



Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvj

Pharmacokinetics and effects of cetirizine in horses with insect bite hypersensitivity

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ARTICLE INFO

Article history:

Accepted 23 December 2009

Available online xxxx

Keywords:

Horse

Antihistamine

Cetirizine

Pharmacokinetics

Insect bite hypersensitivity

ABSTRACT

Horses with insect bite hypersensitivity (IBH) have difficulty in completely avoiding allergens, so effective treatment options are required. A randomised, placebo controlled and double blinded field study was conducted to determine the pharmacokinetics and efficacy in reducing dermatitis of the antihistamine cetirizine given orally at 0.4 mg/kg twice daily for 3 weeks. The influence of protection blankets and stabling were also investigated.

The estimated maximum plasma concentration (C_{max}) and trough plasma concentration of cetirizine were 135 ng/mL and 18 ng/mL, respectively. There was no difference in dermatitis reduction between the treatment and placebo groups ($P = 0.77$). The findings indicated that cetirizine was of no apparent benefit in treating IBH at the dose rate tested. The use of blankets and stabling were shown to have favourable influence on the dermatitis ($P < 0.05$) and may be the preferred options to prevent this condition.

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Introduction

Insect bite hypersensitivity (IBH), also called 'sweet itch' or 'summer eczema', is a worldwide chronic seasonally recurrent pruritic allergic dermatitis in horses hypersensitive to proteins in the saliva of biting insects, in particular those of the genus *Culicoides* and occasionally *Simulium* spp. (Greiner et al., 1988, 1990; Hallorsdottir et al., 1989; Fadok and Greiner, 1990; Anderson et al., 1991, 1993; Marti et al., 1999; Mullens et al., 2005; Baselgia et al., 2006). Due to the preferred feeding sites of the midges, the mane, tail and ventral midline are the most affected areas of the skin (Townley et al., 1984; Braverman, 1988). The intense pruritus leads to scratching and rubbing and the horses develop papules, crusts, alopecia and lichenification of the skin at the affected sites. Treatment usually involves avoidance of exposure to the insects by overnight stabling, use of blankets and insect repellents. In addition, symptomatic treatment with corticosteroids and antihistamines has been applied, although little is known about the efficacy of antihistamines in the field.

Immunoglobulin (Ig) E plays a role in the pathogenesis of IBH (van der Haegen et al., 2001; Wilson et al., 2001, 2006; Rüfenacht

et al., 2005; Ferroglio et al., 2006; Hellberg et al., 2006; Wagner et al., 2006). Binding and aggregation of allergens to specific IgE antibodies attached to high avidity receptors (Fc ϵ RI) of mast cells in the skin induce degranulation and release of histamine and other inflammatory mediators, such as leukotrienes, interleukins, prostaglandins, tryptase and kinins, causing the clinical signs of the allergy. Histamine exerts its effects via interaction with H₁-receptors, present in most tissues. Histamine H₁-antagonists, such as tripeleminamine, promethazine and chlorpheniramine, have been tested experimentally in horses (Morrow et al., 1986; Foster et al., 1998) and used tentatively in hypersensitivity conditions and urticaria (Rosenkrantz, 1995). However, the effects of antihistamines in insect bite hypersensitivity in horse have not been explored in detail.

Antihistamines may also be used in horses diagnosed with atopic dermatitis, which are IgE-related pruritic reactions to environmental allergens other than biting insects (White, 2005). In these horses, pruritis is also commonly seen during non-insect seasons of the year. There are overlaps in the clinical signs of insect bite hypersensitivity and atopic dermatitis and sometimes the horses can initially show a typical seasonality, which then progresses to continual dermatitis. There are difficulties in discriminating these diagnoses by intra-dermal or serological testing methods

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(Ferroglio et al., 2006; Frey et al., 2008). Conceivably, an effective H₁-antagonist for oral treatment may be used in these conditions in horses, regardless of the aetiology.

Cetirizine, a metabolite of hydroxyzine, is a non-sedative, second generation histamine H₁-antagonist i.e. widely prescribed and with demonstrated efficacy in the treatment of seasonal/perennial allergic rhinitis, pruritis and chronic idiopathic urticaria in humans (Zuberbier and Henz, 1999; Simons, 2001). Cetirizine also displays high affinity and selectivity for cloned human H₁ histamine receptors and has very little or no anti-cholinergic activity (Gillard et al., 2002; Orzechowski et al., 2005).

Species differences limit the possibilities for extrapolating doses between different animals. It is therefore important to examine the fate and effects of drugs in the target species. We have recently studied the pharmacokinetics and the pharmacodynamics of cetirizine in horses given a single oral dose or repeated oral doses of the drug (Olsén et al., 2007, 2008). The results showed that cetirizine has favourable pharmacokinetic and pharmacodynamic profiles in the horse and may therefore be useful in this species. The aim of the present study was to examine the pharmacokinetics and the clinical response of cetirizine in horses with insect bite hypersensitivity under field conditions. The influence of blanket protection and stabling on the dermatitis was also explored.

Materials and methods

Horses

A total of 157 horses with a history of seasonal dermatitis were initially selected to participate in the study. An inclusion condition was that the horse had shown clinical signs of IBH during the insect season for at least two consecutive years prior to the study. The horses were of different breeds, sex and age and were held at 32 different farms throughout Sweden. They were fed, watered and treated as usual during the experiment. The study protocol was approved by the Local Ethics Committee for Animal Experiments, Uppsala, Sweden.

Study design

The study was designed as a double blinded test. There were at least two horses at the same farm and the horses were matched in pairs. To be included in the study each pair had to have a history of approximately the same degree of dermatitis. One of the horses in each pair was randomly selected and treated with cetirizine, and the second animal received placebo. In some cases the matching was performed between three horses in which case one or two of the horses were randomly treated with either cetirizine or with placebo. The horse in each pair was treated in the same way during the treatment period, such as wearing protective blankets or stabling during nights. Cetirizine tablets (cetirizine dihydrochloride, 10 mg) were a kind gift from Merck NM. Tablets containing sodium cyclamate plus sodium saccharine (Suketter, tablets, Cederroth International) were used as a placebo.

The horses were given the tablets mixed with sugar-beet in the morning and in the evening daily for 3 weeks. The dose of cetirizine was 0.4 mg/kg bodyweight (BW) $\times$ 2/day as this dose had been shown to have an excellent antihistamine effect in a previous experimental study performed at our department (Olsén et al., 2008). One criterion to start the treatment (cetirizine/placebo) was that the horses showed clinical signs of dermatitis. Anamnesis and clinical examination were performed by the same veterinarians before and directly after the treatment period. A standardised protocol was used to estimate the degree of dermatitis in each horse.

From each horse, one blood sample (10 mL) was collected into heparinised tubes immediately before the start of the treatment and one after the last cetirizine administration (at time intervals varying from 1 to 42 h) following the 3 weeks treatment period. The blood was centrifuged at 1500 g for 15 min and the plasma was separated and stored at -20°C until analyses.

Analysis of cetirizine

The protocol was adopted from Olsén et al. (2007). Briefly, cetirizine dihydrochloride (Sigma–Aldrich) and internal standard [²H₄]-cetirizine (C/D/N Isotopes) were used as reference standards. Stock solutions of cetirizine and [²H₄]-cetirizine were prepared in methanol–water (50:50) and stored at -20°C until used. The water was of Millipore quality and all other solvents and reagents were at least of analytical grade. Different concentration-intervals in the calibration curve were used for different samples and the limit of quantification was 0.11 ng/mL. For evaluation of the accuracy, the quantification precision as percentage relative standard deviation (RSD) and the linearity of the method quality control (QC) samples in three different concentrations were prepared by spiking blank plasma. The standards and the QC samples were treated in the same way as the plasma samples obtained from the horses given cetirizine.

Pharmacokinetic calculations

The measured plasma concentrations of cetirizine were plotted versus time and the data were analysed using a commercially available software program (Win Nonlin 5.0.1, Pharsight Corporation). A non-compartmental model for sparse data was used for the analysis. The weighting factor of choice was $1/Y$, where Y is the observed concentration. The terminal half-life was determined from $t_{1/2} = \ln 2/\lambda$. The mean maximum plasma concentration (C_{max}) was calculated from the blood samples collected at time points from 1 to 1.5 h after the last administration. The trough plasma concentration was read from the terminal slope at the time point 12 h.

Assessment of the dermatitis

A standardised protocol was used to estimate the degree of dermatitis in each horse. The protocol included 13 different zones on the body of the horse, and in each zone the following categories of symptoms were used: (1) swelling, (2) crust, (3) sore and (4) lichenification. In each zone the respective symptoms were judged and scored by a scale from 0 to 3, where 0 = no signs of dermatitis, 1 = mild, 2 = moderate and 3 = severe signs of dermatitis (Fig. 1), so the extent and severity could be judged when all the scores were put together. Sixteen veterinarians were engaged in the clinical examinations of their own clients' farms and horses. Thus the same person performed the examinations before and after the treatment period. In the protocol it was also noted if the horses were stabled during the nights and/or if they were wearing protection blankets.

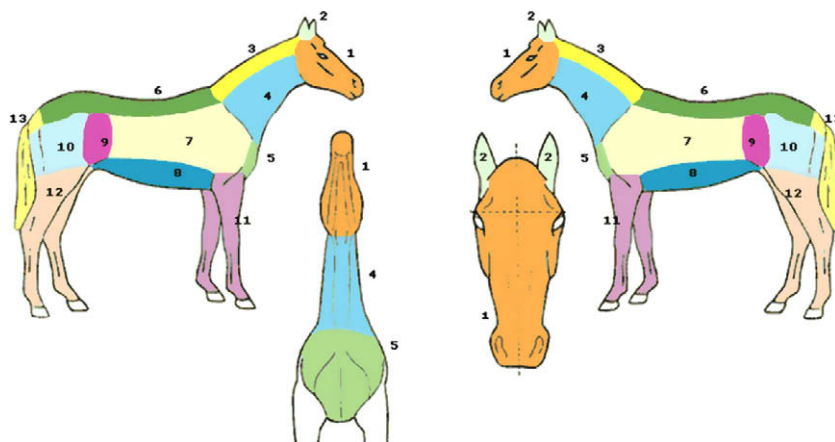


Fig. 1. The 13 different body zones used to estimate the degree of dermatitis. Each zone was judged using a scale of 0–3 and, divided in four different categories: (1) swelling, (2) crust, (3) sore and (4) lichenification. 0 = no signs of dermatitis, 1 = mild, 2 = moderate, 3 = severe signs of dermatitis. Figure modified from web-questionnaire used in Eriksson et al. (2008).

Statistical analysis

For the analysis of the dermatitis data, a general linear mixed model was used to compare the effect of cetirizine and placebo before and after treatment. The value before treatment was used as a covariate. The model included treatment (cetirizine or placebo) as a fixed factor, and farm was used as a random factor. The pairs were used as blocks within the farms. Statistical analysis was performed for all the different symptom-categories (swelling, crust, sore and lichenification) and body-zones, all together producing a dermatitis score. This total estimation of the dermatitis score was used as one parameter to optimise the statistical strength.

We also explored whether covering the horses with protective blankets and/or housing them in a stable overnight would have a beneficial effect on the dermatitis. We estimated the degree of dermatitis before the inclusion of the horses in the treatment groups. The first group, designated 'Blanket and stable', comprised 22 horses that wore blankets during the daytime and were stabled overnight; the second group of 67 horses, designated 'Other', included: (1) horses that were neither stabled at night nor wore blankets ($n = 21$), (2) horses wearing blankets but not stabled overnight ($n = 37$), and (3) horses without blankets and stabled overnight ($n = 9$). The SAS (2004) procedure 'Mixed' was used for the analyses and P values <0.05 were considered significant. Data were also described using means, standard deviations, medians and ranges.

Results

Criteria for exclusion

Of the initial 157 horses selected to participate in the study, a total of 68 horses (34 pairs) were excluded for different reasons. e.g., a horse owner interrupted the treatment, a mare was pregnant, the animal never developed dermatitis during the season, the owner had mixed cetirizine and placebo or had failed to send in the protocols. Eight horses treated with placebo had detectable levels of cetirizine in the plasma (0.2–0.74 ng/mL) following the treatment period. These horses, together with the matching pair-horse (total 16 horses) were also excluded from the study.

Two horses (located at different farms and at different time points) treated with cetirizine suddenly exhibited an aggravation of the dermatitis. Since it could not be excluded that the symptoms were due to an adverse reaction to the drug, the treatment was stopped, and the horses were given glucocorticoids and recovered within a few days. The remaining 89 horses at 32 farms were divided in 42 groups (37 pairs and five groups of three horses). Forty-five of these horses were given cetirizine and 44 were given placebo. Of the 89 horses, 49 were mares, 39 geldings and there was one stallion. The numbers of horses from the different breed groups are presented in Fig. 2 and the age distribution is shown in Fig. 3. No other adverse effects of the drug were noticed during the treatment.

Pharmacokinetics

The accuracy of the drug analysis was 97–101%; RSD varied between 1.9% and 5.2% ($n = 10$ for each QC level); the correlation was 0.9994–0.9997. A total of 153 blood samples were analysed.

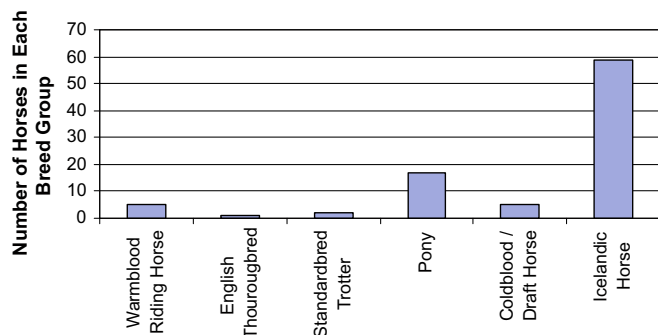


Fig. 2. Number of horses in different breed groups from a total of 89 horses with allergic dermatitis included in the statistical evaluation.

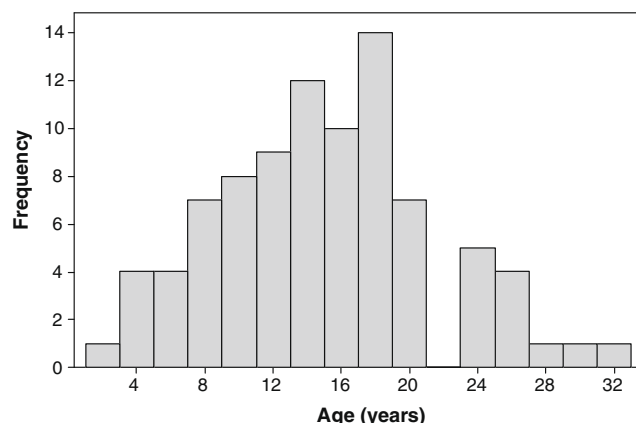


Fig. 3. Age distribution of the 89 horses with allergic dermatitis included in the statistical evaluation.

Among these, 15 (randomly chosen) blood samples were from animals sampled before the treatment started. The other 138 blood samples were from the blood sampled after the 3 weeks treatment period.

All blood samples taken from horses treated with cetirizine had detectable levels of the drug (the time intervals at which the blood samples were taken varied from 1 to 42 h after the last cetirizine administration). Due to the exclusions mentioned above, 42 of the blood samples from horses treated with cetirizine were included in the kinetic study. In these horses the blood samples were taken at time intervals from 1 to 24 h after the last cetirizine administration. None of the 15 analysed blood samples collected before start of the treatment period had any detectable levels of cetirizine.

The plasma concentrations of cetirizine observed following oral administration to the 42 horses with IBH twice daily for 3 weeks are shown in Fig. 4. The following pharmacokinetic parameters were estimated for the cetirizine: $t_{1/2}$: 3.5 h; time to maximum plasma concentration (T_{max}): 1.25 h; mean C_{max} : 135 ng/mL (at 1–1.5 h) and trough plasma concentration at 12 h: 18 ng/mL. The correlation coefficient, r , was 0.98 for the predicted curve of the terminal $t_{1/2}$.

Dermatitis

There were no differences in clinical signs of the dermatitis between the two treatment groups after the 3 weeks treatment with cetirizine ($P = 0.77$; Fig. 5).

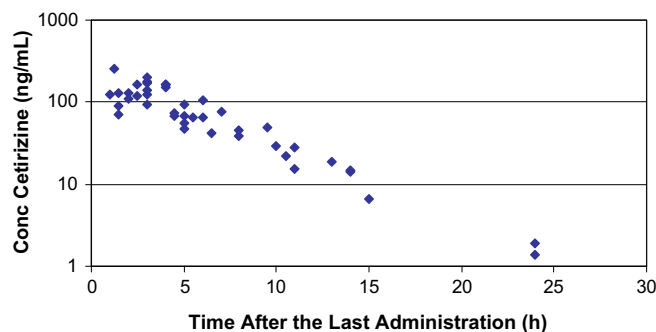


Fig. 4. Plasma concentrations of cetirizine administered orally twice daily (0.4 mg/kg bodyweight $\times$ 2) during 3 weeks to 42 horses with insect bite hypersensitivity. The dots represent the plasma concentrations in the individual horses sampled after the last cetirizine administration.

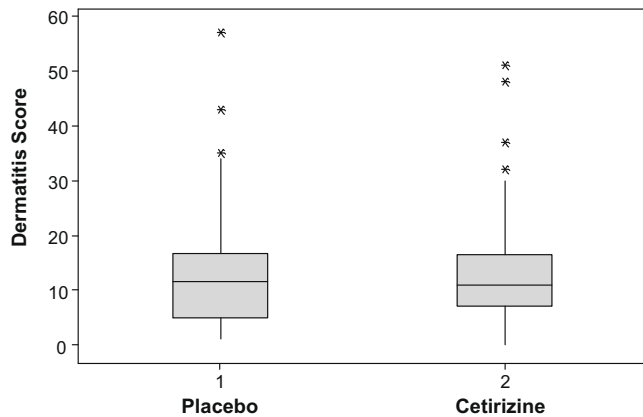


Fig. 5. Dermatitis-scores after 3 weeks treatment with placebo (1, $n = 44$) or cetirizine (2, $n = 45$) from a total of 89 horses with insect bite hypersensitivity. The boxes represent the middle half of the data, the whiskers extend to the extreme values; the asterisks represent the outliers and the lines inside the boxes represent the medians. There were no differences between the placebo group and the cetirizine group (Mixed Procedure Type 3 Tests of Fixed Effects; $P < 0.77$).

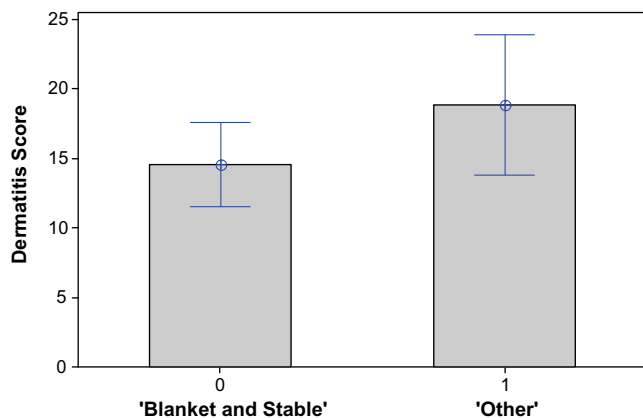


Fig. 6. Dermatitis scores (mean $\pm$ SD) in horses with insect bite hypersensitivity, determined before the start of treatment with placebo or cetirizine. The group 'Blanket and stable' had significantly lower estimated dermatitis scores compared to the 'Other' group. Group 0 'Blanket and stable': were horses wearing a blanket during the daytime and being stabled overnight ($n = 22$). Group 1 'Other' ($n = 67$) were horses that were not stabled at night and did not wear blankets ($n = 21$), horses with blankets but were not stabled overnight ($n = 37$) and horses without blankets that were stabled overnight ($n = 9$). Statistical analysis was performed by the Mixed Procedure Type 3 Tests of Fixed Effects ($P < 0.05$).

Group 'Blanket and stable' (horses which were covered with a blanket and were kept in a stable overnight) showed a significantly less severe dermatitis compared to the 'Other' group (horses with blanket alone, those only stabled overnight, and those not subjected to any treatment, $P < 0.05$; Fig. 6).

Discussion

Horse owners showed considerable interest in this study. We were therefore initially able to include 157 horses with a history of IBH although about 40% of these horses were subsequently excluded from the analysis. Thirty-four horses were directly excluded, but since these were divided into pairs or groups of three horses in the study design, the second horse in the pair also had to be disqualified. Therefore a total of 68 horses were excluded.

The two horses that suddenly became worse during the treatment were suspected to have had an allergic adverse reaction. In

other horses, the treatment was interrupted when the horse owners realised that they had mixed up cetirizine and placebo. Eight horses treated with placebo had detectable levels of cetirizine in the plasma. In these cases, we chose to exclude all these pairs of horses from further statistical analysis, even if the level of cetirizine was low and might have been caused by contamination, such as if the treatment was administered by the same manger.

The majority of the horses were Icelandic horses and ponies suggesting that the incidence of summer eczema is high in these breeds in Sweden. The bias for certain breeds may in addition reflect the fact that the owners of these horses had been informed about the study to a greater extent than other owners, as certain magazines concerned with Icelandic horses had published some advance information about our work.

In spite of the fact that the pharmacokinetic analysis was based on population data from the individual horses treated with cetirizine for 3 weeks, the results showed low variability when the plasma concentrations in the horses were plotted against the time intervals at which the blood samples were collected. In our previous study, the EC_{50} level (concentration of cetirizine in plasma to produce half the maximal antihistaminic effect) was about 18 ng/mL (Olsén et al., 2008). In the present study, the estimated trough plasma value was 18 ng/mL, which indicated that the plasma levels were maintained over EC_{50} for at least 12 h. This should have provided satisfactory antihistaminic effects throughout each day.

The terminal half-life of 3.5 h was similar to that observed in our previous study in which horses were given a single oral dose of cetirizine (median 3.4 h; Olsén et al., 2007), but shorter than in our previous study in which horses were given repeated oral doses of the drug (median 5.8 h; Olsén et al., 2008). In the present study, the pharmacokinetic analyses were based on rather few samples at time points beyond 12 h which might have led to an underestimation of the $t_{1/2}$. The C_{max} of 135 ng/mL was similar to that observed in our previous study when horses were given repeated oral doses of 0.4 mg/kg of the drug (132 ng/mL; Olsén et al., 2008).

We chose to combine the data from all body zones in the statistical analyses since the sites at which the dermatitis occurred were very different in different horses, leading to several 'zeros' in the protocols. Combining the data increased the statistical power and made analyses more robust, although no difference between cetirizine and placebo-treated horses could be observed. The allergic reaction related to IBH is predominantly a type-I hypersensitivity mediated by IgE and possibly IgG isotypes (Hellberg et al., 2006; Wagner et al., 2006). It has also been shown that the antibody-dependent reaction occasionally is followed by a delayed response (type-IV allergy) induced by allergen-specific T cells (Ferroglio et al., 2006).

It would appear that although IgE is known to be important for the development of the dermatitis related to IBH (van der Haegen et al., 2001; Wilson et al., 2001, 2006; Rüfenacht et al., 2005; Ferroglio et al., 2006), the role of histamine is complex and not yet fully understood; moreover several other mediators may also be involved. H_4 -receptors may play a dominant role in pruritic responses (Dunford et al., 2007) and H_1 -antagonists, such as cetirizine, may be less effective. Nevertheless the major finding in the present study suggested a limited role of histamine in the pathogenesis of IBH.

Histamine has greater prominence in the initial phase of the pruritic diseases and antihistamines may therefore be more efficacious at this time. The horses can also aggravate their dermatitis by scratching and rubbing, which may lead to serous effusion, hair loss and secondary skin lesions. As crusts develop and, later on, sores and alopecia, then lichenification, it would be difficult in some cases to see improvement in 3 weeks, and in any case antihistamines may be less effective at this stage

although they can be useful in relieving pruritis and reducing scratching and rubbing.

There was a significantly lower dermatitis score (estimated before the treatment started) in horses that were covered with a blanket and stabled overnight in comparison with horses that were only covered with blankets, only stabled at night, or left without either of these treatments. Thus, an effective way to alleviate symptoms is to take steps to minimise exposure of the horses to insects, although this is clearly difficult to achieve. However, *Culicoides* spp. are vectors of other mammalian pathogens and other insect species may induce IBH in different regions. For example, a recent study in the Netherlands showed that the most common species causing problems for horses is *C. obsoletus* (van der Rijt et al., 2008). Although there were great variations in the number of midges in different locations due to environmental conditions (such as the presence of standing water), the peak of activity at sunset and sunrise suggested that stabling overnight was a useful practice in order to reduce exposure to allergens.

Conclusions

Cetirizine, a potent and effective antihistamine, was not effective in treating recurrent IBH in horses despite being administered orally at 12 h intervals. Increased knowledge about the different insect species, their activity, environment and host preferences, ambient temperatures and the ability to induce disease is recommended.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

Acknowledgements

This study was supported by the Swedish Foundation for Equine Research and by the Hippocampus program of the Swedish University of Agricultural Sciences (SLU).

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