Foot-and-Mouth Disease: Diagnosis and the Carrier Problem

James McHaffie

Writer's Comment: Frequent trips to the zoo with my grandfather, daily recreation with my family's canine companion, and school lessons about rain forest animals all played roles in igniting my passion for animal health at an early age. Throughout my childhood, I envisioned myself performing elaborate surgeries like those depicted on the show "Emergency Vets." Once at UCD, however, I shifted my veterinary interest to infectious diseases. Required to find a topic for both a review article and lay paper in UWP 104E, I chose the topic of foot-and-mouth disease, because I saw that it would give me ample opportunity to enhance my research skills and explore my new interest in livestock animals. After a month of mind-bending research and two weeks of relentless revision, I gained confidence in my research abilities while enhancing my interest in both diseases and livestock. I intend to take a similar opportunistic approach to my upcoming internship at the UC Davis Beef Barn and to my post-collegiate path to becoming a veterinarian. Thanks to Professor Victor Squitieri for encouraging me to make some difficult changes to my writing style that significantly enhanced the readability of this review.

—James McHaffie

Instructor's Comment: When James McHaffie—"Jake" as we knew him in our UWP 104E Scientific & Technical Writing class—first proposed writing a scientific literature review on some then as yet undetermined aspect of foot-and-mouth disease, I suspect that neither he nor I quite realized just what we were getting ourselves into. By his own admission, Jake—at that time—had only a slight familiarity with that notorious disease. As an aspiring disease research veterinarian, however, Jake already possessed substantial knowledge of infectious diseases in general and could discourse eloquently on how viruses spread and immune systems respond. I gave the green light, Jake immersed himself in the cutting edge experimental literature on foot-and-mouth disease, and the impressively thoughtful and informative review paper you are about to read amply attests to the quality and the seriousness of the effort he expended.

—Victor Squitieri, University Writing Program

Summary

Foot-and-mouth disease, a viral infection of cloven-hoofed animals, is among the most economically devastating diseases in the world today. The virus, *A. picornaviridae*, has seven distinct serotypes and over sixty sub strains. This variability makes both proper diagnosis and vaccination formidable challenges. Prompted by the outbreaks of this past decade, scientists have performed studies with the aim of improving the efficiency of diagnostic techniques and reducing the impact of carrier animals on the propagation of outbreaks. Most FMDV diagnostic tests available today are serotype specific and take considerable time to perform. A diagnostic test that confirms the presence of FMDV regardless of serotype is a topic of considerable research today. Carrier animals can arise either through survival of symptomatic infection or through vaccination in an area the live virus frequently inhabits. The present research of rapid and accurate identification of carrier animals will soon become the most influential factor in the reduction of FMD infections across the globe. Current methods of detecting carriers include virus isolation by probang sampling, RT-PCR, and NS-ELISA.

Keywords: foot-and-mouth disease; carrier animals; diagnostic techniques

Introduction

Foot-and-mouth disease is a highly contagious viral infection of cloven-hoofed livestock that is caused by the virus *Aphthovirus picornaviridae* (Alexandersen *et al.,* 2003). Animals that fit into this category include cattle, pigs, sheep, goats, and buffalo. Clinical signs include fever, loss of appetite, vesicular lesions in and around the nose, mouth, and feet, and in some younger animals, cardiac arrest (Alexandersen *et al.*, 2003). *A. picornaviridae*'s genetic variability is the primary factor that determines its rapid and progressive nature. The virus has seven different serotypes, each with multiple sub strains; both diagnosis and vaccination present scientists with a formidable challenge. Standard diagnostic procedures are specific to serotype and often require extensive laboratory analysis (Muller *et al.*, 2008).

Animals that have survived through symptomatic infection and those that have been vaccinated can carry the live virus for an extended period without displaying any clinical signs. This constitutes the carrier problem, which can cause farmers both frustration and considerable economic loss.

This review will investigate research done from 2003 to 2010 concerning the recent advances in diagnostic techniques as well as those in the identification of carrier animals. Specifically, I will analyze two

categories of diagnostic techniques, serotype-specific and serotype-collective. Finally, I will discuss the different carrier identification procedures used today for both vaccinated and post-symptomatic non-vaccinated carriers.

Diagnosis

Serotype-Specific Detection

Current diagnostic tests for FMDV are serotype specific and often require hours of laboratory analysis to reach a definitive conclusion regarding a tissue or fluid sample from an infected animal (Muller *et al.*, 2008). Probang sampling, or collection of esophageal/pharyngeal fluid, is a typical preliminary step to a variety of diagnostic tests that isolate one serotype of *A. picornaviridae*. One study froze cattle probang samples in equal volumes of MEM and thawed them for later testing (Golde *et al.*, 2005). When Golde and his colleagues warmed the samples, they added TTE to extract any antibodies before confirming the presence of FMDV serotype O-Manisa with an ELISA (Golde *et al.*, 2005). Additionally, a 2008 study confirmed the ELISA's reliability when used to diagnose FMD cases in cattle and buffalo (Maroudam *et al.*, 2008). Both animals injected with the live virus as well as those infected by contact revealed, through antigen ELISA, presence of active FMDV serotype O in their vesicular fluid samples (Maroudam *et al.*, 2008).

RT-PCR (reverse transcription polymerase chain reaction) also serves as a serotype-specific diagnostic tool for FMDV. The reverse transcription generates the cDNA strand from a target sequence of the viral RNA; this is followed by PCR of the cDNA for comparison to the cDNA established in databases for each specific serotype (Zhang and Alexandersen, 2003). One particular experiment demonstrated the occasionally inconclusive results of this technique using probang samples from cattle and sheep (Zhang and Alexandersen 2003). Standard RT-PCR without necessary modifications cannot always be sensitive enough to give a specific diagnosis unless used in combination with other tools (Alexandersen *et al.*, 2003).

Serotype-Collective Detection

Scientists currently studying *A. picornaviridae* are pursuing the development of a serotype-nonspecific method of the virus's detection

that would be quicker and more efficient than the serotype-specific methods used today. One method that shows promise of meeting this goal is infrared thermography. Recent investigation of infrared thermography has the potential to reduce the paranoia during an outbreak that often leads to excessive slaughter. Infrared thermography measures maximum foot temperature during all stages of infection using an infrared heat-sensing camera (Rainwater-Lovett *et al.*, 2008). In a recent study, researchers used this technique to predict the clinical onset of serotypes A, O UK, and O Manisa-Turkey in cattle (Rainwater-Lovett *et al.*, 2008). Among twelve cattle directly injected with live virus, average hoof temperature increased 4.7 degrees C during the pre-symptomatic stage and an additional 2.5 degrees C at the onset of symptoms (Rainwater-Lovett *et al.*, 2008). Infrared thermography could facilitate more effective quarantine and diagnostic strategies based on rising foot temperatures. Should this technique prove a reliable predictor of a positive diagnosis during an outbreak, severity of slaughter could be significantly reduced.

Another important advance toward nonspecific FMDV detection involves the use of an RNA sequence homologous to a viral structural protein 1AB, which can be found in every serotype of *A. picornaviridae* (Muller *et al.,* 2008). One recent experiment generated large quantities of the antibodies to this protein by means of extracting various excretory fluids from chickens (Muller *et al.*, 2008). Chickens have shown immune responses to viruses containing protein 1AB. The chickens' antibodies to 1AB were found to attack all seven serotypes of FMDV, as confirmed by an antigen capture ELISA (Muller *et al.*, 2008). FMD researchers are continuing their search for other monoclonal antibodies common to all FMDV serotypes. Discovery of these antibodies will reduce the tedium of laboratory analysis in some of today's serotype-specific diagnostic procedures.

Identification of Carrier Animals

Vaccinated Carriers

When livestock farmers in an FMD endemic area decide on a specific vaccination strategy for their animals, they must take into account variables such as location of a recent outbreak, time since the outbreak began, and species an outbreak has seized. As Golde *et al.*'s 2005 study suggests, animals vaccinated at least seven days before coming into contact with live FMDV may never develop symptoms despite harboring

the antigen (Golde *et al.*, 2005). Deciding to vaccinate only a fraction of animals on a farm is unwise since non-vaccinated individuals are still susceptible to infection through their vaccinated neighbors. Until carrier detection methods are more sensitive and efficient, vaccinating all animals on a farm while restricting their contact with non-vaccinated animals will be critical to keeping infection at bay.

Although scientists have not yet developed an ideal vaccinated carrier detection method, some noteworthy progress must be acknowledged. One particular study revealed the accuracy of an antibody NS ELISA, which identifies antibodies to specific non-structural proteins in FMDV (Moonen *et al.*, 2004). Researchers vaccinated fifteen juvenile cattle with a serotype A Turkey vaccine, then injected them with the active virus four weeks later (Moonen *et al.*, 2004). Probang samples of these cattle subjected to NS ELISA close to five weeks post-infection revealed significant concentrations of antibodies in all fifteen cattle, meaning they all became carriers (Moonen *et al.*, 2004). Should post-symptomatic cattle also produce antibodies to non-structural FMDV proteins, NS ELISA would become a welcome addition to cattle in outbreak prone areas.

Post-Symptomatic Non-Vaccinated Carriers

Recent work with a modified RT-PCR in cattle and sheep has generated a carrier detection of serotype O UK based on a Ct value (Zhang and Alexandersen, 2003). A probang sample's Ct value corresponds with the percentage of test cycles in which a serotype's target nucleotide sequence can be identified (Zhang and Alexandersen, 2003). These values, when gathered from samples at least twenty-eight days post infection, determine whether the animal in question is emptying the virus from its pharynx or maintaining high levels similar to clinical infection. Values below 36 indicate a carrier; those above 44 indicate a non-carrier, while those between 36–44 yield inconclusive results (Zhang and Alexandersen, 2003).

Another promising method for diagnosing carrier status in post-symptomatic animals involves the analysis of nasal secretions for mucus specific antibodies (Maddur *et al.*, 2008). An experiment in India with cattle infections of Serotype A1 revealed markedly higher concentrations of anti-FMDV A1 antibodies in the nasal mucus of carriers vs. non-carriers (Maddur *et al.*, 2008). Researchers in this study also demonstrated that carrier antibody levels in nasal mucus remain high even after

antibody concentrations in probang samples begin to decline (Maddur *et al.*, 2008). Should the same hold true for other serotypes of *A. picornaviridae*, then veterinarians and diagnosticians using nasal mucosal analysis could provide a more accurate assessment of carrier risk during future outbreaks.

Conclusion

Until recently, the genetic diversity of the foot-and-mouth disease virus has kept scientists from discovering a consistent diagnostic test that can limit the intensity of an outbreak. Development of a serotype nonspecific detection provides potential for quicker and more efficient diagnosis. Should serotype-independent diagnostic tests prove consistently effective in experiments during the next five years, nations that have experienced costly outbreaks could reduce their slaughter numbers significantly.

Research within the past eight years has enhanced our comprehension of the carrier problem. For farmers residing in endemic regions of foot-and-mouth disease, it is crucial to gain access to scientists with the facilities to perform the carrier identification tests mentioned. Countries looking to generate a disease-free status after a severe outbreak are advised to implement a nationwide use of RT-PCR and NS-ELISA. Scientists studying the carrier problem have made great strides towards the prevention of future national scale outbreaks of foot-and-mouth disease. However, due to the complexity and variability of *A. picornaviridae*, the task of carrier detection still remains daunting.

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