that have endemic FMD, sheep may be included in regular vaccination campaigns, although they are rarely vaccinated more than once a year. Sheep and goats are usually given half or a third of the cattle vaccine dose, and either oil or aluminium hydroxide/saponin adjuvants can be used. The duration of immunity against disease will depend on the severity of challenge with live virus in the field, the antigenic relationship between the vaccine strain and the field virus and the potency of the vaccine used. The often silent nature of the disease in adult sheep and goats can also give the impression that the vaccination programme has been successful, when in reality the virus is circulating freely. The ability of the particular strain to maintain itself in the small ruminant population becomes critical in these situations, but, as discussed above, has so far received little attention.

Attempts to vaccinate the national sheep population are usually hindered by the numbers involved, the cost of vaccination, and the resources required to administer the vaccine. The initial intention to include sheep and goats in the vaccination campaign throughout Turkey was recognised as unrealistic. Vaccination was therefore targeted at all susceptible stock in European Turkey (Thrace) and only certain areas of Anatolia. Similarly, Morocco concentrated on vaccinating sheep and goats on the border with Algeria, together with strategic vaccination around affected areas during the last outbreak of FMD in the country. Uruguay vaccinated only the cattle population during the final stages of the successful FMD eradication programme undertaken in the early 1990s, and left the approximately 20 million sheep unvaccinated during this period.

Conclusion

The recent UK experience has demonstrated the importance of sheep in the epidemiology of FMD caused by certain strains of virus. The difficulties encountered in identifying clinical disease resulted in large numbers of healthy animals being slaughtered, while other infected flocks went unrecognised, allowing the virus to spread. While some would argue that the policy adopted was effective and the overkill justified (10), a more traditional approach of methodical tracing and laboratory testing may have been equally effective, less expensive and morally more defensible. Both sides of the argument would however probably agree on the need for more rapid and reliable on-farm diagnosis, and a better understanding of the natural history of FMD in small ruminants.
Variation des signes cliniques de la fièvre aphteuse chez les ovins et les caprins

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Résumé
Alors que les manifestations de la fièvre aphteuse sont souvent peu ou non apparentes chez les ovins et caprins adultes, cette maladie peut provoquer une forte mortalité parmi les jeunes sujets. L’épizootie de fièvre aphteuse qu’a récemment connue le Royaume-Uni a souligné l’importance du mouton dans l’épidémiologie de la maladie, même si la responsabilité des petits ruminants dans l’introduction de la fièvre aphteuse en pays indemnes avait été maintes fois établie par le passé. Les difficultés que rencontre le diagnostic clinique devraient inciter à mettre au point des épreuves de dépistage plus rapides, qui deviendraient de précieux auxiliaires dans les futurs programmes de prophylaxie.

Mots-clés

Variación de las manifestaciones clínicas de la fiebre aftosa: ovinos y caprinos

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Resumen
Aunque en los ovinos y caprinos adultos suela revestir carácter benigno o subclínico, la fiebre aftosa puede provocar una elevada mortalidad entre animales jóvenes. El reciente brote en el Reino Unido puso de relieve la importante intervención de las ovejas en la epidemiología de la fiebre aftosa, aunque antes ya se habían producido muchos episodios en que pequeños rumiantes introdujeron la fiebre aftosa en países hasta entonces indemnes. Las dificultades inherentes al diagnóstico clínico deben servir de aliciente para crear pruebas de detección más rápidas y ponerlas al servicio de futuros programas de control.

Palabras clave
References


