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# The use of infrared thermography as a non-invasive method for fever detection in sheep infected with bluetongue virus

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ABSTRACT

Fever, which is closely linked to viraemia, is considered to be both the main and the earliest clinical sign in sheep infected with bluetongue virus (BTV). The aim of this study was to evaluate the potential use of infrared thermography (IRT) for early detection of fever in sheep experimentally infected with bluetongue virus serotype 1 (BTV-1) and serotype 8 (BTV-8). This would reduce animal stress during experimental assays and assist in the development of a screening method for the identification of fever in animals suspected of being infected with BTV.

Rectal and infrared eye temperatures were collected before and after BTV inoculation. The two temperature measures were positively correlated ($r = 0.504$, $P < 0.05$). The highest correlation between rectal and infrared temperatures was observed when temperatures were above physiological levels. IRT discriminated between febrile and non-febrile sheep with a sensitivity of 85% and specificity of 97%. The results showed that eye temperature measured using IRT was a useful non-invasive method for the assessment of fever in sheep infected with BTV under experimental conditions. Further research is required to evaluate the use of IRT under field conditions to identify potentially infected animals in bluetongue surveillance programmes.



## Introduction

Infrared thermography (IRT) is a temperature measurement tool based on the ability of all objects to emit characteristic infrared radiation as a function of their temperature (Kastberger and Stachl, 2003). This radiation is proportional to the surface temperature of the body (Bitar et al., 2009), and is evaluated using a thermographic camera, which produces images with different colour patterns depending on the temperature of the imaged objects. The temperature values in the images are strongly related to the emissivity of the object, which is defined as the relative ability of a surface to emit and absorb radiation (Rodríguez-Prieto et al., 2013). As up to 60% of heat loss of an animal may occur in the infrared range, radiated heat loss could be used as an early indicator of fever in pathological conditions (Schaefer et al., 2007).

Since the late 1950s, IRT applications in human medicine have been varied, with one of the first uses being the detection of temperature increasing in the skin over a breast tumour (Jiang et al., 2005). More recently, during the 2003 severe acute respiratory syndrome (SARS) outbreaks, thermal scanners were used in airports to detect suspected febrile cases (Chiu et al., 2005). In veterinary sciences, IRT has been used as a detection method for lameness in horses (Eddy et al., 2001) and cattle (Nikkhah et al., 2005), as well as for the evaluation of dermatological lesions in wild animals (Arenas et al., 2002). Other studies have used IRT for early detection of different infectious diseases in livestock (Schaefer et al., 2007; Rainwater-Lovett et al., 2009).

Ideally, collection of temperature data for fever detection should be rapid and non-invasive (Ng et al., 2004), particularly in veterinary practice where reduction in the stress caused by capture and handling is important. The ability of the IRT to detect febrile animals with minimal handling suggests its potential as a useful tool in both veterinary preventive medicine and experimental diseases models (Schaefer et al., 2004, 2012; Rainwater-Lovett et al., 2009).

Bluetongue (BT) is a non-contagious, vector-borne disease of domestic and wild ruminants. It is caused by an orbivirus belonging to the family Reoviridae; so far 26 different serotypes have been identified (Hofmann et al., 2008; Maan et al., 2011). The

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disease is of economic importance due to losses associated with mortality and morbidity, veterinary treatment, implementation of control and eradication programmes and commercial restrictions in infected areas (Velthuis et al., 2010). The severity of BT varies according to species, breed and bluetongue virus (BTV) serotype. Sheep have been considered the most severely affected livestock species and typical clinical symptoms include apathy, generalized subcutaneous oedema (mainly observed on the head, intermandibular area and neck), nasal discharge, oral lesions, cyanotic tongue, dyspnoea and lameness (Schwartz-Cornil et al., 2008; Maclachlan et al., 2009). However, fever is the main clinical sign of BT in sheep and the earliest to be detected (Hamblin et al., 1998; Worwa et al., 2010; Perez de Diego et al., 2011).

Since BT is classified as a notifiable disease by the World Organisation for Animal Health (OIE), and is being considered as a re-emerging disease (Maclachlan and Guthrie, 2010), many surveillance and control programmes have been implemented worldwide for early detection and control of the disease. These surveillance programmes require significant economic and human resources and can stress the animals that are being monitored (i.e. sentinel or suspect animals). As a consequence, the development of a rapid, non-invasive and low stress method for detecting suspect cases is required. IRT provides a potential tool for this purpose.

The aim of this study was to evaluate the use of IRT for early detection of fever in sheep experimentally infected with BTV serotype 1 (BTV-1) and serotype 8 (BTV-8), in order to reduce animal stress during experimental assays as well as to develop a screening method for the identification of fever in animals suspected of having BT.

## Materials and methods

### *Animals, virus and experimental design*

All procedures were approved by the Animal Experimental Committees from Córdoba University and the Complutense University of Madrid.

A total of 19 male Merino sheep, 9 months of age, were obtained from a flock declared free of brucellosis, paratuberculosis and maedi-visna according to the Spanish surveillance programmes. During the experiment sheep were housed in the Biosafety Level 3 containment of the Animal Health Surveillance Centre (VISAVET, Complutense University of Madrid, Spain), which is a temperature-controlled facility (20–21 °C).

The sheep were given an adjustment period of 7 days before the experiment started. Prior to inoculation, all animals were treated with anthelmintics and tested negative for BTV RNA and BTV antibodies by real-time RT-qPCR (Toussaint et al., 2007) and a double recognition ELISA (INGEZIM BTV DR 12.BTV.K0, Ingenasa), respectively. The sheep were then divided into three groups and inoculated as follows: (1) BTV-1 group comprised eight sheep (numbered from S1.1 to S1.8) that each received a subcutaneous (SC) inoculation in the axilla of 2 mL containing $10^{6.0}$ tissue culture infective dose 50% ($TCID_{50}$) per mL of BTV-1/AGL2006/01 strain; (2) BTV-8 group comprised eight sheep (S8.1–S8.8) that were each injected SC with 2 mL containing $10^{6.0}$ $TCID_{50}$/mL BTV-8/BEL2006/01 strain; (3) the Control group contained three sheep (C1, C2, and C3) which were each injected SC with 2 mL virus dilution medium (Dubelcco's Modified Eagle's Medium, Sigma–Aldrich).

### *Temperature collection*

The day of inoculation was defined as day 0. Prior to virus inoculation (from day −3 to day 0), rectal temperatures were monitored with a digital thermometer (Catalogue number 291110, Kruuse). At the same time, infrared images were obtained with an infrared thermographic camera (ThermaCam E45, FLIR). After inoculation, alongside a clinical examination, rectal temperatures and infrared images were taken daily throughout the study period. Images were collected at the same hour every morning after the rectal temperatures had been taken and before the animal rooms were cleaned so as to avoid temperature variations induced by the presence of standing water.

The camera was placed 0.5–1 m from the head of the animals. The thermal images were analysed by the ThermaCam Quick View 1.0 and ThermaCam Quick Report 1.0 software. The thermographic temperature used in this study was the maximal eye and surrounding skin area infrared temperature, which was defined as the highest temperature identified by the software utilities on the orbital surface (Schaefer et al., 2007). Since environmental temperature and emissivity could influence the temperature values obtained by IRT, these factors were adjusted in each thermal image. The emissivity of the eye surface was estimated as 0.98 (Girardin et al., 1999), while the environmental temperature was set at 20 °C. In total, 208 rectal temperatures and 208 IRT pictures were collected (Table 1).

### *Blood sample collection*

Blood samples were taken from all sheep from the second day post-inoculation (dpi) onwards and used to confirm the presence of BTV by real-time RT-qPCR (Toussaint et al., 2007). Infected sheep were sedated and euthanased in batches of four at 3, 6, 12 and 15 dpi. The control group sheep were euthanased at the end of the experiment.

### *Statistical analysis*

Data were analysed using the statistical analysis programs GraphPad InStat version 3.0 (GraphPad Software.), SPSS 19 (IBM) and Win Episcope 2.0. The sheep in the uninfected control group showed no significant changes in rectal temperature throughout study (range 39.3–39.6 °C), so their data were not included in the statistical analysis.

Rectal and infrared temperatures were not normally distributed (Kolmogorov–Smirnov test; $P < 0.001$), so Spearman's rank correlation coefficient was used to determine the relationship between rectal and infrared temperatures. Taking into account that the temperature data used were repeated measures, the correlation test was performed using just 16 pairs of data corresponding to the means of temperatures from each animal. Wilcoxon signed-rank test was used assess whether there were differences between the median values of rectal and infrared temperatures.

Previous studies have reported that radiated temperature is generally lower than conductive core temperature (Schaefer et al., 2007), so a cut-off value for the identification fever using IRT was identified by determining the mean difference between the rectal and infrared temperatures and then subtracting this value from 40 °C, which is the rectal temperature considered as fever (Hamblin et al., 1998; Worwa et al., 2010). The cut off value was then used to create a contingency table for a kappa ($\kappa$) analysis to assess the concordance between both techniques as well as to determine the sensitivity and specificity of IRT.

## Results

### *Confirmation of infection status*

Viral RNA was detected by real-time RT-qPCR from 2 dpi in sheep inoculated with BTV-8 and from 3 dpi in sheep inoculated with BTV-1. Most animals were positive from 4 dpi onwards. In addition, moderate clinical signs and lesions characteristic of BT (conjunctivitis, ocular discharge, skin congestion in peri-orbital areas and nostrils, serous nasal discharge and crusts in nasal mucosa) were also observed. Neither viraemia nor clinical signs were detected in uninfected control sheep.

### *Analysis of rectal and thermographic temperatures*

Differences in the thermal images obtained on the orbital surface prior to and after virus challenge were observed (Fig. 1). These changes were associated with a rectal temperature increase (Fig. 2). A positive correlation ($P < 0.05$) between rectal and infrared temperatures was obtained ($r = 0.504$) when data were analysed by Spearman rank test.

### *Cut off value, kappa coefficient, sensitivity and specificity*

Infrared temperatures were lower than rectal temperatures ($P < 0.01$). The mean difference between the two measures was 1.46 °C ± 0.05 °C ($P < 0.005$). Thus, a rectal temperature value of 40 °C was equivalent to 38.54 °C using IRT on the eye surface.

The cut off value (38.54 °C) was used to determine the true positive and true negative cases, as well as the false positive and false negative cases (Table 2). The 11 incorrect diagnoses involved seven animals, four in the BTV-1 group (S1.2, S1.3, S1.5, S1.6) and three in the BTV-8 group (S8.5, S8.6, S8.8). Four of the 11 incorrect diagnoses were identified before virus inoculation and seven after virus