The relationship between age and the likelihood of persistence in FMDV infected cattle

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Abstract:
Introduction: After the acute phase of infection a proportion of cattle infected with foot-and-mouth disease virus (FMDV) may become persistently infected and asymptomatically carry the virus in their pharyngeal regions for months or years. The host factors that enable this state are not known, but several experiments done previously in our laboratory with cattle of different ages in experiments designed for other purposes have indicated that the percentage of carriers in cattle may be lower in younger than in older animals. The present paper provides evidence of age at the time of infection as a factor in the likelihood of persistence of FMDV. Materials and Methods: Nineteen cattle that were between 78 and 176 days old at the time of virus exposure and 13 cattle that were between 221 and 355 days old at the time of virus exposure old were infected with the type O UKG/34/2001 either by intradermo-lingual injection or by direct contact. Clinical observations were performed and the presence of virus in pharyngeal regions (probang samples) assessed by virus isolation on BTY cells and/or fluorogenic PCR (Taquin) assays. Results: All animals were infected with FMDV and had virus in their probang samples for at least 7 days after virus exposure. Virus was cleared from the pharynx of cattle at different rates. About 46% (5/11) of animals that were older than 220 days when exposed had virus in their pharynx 28 days after exposure to virus compared to about 7% (1/15) of cattle younger than 180 days. Discussion: When exposed to FMDV, cattle younger than about 6 months old appear to resist persistent infection, but those older than 7 months are more likely to become carriers. These observations provide opportunities to study the mechanisms for the establishment of persistent infections comparing FMDV infection in these groups of cattle.

Introduction:
Foot-and-mouth disease (FMD) is a severe vesicular disease of cloven-hoofed animals and pigs and has a reputation for rapid and extensive transboundary spread and severe economic consequences for the countries affected (Coetzer et al., 1994). The virus that causes FMD belongs to the Aphthovirus genus of the Picornaviridae family that are non-enveloped, icosahedral viruses with positive sense RNA genomes. Although the genus consists of 7 serotypes of FMDV O, A, C, Southern African Territories (SAT) 1, SAT 2, SAT 3 and Asia 1, they produce the same disease. Infection with any strain may lead to the production of a proportion of persistently infected ruminants that are symptomless carriers of disease (Sutmoller and Gaggero, 1965; Alexandersen et al., 2002a). In natural infections initial FMDV replication most likely occurs in the epithelia of pharyngeal regions (Burrows et al., 1981; Zhang and Kitching, 2001). A viraemia and generalized infection follow that lead to viral replication and typical lesion formation in the squamous epithelia of the foot, mouth and tongue. This is followed by an antibody mediated clearance of virus from the body. However, in some animals complete elimination does not occur from all organs at the same rate and persistently infected animals that have no detectable virus in any other tissues have virus in their oesophageal-pharyngeal (OP) fluids later than 28 days after infection. The host factors that enable this state are not known. Rates of persistence in different groups of non-vaccinated cattle experimentally infected with Type O FMDV have varied between 17 to 77% (Salt et al., 1996; Zhang et al., 2004). Rates of persistence may also vary with species and the maximum duration of the carrier state ranges from 2 months in water buffalo to 5 years in African buffalo, and 3.5 years in cattle (Alexandersen et al., 2002a). Several experiments done previously in our laboratory with cattle of different ages in experiments designed for other purposes indicated that the percentage of carriers in cattle may be lower in younger than in older animals, and recent studies have indicated that the rate of clearance of FMDV from the pharyngeal region may be related to the likelihood of persistence (Zhang et al., 2004). This paper provides targeted evidence of age at the time of infection as a factor in the likelihood of persistence of FMDV.

Materials and Methods:
In two separate experiments a total of 32 animals of which 19 were younger than 180 days old, called the young group, and 13 that were older than 220 days old called the old group, at the time of viral exposure were used to study persistence of FMDV type O strain UKG 34/2001. In two separate but similar experiments 4 groups of 4 cattle were housed in each room of the microbiologically secure isolation unit. Two cattle in each room were intradermo-lingually inoculated with 0.5 ml of inoculum containing 10^{5.9} TCID_{50} of virus as described previously (Alexandersen et al., 2002b). The other cattle...
were allowed free and direct contact with the inoculated cattle. The clinical progress of animals was monitored and scored daily as described by Quan et al., (2004) and Alexandersen et al., (2003); as were rectal temperature measurements, the presence of viraemia, and the presence of infectious virus in OP fluid (probang) samples. Some cattle were removed from the groups at 7, 35 and 59 days after exposure for the collection of tissues for other studies and cattle removed 7 days post infection (dpi) are not included in the calculations.

Blood was collected periodically in 15 ml vacutainer tubes and serum was separated for testing. OP fluid samples were collected with probang cups into equal volumes of 1x MEM with glutamine and 5% foetal calf serum, pH 7.2. Both serum and OP fluid samples were stored at −70°C before use. The infectious viral loads in OP fluid samples were quantified by virus isolation in primary bovine thyroid (BTY) cells (Snowdon, 1966). The magnitude of viraemia was quantified by fluorogenic real-time RT-PCR (Taqman) assays essentially as described previously (Alexandersen et al., 2002c; Zhang and Alexandersen, 2003), as they were in OP fluids samples.

**Results:**

All experimental cattle became infected with FMDV and developed some clinical signs of disease. Inoculated animals developed disease 1-2 days after inoculation while animals in contact with them developed signs about 3-4 days later. The mean clinical scores of older inoculated cattle peaked at 2 days after inoculation (score=3) and, in the same age group, the animals that were in direct contact peaked 6 days after exposure (score=3) to inoculated cattle. In contrast mean peak clinical scores were seen on day 5 for both the inoculated and the direct contact cattle in the younger cattle although the inoculated scored higher at 4 than the direct contacts which scored 2. Mean rectal temperatures in young cattle that were inoculated peaked 2 days after infection at 40.2°C while those in contact peaked at day 4 at 39.2°C. In older inoculated cattle the mean peak temperature was 40.9°C and occurred on day 2 while the older cattle in direct contact peaked on day 3 at 39.4°C.

Most cattle had virus in their OP fluid samples for at least 5 days after virus exposure. After the initial deposition of virus by inoculation, peak viral amounts in probang samples were detected 2-4 dpi for inoculated and 3-6 dpi for contact animals. Persistent infections in carrier animals were confirmed by the demonstration of infectious virus in the OP fluid samples taken 28 dpi or later. About 46% (5/11) of animals that were older than 220 days when exposed had virus in their pharynx for more than 28 days after exposure to virus compared to about 7% (1/15) of cattle younger than 180 days.

In one of the experiments, mean peak viral loads in OP fluid samples from inoculated cattle regardless of the age group was 2 days post inoculation, but mean peak viral load of young contact infected animals of which none were carrier was 5 days post inoculation compared to 3 days post inoculation for similar older cattle. In these groups virus was cleared from the pharynx at different rates where young cattle had a mean clearance rate of 0.047 with a half-life of virus presence of about 15 hours compared to 0.016 and 43 hours, respectively, for older cattle.

**Discussion:**

When exposed to FMDV, cattle younger than about 6 months appeared to resist persistent infection, but those older than 7 months were more likely to become carriers. Clinical and experimental factors were examined in order to provide some explanations for these differences, and overall, the onset of disease was more rapid and more severe in the older cattle which were more evident in the cattle infected by direct contact. Younger cattle eliminated the virus more efficiently than older cattle, but this could also represent either the inability of the virus to influence this environment towards persistent infection or a host factor that inhibits persistence. These observations and other work presented at this meeting by our research group suggest that host factors and viral factors may be important in the establishment of persistence, and as more data becomes available in the course of other experimental work, the definition between old and young cattle and the likelihood of persistence will become clearer. This phenomenon will provide opportunities to study the mechanisms for the establishment of persistent infections comparing FMDV infection in these groups of cattle.

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**References:**


