Environmental heat and airborne pollen concentration are associated with increased asthma severity in horses

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Summary

Reason for performing the study – Clinical exacerbations of severe equine asthma are more frequently reported during winter, when horses are exposed to airborne dusts during stabling. However, we have also observed a worsening of clinical signs on days of heatwave.

Objectives – We sought to investigate the association between environmental temperature and humidity and clinical signs of asthmatic horses during clinical exacerbation of the disease.

Study design – Retrospective longitudinal study.

Methods – Historical data of 14 severe asthmatic horses exposed to a dusty environment and evaluated using a previously validated clinical score system were analyzed. Barn temperature and relative humidity values were obtained, and air enthalpy (h) was calculated. Correlation tests were used for studying the relationship between mean daily clinical scores of horses and environmental variables. Lung function parameters recorded at 4-day interval during hot (25°C) and warm (18°C) barn conditions were compared using paired t-test.

Results – Significant positive correlations were observed between the mean daily clinical score and temperature ($r=0.58$, $p=0.01$) and air enthalpy ($r=0.55$, $p=0.02$). Maximal daily temperature correlated with airborne pollen concentrations ($r=0.51$, $p=0.0002$). Higher barn temperature and enthalpy, in absence of changes in the management of horses, were associated with increased transpulmonary pressure ($p=0.005$), pulmonary resistance ($p=0.008$), and elastance values ($p=0.005$).

Conclusions – Providing a cold environment could help attenuating the severity of airway obstruction in uncontrolled exacerbation of severe equine asthma. Furthermore, variations in environmental heat and associated pollen concentrations should be taken into account when evaluating the response to therapy in clinical or research settings.

Introduction

Severe equine asthma (also known as Recurrent Airway Obstruction, RAO, or heaves) is a chronic obstructive respiratory condition affecting 15 to 20% of adult horses living in temperate climate [1]. The risk of disease exacerbation increases during winter months [1; 2], when horses are stabled for extended periods of time and exposed to endotoxins, molds, mites, as well as other dust particulate matters present in hay and straw [3; 4]. Although not described in asthmatic horses, cold-induced bronchoconstriction could also play a role [5].

We have occasionally observed unexpected worsening of clinical signs in severe asthmatic horses during hot summer months, driving the hypothesis that hot environmental conditions could negatively affect lung function in affected horses. A cholinergic-mediated reflex inducing bronchoconstriction in response of breathing hot humid air has been shown in asthmatic patients [6] and could be present also in the equine form of the disease, given the similarities among the two conditions [7]. Alternatively, the increased respiratory effort observed could have been secondary to thermoregulation strategies leading to altered breathing patterns with minimal changes in lung function (i.e. pulmonary resistance and elastance). Finally, pollens have been implicated as triggers for clinical manifestations of the summer form of equine asthma (SPAOPD) [8]. While evidence linking severe equine asthma exacerbations to these antigens is lacking [9], they could act as non-specific irritants for the reactive airways of affected horses. This study was therefore undertaken with the aim to investigate retrospectively the short-term effect of environmental temperature, humidity, and antigenic load (airborne pollens and spores) on the clinical status of asthmatic horses during clinical exacerbation of the disease.

Methods

All the procedures described were performed as part of another study and approved by the local Ethics Committee (Rech-1324). Environmental data were obtained and analyzed retrospectively. Fourteen severe asthmatic horses aged 15.1±4.4 years (mean±SD; range: 7-30) and weighting

519±64 kg were studied. There were 5 Quarter Horse, 4 Standardbred, 2 Canadian, 2 Paints and 1 Arab mixed breed, of which 4 were geldings and 10 females. Study design is summarized in **Fig 1**. All horses had been kept at pasture for at least 4 months before the beginning of the experimental phase of the study. Antigen exposure started on the 15th of April 2014 and was protracted for 6 weeks. During this period, horses were stabled and fed hay. Stabling conditions (bedding, ventilation, number of animals kept within the facility, hay type/batch and quantities administered) remained the same for the duration of the study. Horses were turned out in a paddock 2 to 6 hours/day in the afternoon. An 8-point clinical score previously validated in horses and ranging from 1 (normal) to 4 (severe effort) for both nasal and abdominal effort during breathing [10] was performed between 8:00 and 10:00 a.m. during the first 5 weeks of antigen exposure. Scoring was made by one of 3 trained operators in optimal agreement (interclass correlation coefficient>0.8), as this is part of the antigen challenge monitoring protocol of our laboratory. During the 6th week, pulmonary mechanics were performed in the stable where horses were housed between 8:00 and 10:00 a.m., on Monday (retrospectively considered a "hot" day based on the average temperature for this time of the year in our geographical area: 25°C, 60% relative humidity, RH, 55.27 kJ/kg enthalpy (h), indoor values at 8:00 a.m.; versus 19°C, 71% RH, 43.65 kJ/kg h outdoor) and on Friday (retrospectively considered a "warm" day: 18°C, 61% RH, 37.82 kJ/kg h indoor at 8:00 a.m., versus 15.5°C, 77% RH, 36.82 kJ/kg h outdoor). Briefly, transpulmonary pressure was measured with an esophageal balloon catheter connected to a pressure transducer, and breathing flow signals obtained from a heated pneumotachograph connected to a mask. Pulmonary resistance and elastance values were derived using the flexiWare 7.6 software[a].

Temperature and relative humidity outside and within the stable at 8:00 a.m. were obtained from www.meteoblue.com and from the archives of the barn in which horses were housed, respectively. Temperature and humidity in the stable are recorded twice daily (8:00 a.m. and 4:00 p.m.). The concentrations of outside airborne pollens and spores were obtained from the Aerobiology Research Laboratories[b] information service. Measurements were performed at a station located 50 km west

from the stable where the horses were kept. A complete list of the airborne allergens tested is provided online (Supplementary item 1). Enthalpy (h, expressed in kJ/kg) of the ambient air was calculated using the formula: $h = T + x\ (2500 + 1.9T)$, where T is temperature and x is the specific humidity (or moisture content) of humid air. Further details on enthalpy calculation are provided online (Supplementary item 2). Enthalpy was chosen as it approximates to which extent a given combination of temperature and humidity affects heat dissipation.

Statistical analyses were performed with SAS/STAT software[c] and Prism 5[d]. A regression model was used to identify data to be included in the analysis. In order to avoid biases due to the concomitant effects of antigen exposure and season-related increase in temperature on the horses' clinical scores, a piecewise regression model was employed for differentiating the initial raising phase of the clinical score curve, where barn antigen exposure is likely to exert a predominant effect, from the following plateau, where the effect of antigen exposure has reached a stable phase. We fitted a model including two different slopes and an inflexion point. The equation for the first segment before the inflexion time is *score = a + b*time* and the equation for the second segment is *score = a + b*time + c*(time-inflexion time)*. Only data obtained during the second segment of the curve (stable phase) were studied. The effect of the environmental variables on the mean daily clinical score obtained from the horses was analyzed using Pearson or Spearman correlation test, depending on data distribution. Indoor and outdoor meteorological variables were compared with Pearson correlation tests. The effect of hot vs warm environment on lung function was assessed with paired t-tests. Pearson correlation coefficient was also calculated to determine whether the pairing was effective (that is, whether the direction and magnitude of the variation induced by the warm vs the hot conditions were similar in all horses). Normal distribution of data was assessed with the Kolmogorov-Smirnov test. P-values <0.05 were considered significant.

Results

Fig 2 shows the time-trend of the mean clinical score (daily mean of all the horses studied, panel A) together with the environmental variables studied (panel B and C). The non-linear model indicates that the slope of the curve ('*b*') was significantly greater than 0 before the inflexion point (confidence interval not including 0), but it became not different from 0 after the inflexion point (confidence interval includes 0). The estimated inflexion point corresponded to 1st May 2014. These findings provided the rationale for including only the data observed after the first 15 days of antigen exposure into statistical analysis.

From day 15 to 35, significant correlations were observed between the daily mean of 14 individual clinical scores of the horses and the indoor temperature (r=0.58, p=0.01, **Fig 3A**) and enthalpy (r=0.55, p=0.02, **Fig 3B**). There was also some evidence of a correlation between the mean clinical score and the indoor RH, but it was not statistically significant (r=0.44, p=0.08, **Fig 3C**). Indoor and outdoor temperature (r=0.94, p<0.0001) and RH (r=0.62, p=0.002) recorded at 8:00 a.m. during the whole study period as well as indoor and outdoor enthalpy values (r=0.85, p<0.0001) were strongly correlated.

Overall, during the period studied, daily airborne pollen concentrations correlated strongly with outdoor maximal daily temperature (r=0.51, p=0.0002), while spore concentrations correlated with minimal daily temperature and RH (r=0.44, p=0.002, and r=0.29, p=0.047, respectively). The most abundant outdoor airborne pollens during the period studied were tree pollens (deciduous trees > coniferous trees), with only limited concentrations of grass pollens. Most of the airborne spores were produced by ascomycetes (i.e. *Oospora spp*) and fungi imperfecti (i.e. *Alternaria spp*, *Aspergillus spp*). Mean clinical scores of the horses were not correlated with the total concentrations of airborne pollens (r=0.35, p=0.15) or spores (r=0.30, p=0.23) of the same day. However, a significant correlation was observed with total pollen but not with spore concentration of the previous day (r=0.5, p=0.03; and r=0.21, p=0.41, respectively). Significant correlations were observed between mean clinical score and specific airborne concentrations of pollens (mainly from

Pinaceae (pine, fir, spruce), *Betula* (birch), and *Morus* (mulberry)) and spores from *Oospora spp* (powdery mildew). Further details are provided online (Supplementary item 1).

Lung function significantly worsened on the hot compared to the warm day, as demonstrated by the reduction of transpulmonary pressure (p=0.005), pulmonary resistance (p=0.008) and elastance values (p=0.005, **Fig 4**). On average, a 32%, 27%, and 36% decrease was detected for transpulmonary pressure, pulmonary resistance, and pulmonary elastance, respectively. The statistical pairing was effective for all 3 parameters (r=0.56, p=0.03 for transpulmonary pressure; r=0.69, p=0.007 for resistance; and r=0.75, p=0.002 for pulmonary elastance), indicating that a similar improvement in lung function occurred proportionally in all subjects when environmental heat was reduced. Respiratory rate (p=0.48) and tidal volume (p=0.12) were not significantly affected by temperature and RH variations. The pairing was effective for tidal volume (r=0.6, p=0.02) but not for respiratory rate (r=0.3, p=0.18). As environmental conditions on the days preceding the lung function test could have exerted a carryover effect, their description is provided in **Table 1**.

Discussion

Winter is considered a risk factor for exacerbations of severe equine asthma [1; 2], as horses spend more time in stables during this season, inhaling increased concentrations of molds and dusts. However, worsening of clinical signs of affected subjects has been reported also during summer months [11], even when horses were kept outdoor for most of the time [12]. During 2 consecutive years, on periods of high environmental temperatures for our geographical area, we observed a worsening of the clinical signs of asthmatic horses kept at pasture (8 weeks post-exacerbation) or stabled and contemporarily treated with inhaled corticosteroids or bronchodilators. Results from this study indicate that an increase of environmental temperature and humidity (determinants of humid air enthalpy and strongly associated with the pollen and spore air content) negatively affects the lung function of asthmatic horses during disease exacerbations, further worsening airway

obstruction. Pulmonary transpleural pressure, resistance, and elastance values significantly improved over few days as a consequence of a reduction in environmental heat, in spite of unchanged breathing strategy or hay and bedding dust exposure.

Increased environmental temperature and humidity, especially if sudden, hinders heat dissipation in animals, which in turn induces changes in their breathing strategy as a physiological response to avoid hyperthermia. Heat dissipation in horses occurs by evaporative cooling mainly from the skin and in part from the upper respiratory tract [13]. We initially postulated that the apparent deterioration of clinical conditions observed in asthmatic horses during hot environmental conditions would be the result of heat-induced thermoregulatory mechanisms altering their breathing pattern. A significant increase in respiratory frequency is indeed observed in horses in response to heat stress, and prevents hyperthermia during resting conditions [14; 15]. Asthmatic horses in exacerbation already have an increased respiratory rate compared to healthy animals, and mucus often covers an important portion of the tracheal mucosa, possibly hampering adequate thermoregulation in these animals. Furthermore, severe asthmatic horses are usually aged [2], which could further reduce their thermoregulatory ability [16] and increase the risk of hyperthermia even during resting conditions compared to healthy animals. However, contrarily to our initial hypothesis, the worsening of the horses' clinical conditions observed with increased temperatures was not associated with an altered respiratory strategy to improve thermoregulation, as breathing frequency or tidal volume were similar during warm and hot days.

Breathing hot humid air increases bronchial temperature and causes bronchospasm in many species, especially in presence of airway inflammation [6; 17; 18], as occurring in equine asthma. Interestingly, breathing hot humid air at increased respiratory frequencies induces a cholinergic-mediated bronchoconstriction also in human asthmatic patients [6], a condition that shares many pathophysiological similarities with equine asthma [7]. In our study, the significant correlation

observed between environmental enthalpy and clinical scores, and significant increase in pulmonary resistance and elastance observed on the hotter day suggest that airway obstruction worsen when heat dissipation is prevented by increased temperature and/or RH, supporting the implication of heat-induced bronchospasm in heaves pathobiology. The rapid development of severe airway obstruction after stabling a cohort of horses previously kept outdoors during winter in Quebec [19] and the identification of spending <15h/day outdoors during winter months as a risk factor for equine asthma exacerbation [1] provide further evidence for the occurrence of heat-induced bronchospasm in diseased horses. It also stresses the importance of even moderate temperature increases as bronchoconstriction triggers rather than absolute cutoffs. However, further studies are needed to confirm this theory and the mechanisms implicated.

Within the range of environmental conditions studied, heat dissipation is prevented to a greater extent by increases in temperature than in RH (i.e. RH should increase of 7-8% in order to produce the same effect on enthalpy as a 1°C-increase in temperature), which could explain why a more severe airway obstruction was detected on the hot day compared to the warm in presence of similar RH but different temperature values. Furthermore, the correlation between RH and clinical scores did not reach significance at the 5% level but there was some weak evidence of a relationship, and this in spite of a significant correlation of the scores with temperature and enthalpy, which further highlight the great effect of temperature on heat dissipation. The study power was, however, only 0.54 for RH, and doubling the time points studied would have been necessary in order to raise the power to 0.8 with the same alpha level (0.05). However, as enthalpy is determined by the integration of temperature and RH, both of them can be considered as causal factors associated to environmental heat.

Increased temperature during spring and summer months is associated with increased airborne pollens and molds [8]. Pollens are considered triggering factors for exacerbations of SPAOPD [8],

but evidence directly linking severe equine asthma exacerbations to these antigens is lacking. Nevertheless, they could act as non-specific irritants for the reactive airways of affected horses, and it has been estimated that up to approximately 30% of the variance in equine asthma prevalence in veterinary hospitals could be explained by the sum of climatic factors and their effect on aeroallergen concentrations in ambient air [11]. As the horses studied spent a few hours per day at pasture, we investigated whether airborne concentrations of pollens and spores could have affected disease severity. Our findings confirm and even strengthen the evidence for a correlation existing between daily outdoor temperature and RH values and air airborne pollen and spore levels. Airborne pollen but not spore concentrations were correlated with the horses' clinical scores, suggesting that they could play a role in disease severity. It is interesting to notice that the correlation was significant between the clinical scores and the pollen concentration of the previous day, as horses spent their afternoon outside and the scores were performed early in the morning. Also, outdoor concentration of pollens were increased on average 3-fold on the hot compared to the warm day during which pulmonary function tests were performed. In particular, increases in birch (*Betulla*, 5.4-fold increase on the hot day), ash (*Fraxinus*, 12.6-fold), mulberry (*Morus*, 5.5-fold), and oak (*Quercus*, 12-fold) pollens were most marked. The same trend was observed on the 3 days preceding the hot and the warm days. *Alternaria* and *Aspergillus/Penicillum* spore concentrations were also higher (4-fold and 6-fold, respectively) on the 3 days preceding the hot compared to the 3 days preceding the warm day. An association between monthly prevalence referrals for equine asthma exacerbations in veterinary hospitals and pollen counts measured 3 months before was observed for *Quercus*, *Fraxinus*, and *Morus spp* in a previous study, as well as with *Alternaria* spore counts measured during the same month [11]. Although these data would support an association between the increase in airborne pollens and equine asthma pathobiology, it is not possible to separate the specific role of environmental temperature/humidity and inhalable allergens based on our observations. However, the same is true in clinical practice. With this study we have shown that a correlation exists between environmental heat and the severity of clinical signs in

265 severe equine asthma. Albeit both heat-induced bronchoconstriction and airway irritation caused by
266 airborne particulates are likely to act synergically, environmental heat can be more easily predicted,
267 assessed, and, at least partially, contained by means of preventive measures (i.e. improved
268 ventilation).
269
270 In conclusion, our study indicates that high environmental temperature and humidity can worsen the
271 clinical signs of horses with severe equine asthma during disease exacerbation due to impaired lung
272 function. Whether and in which proportion the negative effect of high environmental temperature
273 and RH on lung function is worsened by inhalable pollens and molds, or by other undefined factors,
274 remains to be ascertained. Nevertheless, these findings highlight the necessity of providing a
275 temperate environment to severe asthmatic horses, especially during disease exacerbation or when
276 exposure to stable antigens cannot be avoided. Also, changes in environmental temperature should
277 be taken into account when evaluating the response to therapy in clinical or research settings.
278
279 **Footnotes**
280 [a] SCIREQ Scientific Respiratory Equipment Inc., Montreal, QC, Canada.
281 [b] Aerobiology Research Laboratories, Nepean, ON, Canada.
282 [c] SAS Institute Inc., Cary, NC, USA.
283 [d] GraphPad Software Inc., La Jolla, CA, USA.

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340 **Tables**

341 **Table 1. Environmental characteristics during lung function tests.**

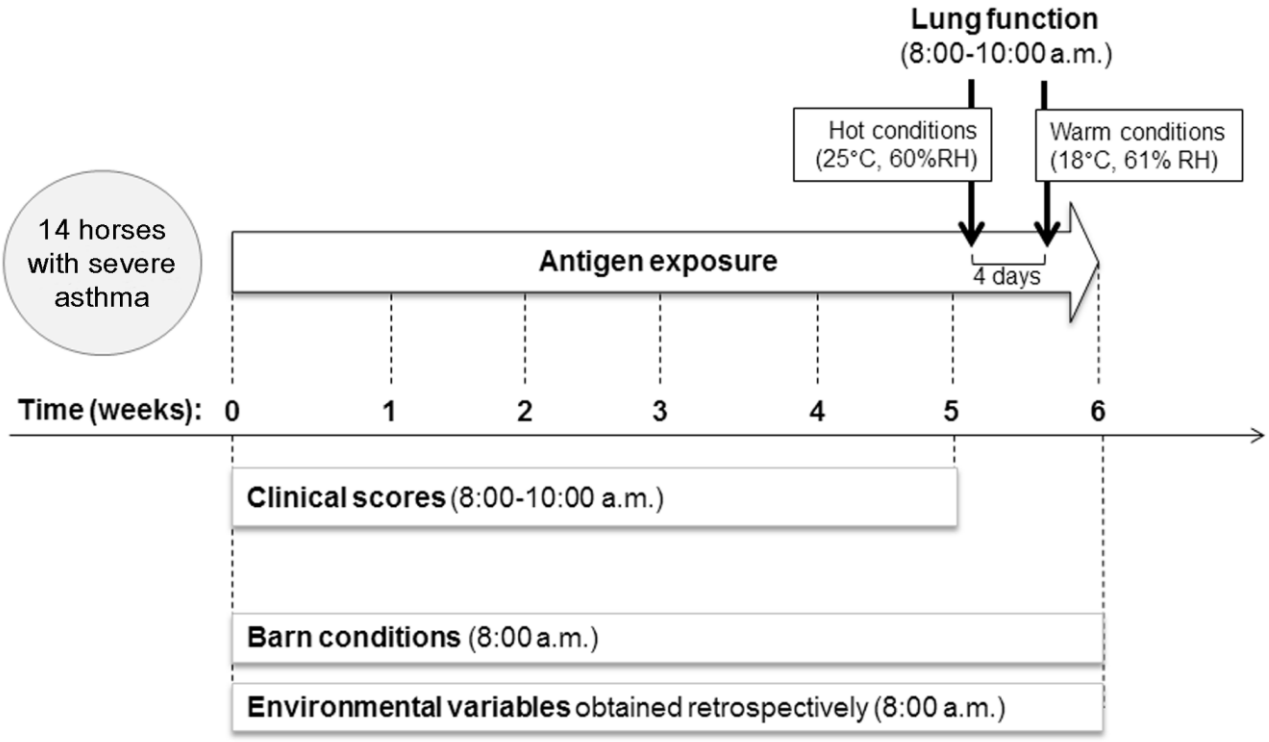
	Hot day	**Warm day**	***p* *(paired t-test)***
Breathing frequency* [Hz]	0.344 (±0.084)	0.304 (±0.123)	0.48
Tidal volume* [L]	5.6 (±0.9)	6.2 (±1.5)	0.12
Indoor temperature (barn) 8h a.m.	25°C	18°C	-
Indoor RH (barn) 8h a.m.	60%	61%	-
Outdoor temperature 8h a.m.	19°C	15.5°C	-
Outdoor RH 8h a.m.	71%	77%	-
Indoor temperature (barn) 8h a.m. (mean previous 3 days)	18.5°C	15.9°C	-
Indoor RH (barn) 8h a.m. (mean previous 3 days)	62.3%	61%	-
Outdoor temperature 8h a.m. (mean previous 3 days)	14°C	11.3°C	-
Outdoor RH 8h a.m. (mean previous 3 days)	88%	83%	-
Pollens [P/m^3]	249.2	85.4	-
Spores [P/m^3]	1737.1	2137.2	-
Pollens [P/m^3] (mean previous 3 days)	102.1	34.8	-
Spores [P/m^3] (mean previous 3 days)	2121.2	4040.5	-

342 RH: relative humidity; P/m^3: particles per cubic meter of air. *: daily mean±SD of individual values

343 observed in horses.

344 **Figures**

345



346

347 **Figure 1.** Experimental design. RH: relative humidity.

348

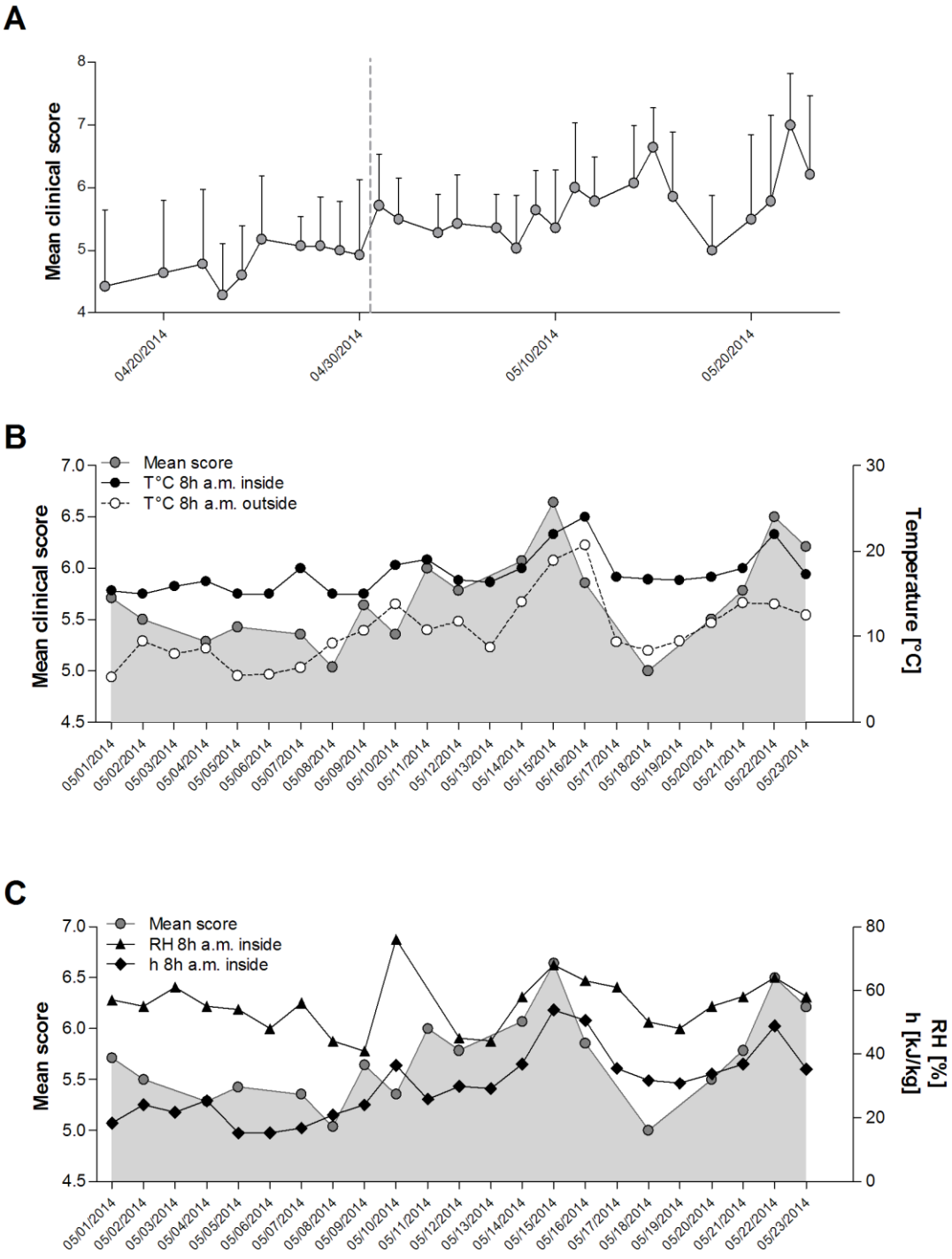
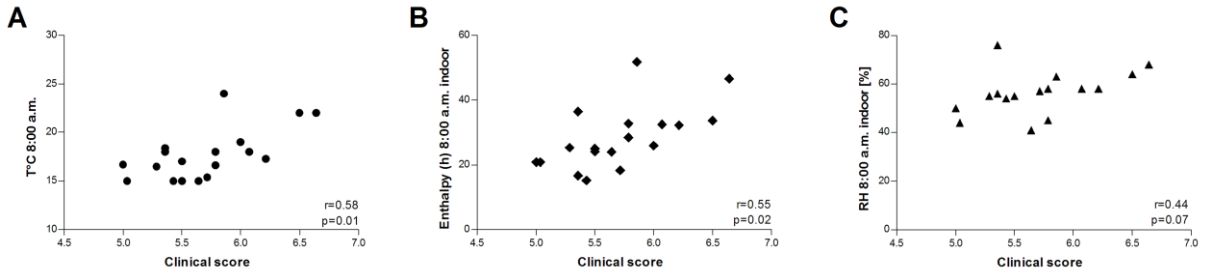


Figure 2. (A) Time trend of daily mean clinical score of the group of horses studied (n=14, error bars correspond to S.D.) for the whole period of antigen exposure. Data on the left of the dashed line were not considered for statistical analysis. (B, C) Time trend of daily mean clinical score, indoor and outdoor temperature, indoor relative humidity (RH) and enthalpy measured at 8:00 a.m. during the period studied.



355

356 **Figure 3.** Correlations of the mean clinical score (daily mean of the clinical scores of the horses
357 studied, n=14) and (A) temperature, (B) enthalpy, and (C) RH measured at 8:00 a.m. in the stable
358 where horses were housed.

359

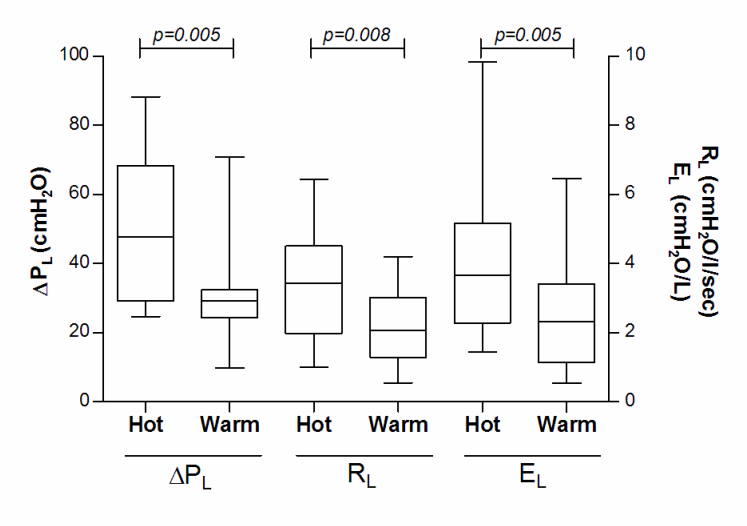


Figure 4. Effect of temperature variation on pulmonary mechanics in asthmatic horses during disease exacerbation. Data are presented as median, 25th to 75th percentiles (boxes), and min-max values (whiskers). ΔP_L: transpulmonary pressure; R_L: pulmonary resistance; E_L: pulmonary elastance.

Supporting information

Supplementary item 1: List of the airborne pollens and spores studied, and results of their correlation with clinical scores of the horses (Bonferroni correction for multiple comparisons was applied).

Supplementary item 2: Details for enthalpy calculation.

Word count: 3894