Randomised positive control trial of NSAID and antimicrobial treatment for undifferentiated calf pyrexia

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Abstract

One hundred and fifty four pre-weaning calves were followed between May and October 2015.

Calves were fitted with continuous monitoring temperature probes (TempVerified FeverTag®, Amarillo, Texas, USA), programmed so a flashing LED light was triggered following 6 hours of a sustained ear canal temperature of ≥39.7°C. A total of 83 calves (61.9%) developed undifferentiated pyrexia, with a presumptive diagnosis of pneumonia through exclusion of other calf diseases. Once pyrexia was detected, calves were randomly allocated to two treatment groups. Calves in Group 1 (NSAID) received 2mg/kg flunixin meglumine (Allevinix, Merial) for three consecutive days, and Group 2 (antimicrobial) received 6mg/kg gamithromycin (Zactran, Merial). If pyrexia persisted 72 hours after the initial treatment, calves were given further treatment (Group 1 received an antimicrobial and Group 2 received NSAID). Group 1 (NSAID) were five times more likely (p=0.002) to require a second treatment (the antimicrobial) after 72 hours to resolve the pyrexia compared to the need to give Group 2 (antimicrobial) calves a second treatment (the NSAID). This demonstrates the importance of on-going monitoring and follow-up of calves with respiratory disease. However, of calves with pyrexia in Group 1 (NSAID), 25.7% showed resolution following NSAID treatment only with no detrimental effect on the development of repeated pyrexia or daily live weight gain. This
suggests that NSAID alone may be a useful first line treatment giving a total of 30.1% reduction of antimicrobial usage between the groups in this sample, provided adequate attention is given to ongoing monitoring in order to identify those cases that require additional antimicrobial treatment.

Introduction

Growth and development of healthy calves through the pre-weaning period is important both for ensuring longevity of the animals produced, and to enable rearing costs to be maintained at an economically viable price. A single case of pneumonia is estimated to cost approximately £43 per dairy calf affected, with approximately 47% of dairy calves and 51% of beef animals in the UK being affected (Esslemont and others, 1998). The main economic costs are due to decreased growth rates (Wittum and others, 1996), the cost of drug treatments (Schneider and others, 2009) and mortality which can range from 0.18% to 3.9% (Elliott and others, 2014).

While early detection of calf pneumonia may improve treatment success and reduce infectious spread, the subtle signs associated with early disease (loss of appetite, depression or raised respiratory rate) are difficult to detect and often missed in the farm situation. Nasal discharge typically appears a median of 19 hours after pyrexia develops, followed by a cough at a median of 65 hours after pyrexia develops (Timsit and others, 2011). However many authors advocate the detection of an undifferentiated pyrexia as an indication for the initiation of treatment (Apley, 2006), with the aim being to introduce therapy before the disease has progressed enough to cause clinical signs.

The time consuming nature of manual restraint and examination of rectal temperatures in calves invariably deters regular monitoring for pyrexia in groups of calves. Remote detection of pyrexia through the use of technology is a growing field, with infrared thermography scans and reticulo-rumen temperature boluses having been trialled, but with limited application in the field due to costs (Timsit and others, 2011). Recent development and advances in technology means continuous
real-time monitoring in calves has become more affordable through a novel ear-canal sensor (TempVerified FeverTags®, Amarillo, Texas, USA). Each tag is a self-contained unit, made up of a temperature probe that is inserted deep into the external ear canal, and a small circuit board with an LED light which is secured in the pinna using a standard ear tag applicator.

With regard to the treatment of pneumonia, NSAIDs have demonstrated efficacy in treatment of pneumonia when used in conjunction with antimicrobials due their effects on reduction of lung consolidation (Lockwood, et al., 2003). However, no studies have assessed the use of NSAIDs as a stand-alone treatment where early detection for calf pneumonia is undertaken. Therefore, a rationale for such a course of action could be that if detection is early enough in the course of the disease and the pneumonia of ‘simple’ viral aetiology, then the use of antimicrobials could be considered unnecessary.

This study aimed to use a randomised trial to directly compare the efficacy of NSAID therapy with antimicrobial therapy for the treatment of undifferentiated pyrexia, with the pyrexia defined as sustained ear canal temperature of 39.7°C detected by TempVerified FeverTags. We hypothesise that the use of an early NSAID treatment will reduce the requirement for antimicrobial usage as determined by resolution of the pyrexia.

**Materials and Methods**

A randomised positive control study design was used to compare the level of antimicrobial usage and growth rates between calves that were treated with an initial course of NSAID and an initial course of antimicrobial. The trial protocol was reviewed and approved by the Royal Veterinary College’s Ethical Review Committee (URN 2015 1317) and was granted a VMD Animal Test Certificate (ATC-S-057) before commencement of the study. A power calculation indicated that treatment
group sizes of 48 would detect a 30% difference in the proportion of calves requiring further treatment after 72 hours. Power was set at $\beta=0.8$, significance at $p \leq 0.05$.

Animals

Two Holstein dairy herds were recruited in the South-West of England. Both herds were closed, with vaccination for BVD, IBR and Leptospirosis in use in the adult herd, and no vaccination in the calves. Calves were kept in large barns with a shared air space using natural ventilation, a range of ages from 0-16 weeks old and an all year round calving pattern. All calves were Holstein-Friesian, with both male and female calves recruited into the study. Both farms fed the same milk replacer (20% whey protein, 18.5% fat, 8.3% ash). Further information on the farm management is detailed in Table 1.

Table 1: Description of farm and management systems on Farm 1 and Farm 2.

<table>
<thead>
<tr>
<th></th>
<th>Farm 1</th>
<th>Farm 2</th>
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</thead>
<tbody>
<tr>
<td>Adult Herd Size</td>
<td>300</td>
<td>200</td>
</tr>
<tr>
<td>Colostrum fed</td>
<td>Total 6L within 12 hours</td>
<td>Total 6L within 12 hours</td>
</tr>
<tr>
<td>Milk replacer fed</td>
<td>900g/day from birth, fed at 150g/L</td>
<td>600g/day from birth to 3 weeks, then 800g/day until weaning, fed at 125g/L</td>
</tr>
<tr>
<td>Calf group sizes</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>Milk feeding method</td>
<td>Group teat bucket</td>
<td>Automatic milk machine</td>
</tr>
<tr>
<td>Additional feed</td>
<td>Ad-lib concentrates, straw bedding</td>
<td>Ad-lib concentrates, straw in racks and bedding</td>
</tr>
<tr>
<td>Weaning age</td>
<td>10 weeks</td>
<td>10 weeks</td>
</tr>
</tbody>
</table>
Calves were allocated to treatment groups by random number generation conducted by SAM. Calves were included if they developed pyrexia related to respiratory disease between the ages of 0 to 10 weeks (pre-weaning calves). The origin of the pyrexia was determined to be due to respiratory infection by exclusion of other common calf diseases (navel ill, joint ill, diarrhoea) through a structured protocol for physical examination carried out by the farmer. Calves were removed from the study if they developed other diseases that required NSAID or antimicrobial therapy such as navel ill and joint ill. Calves that developed diarrhoea and were treated with oral electrolyte fluids (Lifeaid, Norbrook) were retained in the study unless they received additional NSAID or antimicrobial treatment (given at the farmer’s discretion).

Pyrexia detection

External ear canal temperature was measured as a proxy for core temperature every 15 minutes using a temperature probe that was inserted 5 cm deep into the external ear canal, and a small circuit board with an LED light which was secured in the pinna using a standard ear tag applicator (TempVerified FeverTag®, Amarillo, Texas, USA) (Figure 1). When the device detected pyrexia (ear canal temperature ≥ 39.7°C) for a sustained period of 6 hours, an LED light would flash for 6 hours to draw attention to the animal. The device would then enter a monitoring phase with the temperature taken every 15 minutes, and flashing would resume immediately if pyrexia was detected again. Only one other study (McCorkell and others, 2014) has used FeverTags for identification of respiratory disease related pyrexia, although an earlier version of the tag was used which did not have the 6 hour monitoring phase of the TempVerified model.

Treatment protocol
Calves were enrolled into one of two treatment groups: Group 1 (NSAID) received Flunixin meglumine (Allevinix, Merial) at 2 mg/kg via intramuscular injection daily for 3 consecutive days starting when the flashing tag was observed. If clinical signs of acute pneumonia developed after 24 hours (spontaneous coughing, severe nasal or ocular discharge, tachypnoea), or if the FeverTag was still flashing after 72 hours (indicating continued pyrexia), calves were given Gamithromycin (Zactran, Merial) at 6 mg/kg via a single subcutaneous injection. Group 2 (antimicrobial) received Gamithromycin (Zactran, Merial) at 6 mg/kg via a single subcutaneous injection starting when the flashing tag was observed. If clinical signs of acute pneumonia developed after 24 hours, or the FeverTag was still flashing after 72 hours, then calves were given a NSAID treatment with Flunixin meglumine (Allevinix, Merial) at 2 mg/kg via intramuscular injection daily for 3 consecutive days.

Pyrexia for up to 72 hours following treatment without clinical disease was deemed tolerable as this allowed sufficient time for full therapeutic action and to prevent unnecessary secondary treatments.

A repeat case of pyrexia was defined as a temperature of ≥39.7°C for 6 hours which was detected at least 10 days following the last treatment given for the initial case of pyrexia. A 10 day duration was chosen as this is the licensed duration of action of Zactran in calves. Repeat cases of pyrexia were treated using the same protocol as initial cases, and recorded as a repeat case of pyrexia within the statistics.

Calves were weighed at birth and weaning using a calf weigh scale (mechanical calf weighing crate, Bateman, UK). Further data on calf mortality and treatments given to the study animals was collected up to 6 months of age from the farm records.

Statistical analysis

Data was analysed using SPSS (SPSS version 21, Lead technologies 2012). Associations between the efficacy of each treatment group, the sex, the farm and the occurrence of diarrhoea was tested using binary logistic regression. Associations between the treatment group and daily live weight gain was tested using analysis of variance (ANOVA). Associations between the requirement for a second
treatment (continuation of pyrexia 72 hours after the initial drug treatment) and sex, treatment group, farm and development of diarrhoea in the first two weeks of life was tested using binary logistic regression for calves that experienced an episode of pyrexia. Kaplan Meier survival analysis was used to assess the age at which respiratory disease related pyrexia was first detected.

**Results**

A total of 154 calves were enrolled into the study between May and October 2015 (Table 2) with 83 developing pyrexia assumed to be related to respiratory disease. Eight calves were excluded due to pyrexia detected by the FeverTag being caused by navel ill, and 12 calves were excluded due to development of diarrhoea which required antimicrobial or NSAID treatment, although no pyrexia was detected by the FeverTags in these calves.

Table 2: Total number of calves recruited into the trial and their division into the different treatment groups once pyrexia had developed

<table>
<thead>
<tr>
<th></th>
<th>Farm 1</th>
<th>Farm 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of calves recruited</td>
<td>66</td>
<td>88</td>
</tr>
<tr>
<td>Calves that developed pyrexia</td>
<td>43</td>
<td>40</td>
</tr>
<tr>
<td>Calves in treatment group 1 (NSAID)</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>Calves in treatment group 2 (antimicrobial)</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>Undifferentiated pyrexia prevalence (%)</td>
<td>65.2</td>
<td>45.5</td>
</tr>
<tr>
<td>Calves excluded due to navel ill</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Calves excluded due to diarrhoea</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

Of the 83/134 (61.9%) calves with respiratory disease related pyrexia, none developed acute signs of respiratory disease within 24 hours of pyrexia being detected. A total 58/83 (69.9%) calves received
antimicrobials as part of the treatment protocol for the initial case of pyrexia detected (26 calves from Group 1 (NSAID) and 32 calves from Group 2 (antimicrobial)). Additional treatments (Group 1 received additional antimicrobial and Group 2 received additional NSAID) were administered after 72 hours in 42 (50.6%) cases due to continued pyrexia. No calves died while on the study although there was a 10% mortality rate amongst the calves excluded from the study (2/20 excluded calves died).

Calves in treatment Group 1 (NSAID) were five times more likely (p=0.002) to require additional treatment (antimicrobial) to resolve pyrexia compared to Group 2 who received antimicrobials as their first line treatment (Table 3). However 25.7% of calves in Group 1 did recover following NSAID treatment alone. There was no significant difference in the number of calves that developed repeated pyrexia between the two treatment groups (p=0.73). The ANOVA indicated there was no significant difference in the daily live weight gain of the calves between the treatment groups (p=0.632), with a mean daily live weight gain of 0.64kg/day (SE +/- 0.02).

Table 3: The odds ratio and p-values associated with the successful resolution of a case of undifferentiated pyrexia.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>0.47 (0.11-2.05)</td>
<td>0.31</td>
</tr>
<tr>
<td>Treatment group</td>
<td>5.09 (1.84-14.10)</td>
<td>0.002</td>
</tr>
<tr>
<td>Farm</td>
<td>0.24 (0.065-0.88)</td>
<td>0.031</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>0.42 (0.11-1.70)</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Results from the binary logistic regression model (Table 4) indicated that calves were less likely to require a second treatment if in Treatment Group 2 (antimicrobial) (p=0.003) and on Farm 2 (p=0.050). There was no effect on the odds of requiring a second treatment of sex (p=0.363) or having diarrhoea (p=0.976).
Table 4: The effect of different variables on the requirement for a second treatment 72 hours after the initial onset of pyrexia was detected.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>2.31 (0.38-14.0)</td>
<td>0.36</td>
</tr>
<tr>
<td>Treatment group</td>
<td>0.16 (0.05-0.52)</td>
<td>0.003</td>
</tr>
<tr>
<td>Farm</td>
<td>4.78 (0.99-22.93)</td>
<td>0.050</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>1.03 (0.17-6.17)</td>
<td>0.98</td>
</tr>
</tbody>
</table>

The Kaplan Meier survival analysis (Figure 2) for time to development of the first pyrexia experienced by pre-weaning calves produced a median age of 25 days (IQR 15-32 days).

Figure 2: Kaplan Meier survival analysis of the age (days) that respiratory disease related pyrexia developed in pre-weaning calves.

For the post-weaning period until 6 months of age, one calf died for unknown reasons (from Group 2) and two calves required additional treatment for calf pneumonia (of which one calf was from Group 1 and one was from Group 2).

**Discussion**

This randomised clinical trial investigated the efficacy of NSAID only treatment in comparison to antimicrobial therapy for undifferentiated pyrexia in calves, which was considered to be due to respiratory disease though a diagnosis of exclusion. The results indicate that using NSAIDs as the first line treatment resulted in calves being five times more likely (p=0.002) to require an additional treatment (antimicrobials) to resolve pyrexia compared to the use of antibiotics as the first line of treatment. However, the initial treatment group had no significant effect on the daily live weight gain or the prevalence of recurrent pyrexia episodes experienced by the calves. The early use of
antimicrobials led to resolution of 64.7% of the initial cases of pneumonia, compared to a 25.7%
success rate using a NSAID treatment alone (Table 2). None-the-less, this study demonstrates the
potential for reducing antimicrobial usage with NSAID initial treatment, as the delay in receiving the
additional treatment of antimicrobials did not appear to have long term detrimental effects on calf
health up to 6 months of age.

There is an internationally recognised need to reduce current usage of antimicrobials in animals
farmed for food production, especially prophylactic and metaphylactic use which has been common
practice on calf rearing units with pneumonia epidemics (Ives & Richeson, 2015). Multi-drug
resistant bacteria are already present within the population of pneumonia pathogens, with
Pasteurella multocida demonstrating concurrent resistance to three antibiotics in 2.1% of isolates
(Jamali and others, 2014). The World Health Organisation (WHO) has recommended restricting the
use of some antimicrobial classes in food-producing animals, along with tighter regulations in some
European countries, highlighting the need for new methods to control antimicrobial usage in order
to maintain their efficacy and availability (WHO, 2014). Establishing treatment protocols that can
improve the rationale and reduce the use of antimicrobials is an important aspect of a veterinarian’s
role in the food-producing animal sector. Traditionally, poor observer sensitivity to the clinical signs
of pneumonia has resulted in administration of treatments to animals whose initial acute
pneumonia has progressed to a chronic suppurative form (Barrett, 2000), resulting in a poor
response to treatment, chronic weight loss and increased mortality rates (Breeze, 1985).

Whether the initial course of treatment following detection of pneumonia is NSAID or antimicrobial
therapy alone, or the two in combination, early detection and resolution of calf pneumonia may
reduce the amount of antimicrobial usage due to limitation of the severity of the disease, and
reduction in spread of pathogens to other animals in the shared air space and therefore reduced
development of new cases. The use of NSAIDs may be an important strategy for clinicians providing
treatment and safeguarding welfare while withholding antimicrobial treatments. There is a strong rationale for NSAID use in cases of pneumonia, primarily to reduce excessive inflammation associated with cell mediated immunity, cytokines and endotoxin release (Panciera & Confer, 2010).

Despite the fact that many initial cases of pneumonia are primarily of viral aetiology (Tuncer & Yesilbag, 2015) and are only complicated by later/secondary bacterial infection, NSAIDs and antibiotics in combination remain necessary for most cases of calf pneumonia as demonstrated in this sample.

The protocol for early pyrexia detection though the use of FeverTags in this study enabled treatment to be administered much earlier in the disease course compared to what is achievable on most farms, leading to a reduction in the transmission of pathogens to other calves in the same air space, and a reduction in the total amount of antimicrobial administered to the calves. This resulted in a total of only 83/154 calves requiring any form treatment, with only 58/83 of the total treatments given for the initial pyrexia detected being antimicrobial, which is a 30.1% reduction in antimicrobial usage compared to a prophylactic antibiotic treatment strategy, and a 62.3% reduction when compared to a metaphylactic treatment strategy. This suggests the use of NSAIDs as a first line treatment for early onset calf pneumonia as indicated by pyrexia may be a suitable treatment protocol provided sufficient attention is given to continuous monitoring and suitable consideration of the need for an additional treatment with antimicrobials if the pyrexia does not resolve. However definitive conclusions on the efficacy of NSAID treatment is difficult to establish due to it being deemed ethically inappropriate to withhold treatment in a negative control group in this study, although further work could explore the effect of NSAID only treatment for early on-set pneumonia through the use of both positive and negative control groups.

One possible reason for the apparent lack of efficacy of NSAID only treatment may have been that the pyrexia threshold used in this study (39.7°C) was too high. Other sources have indicated that the
The upper range for the normal body temperature of cattle is 39.2°C (Divers & Peek, 2008), with a rectal temperature between 38.9 – 39.4°C being given an abnormal classification (Lago and others, 2006). This suggests that lowering the temperature threshold for activation of the temperature monitors may be more appropriate. This may lead to the introduction of therapy before significant lung damage has occurred; in this study it would appear that the initiating agents had already caused damage to the respiratory clearance mechanisms and lung parenchyma when treatment was initiated, so facilitating secondary bacterial infection in the compromised lung (Taylor and others, 2010) with the resultant need for antimicrobials. This pathogenesis is supported by the high success rate of the antimicrobial therapy in this study, which supports the theory that it is the presence of bacterial pathogens that has caused the pathology in the respiratory tract, resulting in an increased requirement of Group 1 (NSAID) calves (39%, p<0.01) to be treated with antimicrobial due to lack of resolution of pyrexia. Another possible reason for the reduced efficacy of the NSAID treatment is the anti-pyretic nature of this drug type. Respiratory viruses have an optimal body temperature range for their in vivo survival, and the development of pyrexia may actually be beneficial as part of the immune defence system (Apley, 2006). This could mean that antipyretics are not an optimal treatment during per-acute viral pneumonia infection. Further work comparing NSAID treatment with a negative control group would further elucidate this question.

The overall period prevalence of pneumonia detected by this study was 61.9% which is higher than 47% suggested by ADAS (2015), although this study did have a lower population number of calves who were selected due their high risk housing management. Both farms had calves housed in large shared air spaces, without the minimum recommended number of four air changes per hour being met (Bates & Anderson, 1979). This can result in raised airborne bacterial levels, which also occurs with raised stocking densities (Lago and others, 2006). Although the majority of airborne bacteria are non-pathogenic, they can provide an additional burden to the respiratory tract defences (Wathes, et al., 1983). The use of group pens with no solid barriers has also previously been
demonstrated to be a risk factor for calf pneumonia due to reduced exchange of airborne pathogens between pens as well as preventing direct nose to nose contact (Lago and others, 2006). All of these risk factors were present on the study farms, which may account for the high incidence of pneumonia detected. The median age at which pneumonia was detected in this study was 25 days, which is in agreement with Elliott and others (2014) that indicated 53.7% of pneumonia occurred in calves aged between two weeks and two months. This confirms that close monitoring of calves during this time period is important.

The eight calves excluded for developing navel ill all developed pyrexia that was identified by the FeverTags, but only two of the 12 calves excluded for diarrhoea were detected as pyrexic. The clinical exam and diagnosis was carried out by a trained farmer, although no confirmatory diagnostic tests were carried out. This indicates that the temperature monitors can be beneficial for detecting any calf disease that produces pyrexia such as navel ill, with activation of the temperature monitors triggering a general clinical exam of a calf, therefore increasing detection rates of disease. During this study, no cases of otitis or other ear infections were observed to be caused by the placement of the FeverTags, with only some mild inflammation noted around the FeverTag placement in the pinna which would be expected during normal identification ear tag placement.

A significant advantage of the temperature monitors in the study was the constant real-time monitoring of the calves’ health status, aiding in early detection and therefore prompt initiation of treatment. In many conventional systems, continued monitoring of sick calves after treatment is challenging, leading to a further delay in the provision of secondary or repeat treatments for calves that continue to experience pyrexia or progression of clinical symptoms. A total of 42 calves experienced continued pyrexia following initial treatment, with a further 23 calves having a repeat episode of pyrexia, indicating the high requirement for continued monitoring of animals previously identified as sick.
A major limitation of this study was the lack of definitive diagnosis and pathogen identification from pyrexic calves, as this would have allowed more robust conclusions to be drawn regarding the nature of the primary respiratory disease pathogens. Pneumonia in pre-weaned calves can have both viral and bacterial aetiologies, with bacterial pathogens primarily occurring following stressful procedures such as transport, castration or disbudding (Gorden & Plummer, 2010). In this study, the detection of pneumonia was primarily by development of pyrexia, with the exclusion of other causes through a thorough clinical examination since early cases of pneumonia would be unlikely to exhibit typical clinical signs such as raised respiratory rate, altered respiratory character, observed anorexia, nasal discharge, ocular discharge, coughing, and depression (Apley, 2006). This is supported by Apley (2006) who concluded that in order to initiate early treatment, a presumptive diagnosis of pneumonia would often have to occur on the basis of depression and an undifferentiated fever. Combining the use of TempVerified FeverTags with other calf monitoring tools such as the scoring system described by Lago and others, (2006) whereby individual animals are examined and given a clinical score based on temperature, nasal discharge, cough, ocular discharge and ear position may provide the most sensitive and specific method for the early detection and therefore treatment of calf pneumonia.

Conclusion

Calf pneumonia is a costly disease affecting animals both in the dairy and beef industry, with long lasting consequences on growth and productivity. The initiation of early treatment is important for reduction in lung pathology, reducing risk of secondary infection and stopping progression of clinical signs. The targeted use of NSAIDs and antimicrobials in pyrexic calves and continued monitoring post-treatment provides a time-efficient and easy to use method to help stock people ensure high standards of calf welfare and health are maintained. Although prevention of pneumonia will be the
target for calf producers, the use of real-time temperature monitoring systems along with targeted
therapy does allow for very early identification and initiation of pneumonia treatment, along with a
30.1% reduction in antimicrobial usage in this study.

Competing interests

The TempVerified FeverTags were provided to the study by FeverTags LLC®, Amarillo, Texas, USA.
The NSAID Allevinix and antimicrobial Zactran were provided by Merial UK.

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