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Detection of foot-and-mouth disease virus infected cattle using infrared thermography

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Abstract

In this study, infrared thermography (IRT) was assessed as a means of detecting foot-and-mouth disease virus (FMDV)-infected cattle before and after the development of clinical signs. Preliminary IRT imaging demonstrated that foot temperatures increased in FMDV-infected animals. The maximum foot temperatures of healthy ($n = 53$), directly inoculated (DI) ($n = 12$), contact (CT) ($n = 6$), and vaccine trial (VT) ($n = 21$) cattle were measured over the course of FMD infection. A cut-off value was established at 34.4 °C (sensitivity = 61.1%, specificity = 87.7%) with the aim of detecting FMDV-infected animals both before and after clinical signs were observed. Seven of 12 (58%) DI and 3/6 (50%) CT animals showed maximum foot temperatures exceeding the 34.4 °C cut-off before the development of foot vesicles. In contrast, only 5/21 (24%) VT animals displayed pre-clinical foot temperatures above this cut-off possibly indicating partial vaccine protection of this group. These results show IRT as a promising screening technology to quickly identify potentially infected animals for confirmatory diagnostic testing during FMD outbreaks. Further evaluation of this technology is needed to determine the value of IRT in detecting animals with mild clinical signs or sub-clinical infections.
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Keywords: Infrared thermography; Foot-and-mouth-disease; Bovine; FMDV

Introduction

Foot-and-mouth disease (FMD) is one of the most significant animal diseases affecting trade. It has been eradicated from many regions of the world where re-introduction has devastating economic, social and environmental effects (Woolhouse et al., 2001). The causative virus, foot-and-mouth disease virus (FMDV), causes vesicles on the foot, mouth, tongue, and teats of cloven-hooved animals and is one of the most contagious disease agents known. FMD is classified as a reportable disease by the Office International des Epizooties (OIE).

Although rarely fatal in adult animals, the appearance of FMD in a disease-free country results in severe trade restrictions and agricultural losses. For example, the reappearance of FMD in the United Kingdom in 2001 resulted in multi-billion dollar losses associated not only with agriculture, but a wide range of activities including the pharmaceutical and tourist industries (Thompson et al., 2002). In order to re-gain FMD-free status, countries like the UK must demonstrate freedom not only of the disease but also of the virus in their animal population. Therefore, control measures include mass slaughter of animals in premises reporting disease as well as neighboring premises. In 2001, this approach resulted in the slaughter of millions of animals, most of which were not infected, to quickly achieve eradication (Davies, 2002).

Currently, clinical screening for FMD in cattle is time-consuming and labor-intensive since it necessitates the restraint of suspect animals for clinical examination. One of the main problems hampering the diagnosis, control and eradication efforts during the 2001 UK epidemic was

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the need for veterinarians to inspect hundreds, or in some cases thousands of individual animals on suspected infected premises (Davies, 2002). This was particularly difficult as many animals were either at an early stage of infection or not clinically affected by the OUK/2001 FMDV strain.

An often observed sign of FMD preceding development of vesicular lesions is the presence of fever, but in some animals infected with certain viral strains fever can be mild and/or of short duration, or absent. In the absence of overt clinical signs, a pen-side rapid screening test such as infrared thermography (IRT) that measures heat emission could be instrumental in selecting likely infected animals for further testing for FMDV infection either by direct virus detection or using serological methods.

This study was aimed at evaluating IRT as a screening method for FMDV-infected cattle and its potential application in the identification of suspected animals for sampling and confirmatory diagnostic testing during FMD outbreaks.

Materials and methods

Animals and virus

Holstein steers aged 6–8 months of age and weighing 180–270 kg were used in the study. All cattle experiments were performed in biosafety level 3 isolation facilities at the Plum Island Animal Disease Center following protocols approved by the Institutional Animal Care and Use Committee. Infrared images were collected from animals undergoing FMD vaccine trials or pathogenesis studies.

Animals from pathogenesis studies were grouped by route of virus exposure – direct inoculation or contact exposure. Directly inoculated (DI) animals ($n = 12$) were sedated and inoculated intradermalingually (IDL) at four sites with 100 μL per site of virus suspension containing a total of 10^4 bovine infectious doses (BID_{50}) of FMDV.

Two FMDV serotype O viruses (strains O UKG/2001 and O1-Manisa-Turkey/1969) and one FMDV serotype A virus (strain A24 Cruzeiro) were used for inoculation. Contact (CT) animals ($n = 6$) were introduced in groups of two into the room where two DI animals were housed at 24 h post-inoculation. Cattle that were part of a vaccine trial (VT) ($n = 21$) were directly inoculated as described above. The VT group included animals that were protected, partially protected or unprotected after FMDV challenge. The three study groups (DI, CT, and VT), virus strains, and vaccine treatments are detailed in Table 1.

During inoculation and every 24 h thereafter, animals were sedated and visually examined for vesicular lesions on their feet, nose, and mouth.

Table 1
Treatments, challenge virus strain and number of animals used in this study

Group	Challenge virus (number of animals)	Treatment
Directly inoculated [DI] ($n = 12$)	A24 ($n = 8$) O/UKG/2001 ($n = 2$) O1 Manisa ($n = 2$)	Unvaccinated
Contact [CT] ($n = 6$)	A24 ($n = 2$) O/UKG/2001 ($n = 2$) O1 Manisa ($n = 2$)	Unvaccinated
Vaccine trial [VT] ($n = 21$)	O1 Manisa	Unvaccinated ($n = 3$) Inactivated antigen commercial vaccine ($n = 8$) Ad5-IFNα experimental vaccine ($n = 10$)

A numeric scoring system was used to record clinical scores where a point is assigned for lesions on each of the four feet, the mouth and the nostril. The highest score of 6 indicated lesions in the tongue other than the inoculation site, on each foot, and on/in the nostril.

Infrared thermography

Infrared images were obtained always prior to sedation using one of two cameras, namely a FLIRSystems ThermaCAM EX320 or a Fluke IR FlexCam R1. Images were collected before animal rooms were cleaned in order to avoid temperature variations induced by the presence of standing water. The camera was placed 1.5–2 m from the animals to capture all images. Images were downloaded using ThermaCAM QuickView or Fluke SmartView software for analysis. Cameras were surface-decontaminated between each study by wiping all surfaces with a 5% acetic acid solution and a 70% ethanol solution followed by a 30 min ultraviolet light exposure inside a class II biological safety cabinet.

Confirmation of infection status

Infection status was established by clinical assessment and laboratory confirmation of infection. Viremia was determined by virus isolation as previously described with minor changes (Amass et al., 2003). Briefly, whole blood was collected daily and centrifuged at 800*g* for 10 min. Sera was harvested and frozen at −70 °C. Multi-well plates containing 2 cm^2 monolayers of BHK-21 cells (passage level 62–68) in duplicate wells were inoculated with serum to detect FMDV (sample volume of 200 μL). Plates were monitored for cytopathic effects (CPE) for 3 days. All samples without CPE were frozen/thawed and passed two more times as described above to confirm an absence of infectivity. Samples with CPE were confirmed by real-time PCR as previously described (Callahan et al., 2002).

Data analysis

One hundred and six individual observations were collected from 53 healthy, naïve cows before FMDV exposure to generate baseline foot-temperature data. Multiple observations from each animal were separated by at least 24 h in order to incorporate day-to-day variation. After virus exposure, IRT images were collected daily in order to capture three stages of infection: Pre-clinical (1 day before foot lesions identified), Clinical (the first day foot lesions were identified), and post-clinical (1 day after foot lesions were identified). The single maximum data point from each foot of at least three feet of an animal was collected. The maximum foot temperature was defined as the highest temperature identified by the software program in the area from the bottom of the hoof up to the top of the digits. These temperatures were compared using a 2-tailed Student's *t* test in Microsoft Excel; $\alpha < 0.05$ was considered significant. Exclusion from the clinical stage analysis occurred if an animal became injured or did not develop lesions, all stages of infection were not captured, or fewer than three hooves were visible in the IRT image ($n = 34$).

The Screening and Diagnostic Tests/Validity Measures option in the Describe program of WINPEPI (http://www.brixtonhealth.com) was used to generate descriptive statistics for the maximum foot temperatures (Abramson, 2004). WINPEPI (Programs for Epidemiologists for Windows) is a free, downloadable statistics package that provides a wide variety of statistical calculations. The Describe program computes descriptive statistics from manually entered data sets including the appraisal of screening and diagnostic tests. Cut-off values reported here were determined by utilizing the Youden's index (the percent sum of the sensitivity and specificity of a particular cut-off point minus 100).

The maximum floor temperature between an animal's feet, the maximum eye temperature, and the rectal temperature of each animal were collected alongside the maximum foot temperatures. To determine if correlations existed, the single maximum foot temperature of each animal at each stage of infection was plotted against the floor, eye and rectal temperatures in Microsoft Excel and the Pearson's correlation coefficient (r) was obtained.

Results

Site selection

Preliminary IRT imaging demonstrated temperature differences between FMDV-infected cattle that presented fever and viremia from those that did not before vesicular lesions were observed (Fig. 1). These differences motivated further evaluation of IRT as a screening test for FMDV-infected cattle. To identify the best site for FMD screening, maximum foot temperatures, maximum face temperatures, and rectal temperatures were plotted with clinical scores. Foot temperatures paralleled clinical scores better than face and rectal temperatures in 14/17 (82.4%) vaccinated-unprotected cattle (see example in Fig. 2A). In contrast, the foot temperatures of vaccinated-protected animals remained low, reflecting their protective immune status (see example in Fig. 2B). Further confirmation of the foot as an ideal site to screen for FMDV-infected cattle came from correlation analysis of contact ($n = 6$) and directly ($n = 12$) inoculated animals with maximum foot, eye, and floor temperatures and rectal temperatures. Moderate positive correlations between maximum foot temperatures and rectal and eye temperatures were identified ($r = 0.53$ and $r = 0.60$, respectively) as well as between the rectal and eye temperatures ($r = 0.50$). Conversely, a small positive correlation was demonstrated between the floor temperature and foot temperatures ($r = 0.18$) indicating that foot temperatures detected by IRT were not affected by floor temperature under the experimental conditions of this study (Fig. 3). Based on this evidence, all further analyses were focused on maximum foot temperature as determined by IRT.

IRT as a screening tool for FMDV-infected cattle

Two serotype O viruses (O/UKG/2001 and O1 Manisa) and one serotype A virus (A24 Cruzeiro) were used in this study. Only one serotype O virus (O1 Manisa) was used for the challenge of VT animals. Among the DI and CT groups, no significant differences between the two serotypes ($P = 0.48$ and 0.09, respectively) were detected in maximum foot temperatures at the pre-clinical or clinical stages of infection. A significant difference at the post-clinical stage was detected ($P = 0.02$) where animals infected with type O virus had maximum foot temperatures between 39.1 °C and 40.1 °C, while animals with type A virus ranged from 31.6 °C to 39.3 °C. Post-clinical data from DI and CT groups were not available for 3, 2, and 1 animals infected with A24, O1Manisa, and O/UKG/2001 viruses, respectively. For further analysis animals were grouped

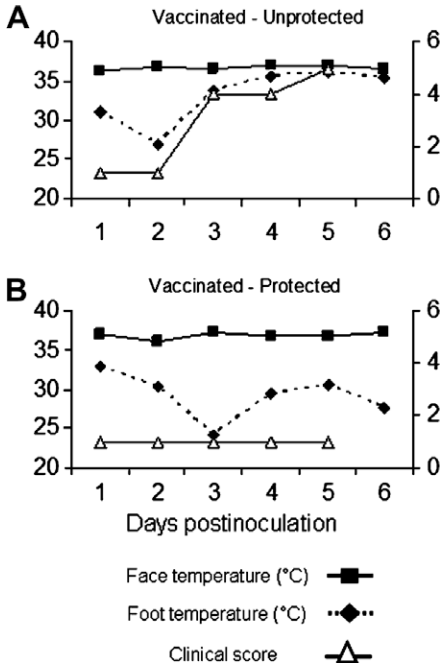

Fig. 2. Example of individual temperatures and clinical sign scores in FMDV vaccinated-unprotected ($n = 15$) and vaccinated-protected animals ($n = 3$). Face and foot temperatures based on IR images; clinical score based on number and distribution of vesicular lesions. Left Y axes indicate temperature in °C, right Y axes indicate clinical scores.

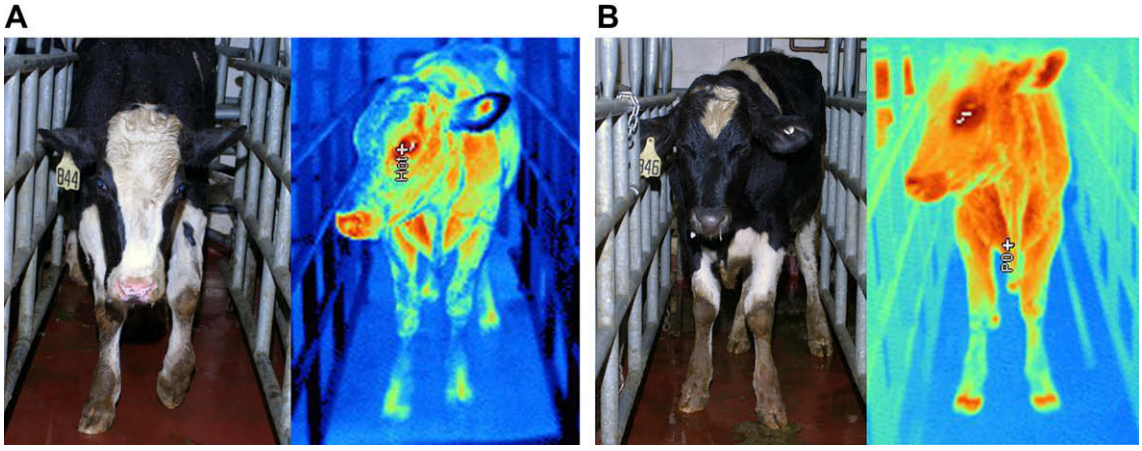

Fig. 1. Digital and infrared images of cattle without (A) or with (B) fever and viremia at 24 h post challenge, before vesicular lesions were observed. Note the lower temperatures (blue–green) in the animal without fever or viremia versus the higher temperatures (orange–red) in the viremic and feverish animal.

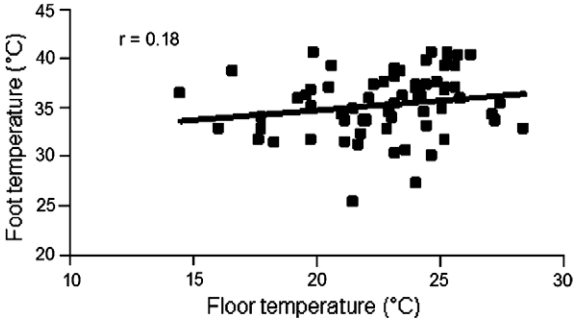

Fig. 3. Comparisons of floor temperatures with maximum foot temperatures observed in 18 FMDV infected animals at various stages of infection.

by route of infection and vaccination status (DI, CT, and VT groups) and temperature differences were observed according to disease stage. The VT group included protected, partially protected and unprotected individuals.

One hundred and six individual baseline observations taken from 53 cows prior to inoculation yielded a mean maximum foot temperature of 30.1 °C (SD 4.1 °C; range 20.3–36.8 °C). Thirty-nine FMDV-infected animals were used in subsequent analyses. After FMDV inoculation, DI animals ($n = 12$) showed a mean increase in maximum foot temperatures of 4.7 °C from the baseline mean to pre-clinical stage and 7.2 °C to clinical and post-clinical stages ($P \leqslant 0.001$) (Fig. 4A). CT animals ($n = 6$) demonstrated similar temperature differences from baseline with 4.8 °C, 7.5 °C, and 8.9 °C increases at the pre-clinical, clinical, and post-clinical stages of infection, respectively ($P < 0.003$) (Fig. 4B).

The ranges of maximum foot temperatures for each group and stage are shown in Table 2. There were no significant differences between DI and CT animals at any stage of infection (pre-clinical, $P = 0.95$; clinical, $P = 0.81$; post-clinical, $P = 0.21$). VT animals ($n = 21$) showed smaller increases from the baseline mean with 0.5 °C at the pre-clinical stage, 5.7 °C at the clinical stage, and 5.2 °C at the post-clinical stage (Fig. 4C). The clinical and post-clinical means from the VT group were significantly different from the baseline values ($P < 0.001$). Increases in maximum foot temperature occurred regardless of strain and differed significantly ($P < 0.03$) between the VT animals and DI and CT animals at all stages of infection (Fig. 4).

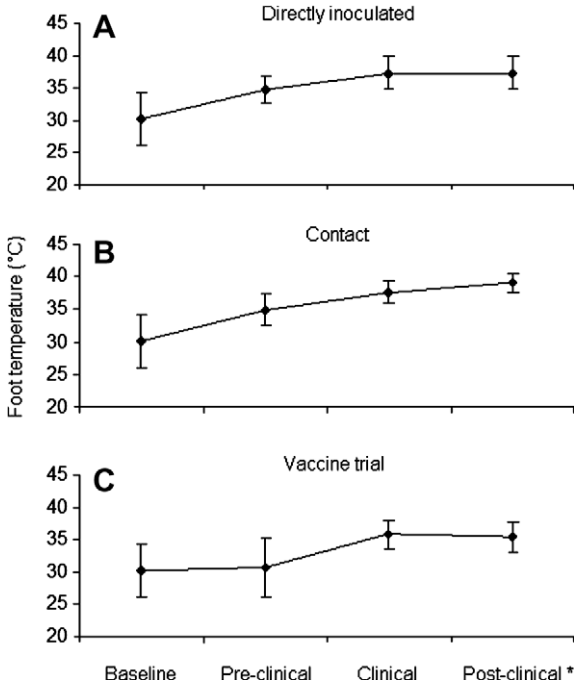

Fig. 4. Mean and standard deviation of maximum foot temperatures at each stage of infection for DI, CT, and VT animals (A, B and C, respectively).

Table 2
Ranges of maximum foot temperatures by group and stage of infection

	Pre-clinical	Clinical	Post-clinical[a]
Contact [CT] ($n = 6$)	31.8–37.1	36.0–40.3	37.3–40.1
Directly inoculated [DI] ($n = 12$)	31.4–38.1	33.9–40.7	31.6–39.5
Vaccine trial [VT] ($n = 21$)	23.1–39.1	31.0–40.6	31.3–42.3

All temperatures are shown in degrees Celsius.
[a] Post-clinical stage data missing on 3, 3, and 1 animals from the CT, DI, and VT groups, respectively.

Sensitivity and specificity

Pre-clinical maximum foot temperatures from all animals ($n = 39$) regardless of the route of virus infection or vaccine status were used to calculate a cut-off value using WINPEPI (Abramson, 2004). The cut-off value generated was 33.0 °C (sensitivity [SE] = 62.5%, specificity [SP] = 73.6%). However, this cut-off yielded a number of false positives as illustrated by the large number of baseline animals falling within the 31.4–34.3 °C range (Fig. 5). Therefore, we established a cut-off value of 34.4 °C (SE = 61.1%, SP = 87.7%), which correctly identified 58% and 67% of pre-clinical CT and DI animals, respectively, while mistakenly identifying only 12% of baseline animals (Fig. 5). Sensitivity and specificity for IRT detection of FMD-infected animals during the clinical stage using this cutoff were 79.5% and 87.5%, respectively. On the second day of clinical disease (post-clinical stage), the SE and SP were 78.1% and 88.4%, respectively. Animals in the VT group were not considered in this analysis since many of them were partially protected and yielded lower foot temperatures.

Clinical sensitivity of IRT

Utilizing the cut-off value of 34.4 °C, we evaluated the ability of IRT to detect animals that would develop clinical FMD signs. Viremia is often used to monitor FMDV

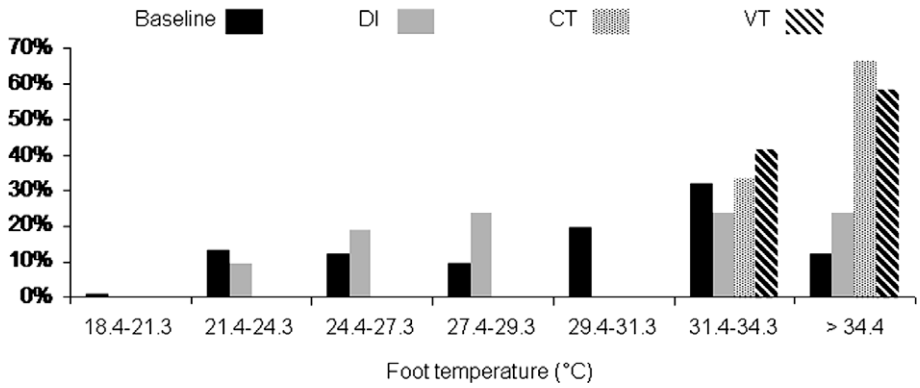

Fig. 5. Proportion of cattle in each foot temperature range from baseline and pre-clinical stage of FMDV infection for the DI, CT, and VT groups.

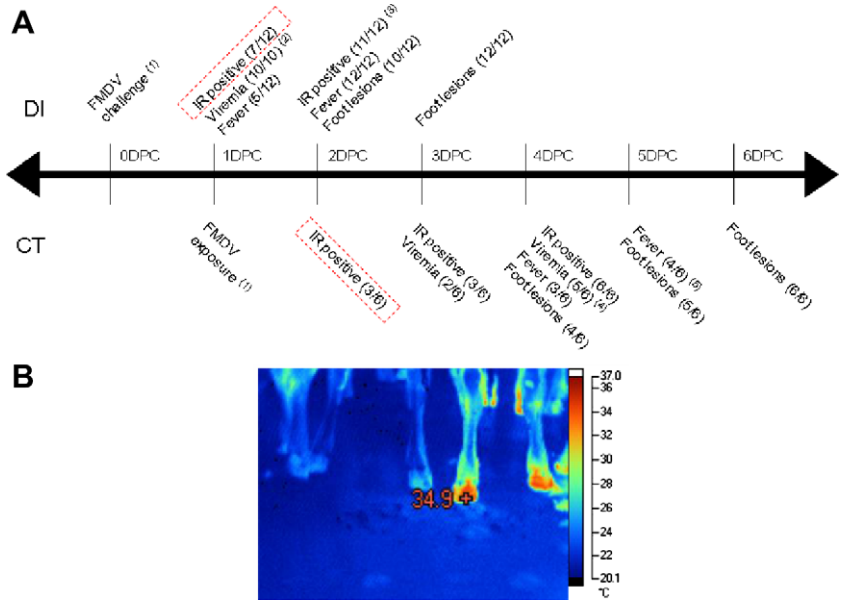

Fig. 6. (A) Timeline illustrating proportion of cattle from DI and CT groups presenting viremia, clinical disease (fever and vesicular lesions) and IRT positive foot temperatures (utilizing a cutoff value of 34.4 C) after FMDV exposure. DPC = days post-challenge. (B) Example of infrared image showing foot-temperature difference between FMDV infected (right) and non-infected (left) cattle.

infection in animals as it frequently precedes the development of clinical signs. In this study, 3/6 (50.0%) CT animals were positive by IRT 1 day prior to having detectable viremia and 2 days prior to the development of foot lesions (Fig. 6A). At 1 day post-challenge (DPC), of the ten DI animals assessed for viremia, 100% were viremic and 7/12 (58.3%) were detected by IRT (Fig. 6A). Foot lesions were identified in these 10 animals the following day. Clinical signs never occurred before viremia for any animals in the CT and DI groups. Eight of 21 (38.1%) VT animals developed viremia and foot lesions by 2 DPC. One animal was detected by IRT at 1 DPC and five were detected the next day (data not shown). An example of a possible application of IRT as a screening test in a group of animals is shown in Fig. 6B where an FMDV-infected animal was easily detected by the increased foot-temperature.

Discussion

Previous studies have assessed the efficacy of IRT for the detection of injury and disease. Human medical applications have included the early detection of breast cancer (Mital and Scott, 2007), quantification of the disease process in herpes labialis lesions (Biagioni and Lamey, 1995), and airport screening for severe acute respiratory syndrome (SARS) (Chiu et al., 2005). Veterinary studies have also been varied. Schaefer et al. identified IRT as a method for early detection of animals infected with bovine viral diarrhea virus (Schaefer et al., 2004) or with bovine respiratory disease using facial scans (Schaefer et al., 2007). Infrared has been identified as a possible detection method for laminitis in lactating dairy cattle (Nikkhah et al., 2005) and chronic pain following tail docking (Eicher et al., 2006). Measurements from IRT have also been used to

recognize orthopedic injuries in dogs and horses (Eddy et al., 2001), rabies virus in raccoons (Dunbar and MacCarthy, 2006), and mange in the Spanish ibex (Arenas et al., 2002). These studies have concluded that while IRT provides an additional perspective on disease and injury, it should complement traditional diagnostics methods. Similarly, the present study assessed the application of IRT as a screening method for identifying potential FMDV-infected cattle for further sampling and laboratory confirmation of infection. This is the first report of IRT as a screening method for FMD in cattle.

Foot temperature was chosen as the area of interest in this study because, unlike face temperature, important temperature changes were seen in animals during the different phases of disease. Although Schaefer et al. (2004) identified increased face temperatures for early detection of bovine viral diarrhea virus, the data presented here did not support this finding for FMDV. The highest face temperature is often identified in the eye, which is believed to reflect internal body temperature (Kastberger and Stachl, 2003). Interestingly, we did not see a strong positive correlation between face and rectal temperatures. While we were able to identify a positive correlation between foot temperatures and rectal temperatures, increases in foot temperatures consistently occurred prior to the development of fever. Furthermore, the presentation of fever occurs in a wide variety of illnesses in cattle but increased foot temperatures have fewer etiologies. We were unable to identify a large correlation between foot and floor temperatures, strongly suggesting that under the conditions of this study floor temperature did not influence the temperature of the foot.

Interestingly, DI and CT animals showed similar increases in foot temperatures regardless of the viral strain or route of FMDV exposure while VT animals did not show significant increases in foot temperature in the preclinical stage and showed smaller increases than DI and CT animals. This difference might be due to the fact that these animals were partially protected, had less of an inflammatory response and therefore, had lower temperatures in the feet. The 33.6 °C cut-off value obtained using WINPEPI maximized the sensitivity and specificity of this test (SE = 72.2%, SP = 82.1%) but misclassified a number of healthy animals. Since this tool is intended for identifying potentially infected cattle for further testing, a high number of false positives would limit the utility of the test. By increasing the cut-off value to 34.4 °C (SE = 61.1%, SP = 87.7%), IRT was able to more accurately identify infected cattle and reduce the number of false positives.

During the 2001 FMD outbreak in the UK, the decision to cull animals was originally based on laboratory confirmation but changed to clinical presentation as the diagnostic laboratory became overwhelmed by large numbers of samples arriving daily (McLaws et al., 2007). IRT could provide a tool for better selecting animals for sampling, resulting in a decreased number of clinical samples submitted for diagnostic confirmation and easing the strain on veterinarians in the field and laboratory technicians during a large FMD outbreak. One of the main problems during this outbreak was the difficulty of detecting clinical signs in sheep (McLaws et al., 2007). The IRT test would need to be evaluated to determine its utility as a screening test in this species.

As illustrated by Fig. 6A, IRT detected foot temperatures above the cut-off value at 1 DPC for DI animals, which was the same day that viremia and fever were first detected but before any lesions were observed. In contrast, IRT identified increased foot temperatures prior to the detection of viremia and foot lesions in CT animals. The ability of IRT to detect animals infected with FMDV by contact (presumably the mechanism of infection during natural transmission) not only in the pre-clinical but even during the pre-viremic phase provides strong evidence that this technology can be very useful in detecting FMDV-infected animals prior to other evidence of infection. This early detection capability can become critical during an FMD outbreak, particularly when suspect animals need to be identified for diagnostic sample collection. Since two-thirds of pre-clinical and 100% of clinical CT animals in this study had a maximum foot temperature above the cut-off of 34.4 °C, it is likely that at least one animal in an infected herd would be detected by IRT during an FMD outbreak.

FMDV-infected animals in the VT group were not as easily detected by IRT during the pre-clinical phase. The fact that only 8/21 (38.1%) VT animals developed viremia supports the hypothesis that the VT animals developed partial protection to FMDV after vaccination. This partial protection might or might not interfere with the inflammatory process, and may make IRT pre-clinical detection of VT animals more difficult. Infected animals without clinical signs might or might not be detectable through inflammatory responses in the coronary band and resultant rise in temperature. This may or may not limit the usefulness of this test in screening for FMD in countries that use FMDV vaccines and where partially protected animals would be common. Another potential limitation of this technology is the cost of the infrared cameras used in this study. However, it is likely that less expensive equipment can be employed to detect maximum foot temperatures and so allow for rapid screening of suspected FMDV-infected cattle.

Further validation of the technology is necessary as we did not have access to a large number of healthy animals under field conditions. Also, it is well established that other pathologies result in inflammation of the feet, mimicking the 'hot feet' seen here, which makes it important to carry out the field validation of this screening test. Collection of foot temperature data using IRT under a variety of environmental conditions, floor surfaces (i.e. grass, mud, concrete), and other variables is necessary for the validation of this technology. The data collected with the infrared camera included up to 25,000 individual temperatures for

each image generated. Therefore, unique patterns or temperature signatures for FMD could be better defined using computer algorithms.

The purpose of this study was to test the feasibility of IRT as a screening tool for detection of FMDV-infected cattle. The use of a quick and reliable tool to screen large numbers of animals without the need for handling or restraining would allow for a more efficient use of valuable resources. An important issue during the 2001 UK epidemic was the 3-day quarantine for veterinarians after visiting a suspected FMDV-infected premise (Kitching et al., 2005). This limitation could be avoided by having veterinary assistants trained in IRT scan the herds with an IRT camera before veterinarians enter the premises. Furthermore, with existing wireless technology, IR images could be transmitted remotely to incident command centers where veterinarians could pre-select animals for further clinical examination and sampling. Other potential uses of IRT technology could be in combination with rapid pen-side diagnostic tests such as real-time RT-PCR or antigen detection methods. By rapidly identifying potentially infected animals, sampling and testing could be done on-site, cutting the time of detection and allowing for faster implementation of quarantines in the control phase or quarantine release during the recovery phase of an FMD outbreak.

Future research should focus on differentiating foot-associated conditions in cattle and developing computational algorithms that assess signature temperature patterns of specific diseases including FMD. This study demonstrated the feasibility of IRT as a screening tool for FMD in cattle that, in combination with other rapid diagnosis tests, could play an important role during the control, eradication, and recovery phases of an FMD outbreak.

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