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Experimental and Basic Research Studies

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

M. BULLONE, R. Y. MURCIA and J-P. LAVOIE*

Department of Clinical Sciences of the Faculty of Veterinary Medicine, Université de Montreal, St-Hyacinthe, Quebec, Canada.

*Correspondence email: jean-pierre.lavoie@umontreal.ca; Received: 22.09.15; Accepted: 15.12.15

Summary

Reason for performing study: Clinical exacerbations of severe equine asthma (formerly recurrent airway obstruction [RAO]) 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 during a heatwave.
Objectives: To investigate the association between environmental temperature and humidity and clinical signs of asthma in horses during clinical exacerbation of the disease.
Study design: Retrospective longitudinal study.
Methods: Historical data on 14 severe asthmatic horses exposed to a dusty environment and evaluated using a previously validated clinical scoring system were analysed. Barn temperature and relative humidity values were obtained and air enthalpy (h) calculated. Correlation tests were used to study the relationship between mean daily clinical scores of horses and environmental variables. Lung function parameters recorded at 4 day intervals during hot (25°C) and warm (18°C) barn conditions were compared using a 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 ($r = 0.51$, $P = 0.0002$). In the absence of changes in the management of horses, higher barn temperature and enthalpy 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 may help attenuate the severity of airway obstruction in uncontrolled exacerbations of severe equine asthma. Furthermore, variations in environmental heat and associated pollen concentrations should also be taken into account when evaluating the response to therapy in clinical or research settings.

Keywords: horse: recurrent airway obstruction; heaves; temperature; relative humidity; enthalpy

Introduction

Severe equine asthma (also known as recurrent airway obstruction [RAO] or heaves, and previously as chronic obstructive pulmonary disease [COPD]) is a chronic obstructive respiratory condition affecting 15–20% of adult horses living in a temperate climate [1]. The risk of disease exacerbation increases during the winter months [1,2] when horses are stabled for extended periods and exposed to endotoxins, moulds, mites, and other dust particulate matters present in hay and straw [3,4]. Although not described in asthmatic horses, cold-induced bronchoconstriction may 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 to breathing hot humid air has been shown in asthmatic humans [6] and could also be present in the equine form of the disease, given the similarities between the 2 conditions [7]. Alternatively, increased respiratory effort 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]. Although evidence linking severe equine asthma exacerbations to these antigens is lacking [9], they could act as nonspecific irritants for the reactive airways of affected horses. This study was therefore undertaken with the aim of retrospectively investigating the short-term effect of environmental temperature, humidity and antigenic load (airborne pollens and spores) on the clinical status of asthmatic horses during clinical exacerbations of the disease.

Materials and methods

Environmental data were obtained and analysed retrospectively. A total of 14 severe asthmatic horses aged 15.1 ± 4.4 years (mean ± s.d.; range: 7–30) and weighing 519 ± 64 kg were studied. There were 5 Quarter Horses, 4 Standardbreds, 2 Canadians, 2 Paints and one Arab mixed breed, of which 4 were geldings and 10 females. The study design is summarised in Figure 1. All horses had been kept at pasture for at least 4 months before the beginning of the experimental phase of the study. Six weeks' antigen exposure started on 15 April 2014. 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 2–6 h/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 08.00 and 10.00 h during the first 5 weeks of antigen exposure. Scoring was performed by one of 3 trained operators in optimal agreement (interclass correlation coefficient >0.8), as was part of the antigen challenge monitoring protocol of our laboratory. During the sixth week, pulmonary mechanics were performed in the stable where horses were housed between 08.00 and 10.00 h 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, 55.27 kJ/kg enthalpy [h], indoor values at 08.00 h vs. 19°C, 71% relative humidity, 43.65 kJ/kg h outdoor) and on Friday (retrospectively considered a 'warm' day: 18°C, 61% relative humidity, 37.82 kJ/kg h indoor at 08.00 h vs. 15.5°C, 77% relative humidity, 36.82 kJ/kg h outdoor). Briefly, transpulmonary pressure was measured with an oesophageal balloon catheter connected to a pressure transducer and breathing flow signals obtained from a heated

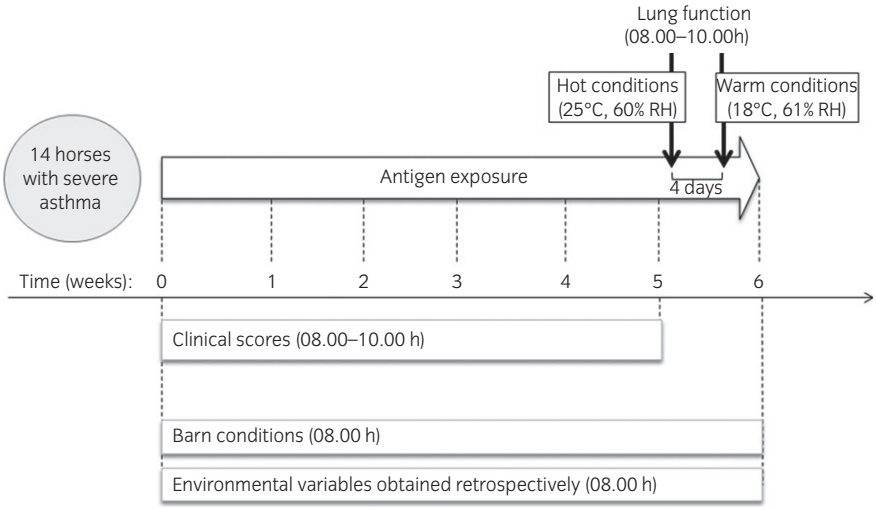

Fig 1: Experimental design. RH: relative humidity.

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 08.00 h 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 were recorded twice daily (08.00 and 16.00 h). 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 in 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 in Supplementary Item 2. Enthalpy was chosen as it approximates the extent to which 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 used to differentiate 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 2 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 $score = a + b*time + c*(time\text{-}inflexion\ time)$. 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 analysed using Pearson or Spearman correlation tests, 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

Figure 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 (panels b and c). The nonlinear model indicates that the slope of the curve ('b') was significantly >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 1 May 2014. These findings provided the rationale for including only the data observed after the first 15 days of antigen exposure into the statistical analysis.

From Day 15 to Day 35 there were significant correlations between the daily mean of 14 individual clinical scores of the horses and 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 relative humidity, but this was not statistically significant ($r = 0.44$, $P = 0.08$, Fig 3c). Indoor and outdoor temperature ($r = 0.94$, $P<0.0001$) and relative humidity ($r = 0.62$, $P = 0.002$) recorded at 08.00 h 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 relative humidity ($r = 0.44$, $P = 0.002$, and $r = 0.29$, $P = 0.047$, respectively). The most abundant outdoor airborne pollens during the period studied were tree pollens (deciduous > 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 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 between mean clinical scores and 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 (Supplementary Item 1) 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).

Lung function was significantly worse on the hot than the warm day, demonstrated by the reduction in 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 ($P = 0.5$) and tidal volume ($P = 0.1$)

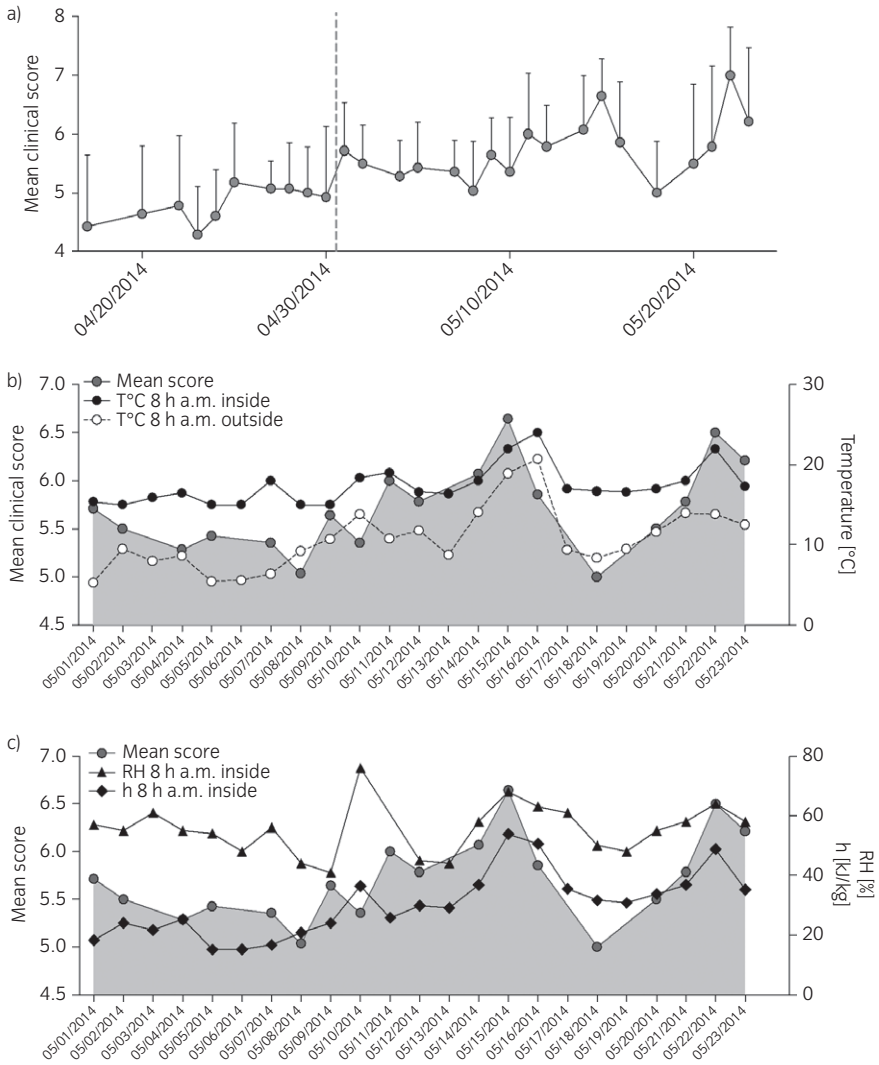

Fig 2: a) Time trend of daily mean clinical scores in the group of horses studied (n = 14, error bars correspond to s.d.) for the whole period of antigen exposure. Data on the left of the dashed line were not considered for statistical analysis. b, c) Time trend of daily mean clinical score, indoor and outdoor temperature, indoor relative humidity and enthalpy measured at 08.00 h during the period studied.

were not significantly affected by temperature and relative humidity variations. The pairing was effective for tidal volume (r = 0.6, P = 0.02) but not for respiratory rate (r = 0.3, P = 0.2). As environmental conditions on the days preceding the lung function test could have exerted a carryover effect, they are described 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 moulds and dusts. However, worsening of clinical signs of affected subjects has been reported also during summer months [11], even when horses were kept outdoors for most of the time [12]. During 2 consecutive years, in 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 treated contemporarily with inhaled corticosteroids or bronchodilators. Results from this study indicate that an increase in 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 a 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, which prevents hyperthermia during resting conditions [14,15]. During exacerbations, the breathing frequency of asthmatic horses is increased compared with healthy animals, and mucus often covers an important portion of the tracheal mucosa, which could possibly hamper thermoregulation in these animals. Also, severe asthmatic horses are usually aged [2], which could further reduce their thermoregulatory ability [16] and increase the risk of hyperthermia, even

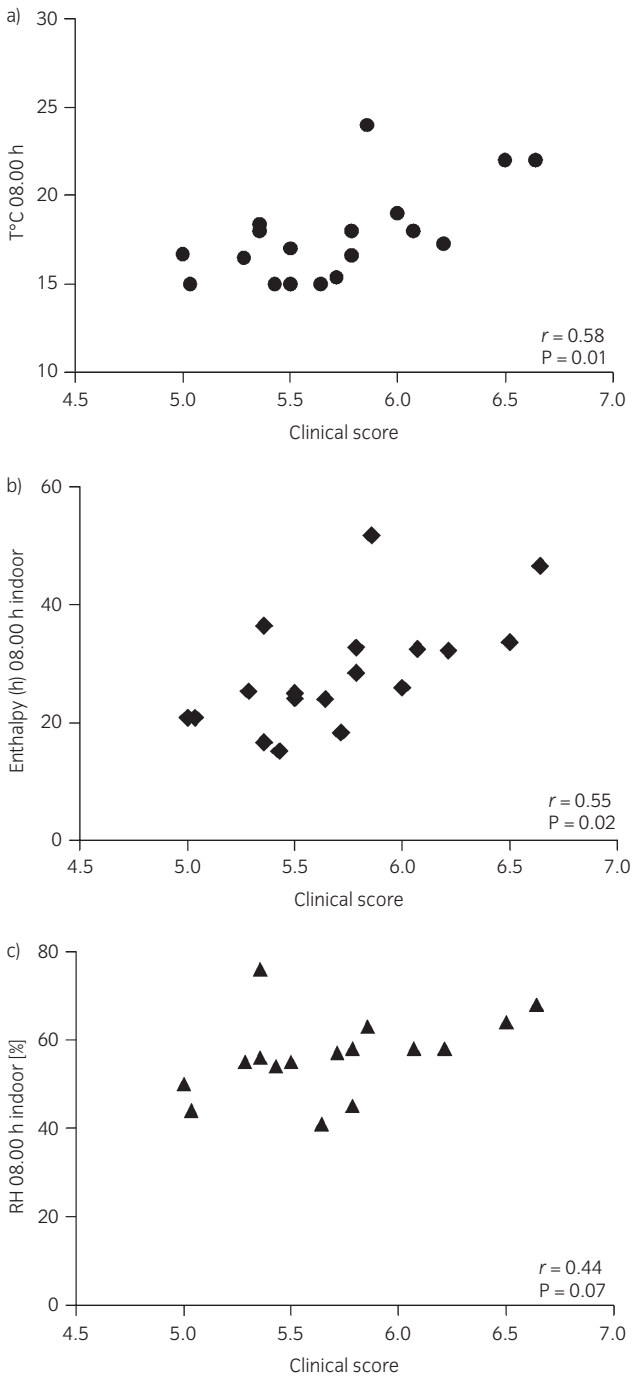

Fig 3: Correlations of the mean clinical score (daily mean of the clinical scores of the horses studied, n = 14) and (a) temperature, (b) enthalpy and (c) relative humidity measured at 08.00 h in the stable where horses were housed.

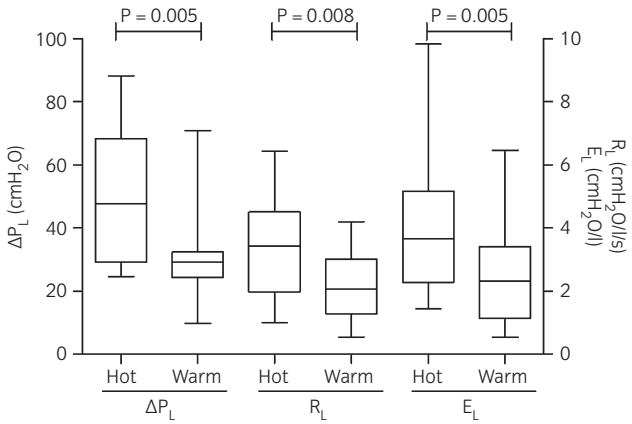

Fig 4: Effect of temperature variation on pulmonary mechanics in asthmatic horses during disease exacerbation. Data are presented as median, 25th–75th percentiles (boxes) and min-max values (whiskers). ΔP_L: transpulmonary pressure; R_L: pulmonary resistance; E_L: pulmonary elastance.

during resting conditions, compared with healthy animals. However, contrary to our initial hypothesis, the worsening of the horses' clinical condition observed with increased temperatures was not associated with an altered respiratory strategy to improve thermoregulation, as breathing frequency or tidal volume were similar during both warm and hot days.

Breathing hot humid air increases bronchial temperature and causes bronchospasm in many species, especially in the presence of airway inflammation [6,17,18], as occurs 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 the significant increase in pulmonary resistance and elastance observed on the hotter day suggest that airway obstruction worsens when heat dissipation is prevented by increased temperature and/or relative humidity, 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 <15 h/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 cut-offs. 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 relative humidity (i.e. relative humidity should increase by 7–8 percentage points 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 than on the warm day in the presence of similar relative humidity but different temperature values. Furthermore, the correlation between relative humidity and clinical scores did not reach 5% significance, 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 highlights the great effect of temperature on heat dissipation. The study power was, however, only 0.54 for relative humidity, 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). As enthalpy is determined by temperature and relative humidity, it is likely that we believe that both factors should be regarded as contributors to the respiratory obstructive response observed in concomitance with environmental heat.

Increased temperature during the spring and summer months is associated with increased airborne pollens and moulds [8]. Pollens are considered to be 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 nonspecific irritants for the reactive airways of affected horses, and it has been estimated that 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

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)	0.5
Tidal volume* (l)	5.6 (± 0.9)	6.2 (± 1.5)	0.1
Indoor temperature (barn) 08.00 h	25°C	18°C	-
Indoor relative humidity (barn) 08.00 h	60%	61%	-
Outdoor temperature 08.00 h	19°C	15.5°C	-
Outdoor relative humidity 08.00 h	71%	77%	-
Indoor temperature (barn) 08.00 h (mean previous 3 days)	18.5°C	15.9°C	-
Indoor relative humidity (barn) 08.00 h (mean previous 3 days)	62.3%	61%	-
Outdoor temperature 08.00 h (mean previous 3 days)	14°C	11.3°C	-
Outdoor relative humidity 08.00 h (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	-

P/m^3 = particles per cubic metre of air. *Daily mean ± s.d. of individual values observed in horses.

evidence for a correlation between daily outdoor temperature and relative humidity values and 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 note that the correlation was significant between the clinical scores and 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 with 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- and 6-fold, respectively) on the 3 days preceding the hot than on 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 *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. In this study we have shown that a correlation exists between environmental heat and the severity of clinical signs in severe equine asthma. Although both heat-induced bronchoconstriction and airway irritation caused by airborne particulates are likely to act synergistically, environmental heat can be more easily predicted, assessed and at least partially contained by means of preventive measures (i.e. improved ventilation).

In conclusion, our study indicates that high environmental temperature and humidity can worsen the clinical signs of horses with severe equine asthma during disease exacerbations owing to impaired lung function. Whether and in which proportion the negative effect of high environmental temperature and relative humidity on lung function is worsened by inhalable pollens and moulds, or by other undefined factors, remains to be ascertained. Nevertheless, these findings highlight the necessity of providing a temperate environment to severe asthmatic horses, especially during disease exacerbation or when exposure to stable antigens cannot be avoided. Also, changes in environmental temperature should be taken into account when evaluating the response to therapy in clinical or research settings.

Authors' declaration of interests

No competing interests have been declared.

Ethical animal research

The experimental protocol of this study has been approved by the Animal Ethics Committee of the Université de Montréal (Rech-1324).

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Authorship

M. Bullone contributed to study design, data collection, analysis and interpretation, preparation and approval of the manuscript. R.Y. Murcia contributed to study execution, preparation and approval of the manuscript. J-P. Lavoie contributed to study design, data interpretation, preparation and approval of the manuscript.

Manufacturers' addresses

[a]SCIREQ Scientific Respiratory Equipment Inc., Montreal, Quebec, Canada.
[b]Aerobiology Research Laboratories, Nepean, Ontario, Canada.
[c]SAS Institute Inc., Cary, North Carolina, USA.
[d]GraphPad Software Inc., La Jolla, California, USA.

References

1. Hotchkiss, J.W., Reid, S.W. and Christley, R.M. (2007) A survey of horse owners in Great Britain regarding horses in their care. Part 2: risk factors for recurrent airway obstruction. *Equine Vet. J.* **39**, 301-308.
2. Couetil, L.L. and Ward, M.P. (2003) Analysis of risk factors for recurrent airway obstruction in North American horses: 1,444 cases (1990-1999). *J. Am. Vet. Med. Ass.* **223**, 1645-1650.
3. Beeler-Marfisi, J., Clark, M.E., Wen, X., Sears, W., Huber, L., Ackerley, C., Viel, L. and Bienzle, D. (2010) Experimental induction of recurrent airway obstruction with inhaled fungal spores, lipopolysaccharide, and silica microspheres in horses. *Am. J. Vet. Res.* **71**, 682-689.
4. Pirie, R.S., Collie, D.D., Dixon, P.M. and McGorum, B.C. (2002) Evaluation of nebulised hay dust suspensions (HDS) for the diagnosis and

investigation of heaves. 2: effects of inhaled HDS on control and heaves horses. *Equine Vet. J.* **34**, 337-342.
5. Davis, M.S., Royer, C.M., McKenzie, E.C., Williamson, K.K., Payton, M. and Marlin, D. (2006) Cold air-induced late-phase bronchoconstriction in horses. *Equine Vet. J.* **38**, *Suppl.* **36,** 535-539.
6. Hayes, D. Jr, Collins, P.B., Khosravi, M., Lin, R.L. and Lee, L.Y. (2012) Bronchoconstriction triggered by breathing hot humid air in patients with asthma: role of cholinergic reflex. *Am. J. Respir. Crit. Care Med.* **185**, 1190-1196.
7. Bullone, M. and Lavoie, J.P. (2015) Asthma "of horses and men" - How can equine heaves help us better understand human asthma immunopathology and its functional consequences? *Mol. Immunol.* **66**, 97-105.
8. Costa, L.R., Johnson, J.R., Baur, M.E. and Beadle, R.E. (2006) Temporal clinical exacerbation of summer pasture-associated recurrent airway obstruction and relationship with climate and aeroallergens in horses. *Am. J. Vet. Res.* **67**, 1635-1642.
9. Tahon, L., Baselgia, S., Gerber, V., Doherr, M.G., Straub, R., Robinson, N.E. and Marti, E. (2009) In vitro allergy tests compared to intradermal testing in horses with recurrent airway obstruction. *Vet. Immunol. Immunopathol.* **127**, 85-93.
10. Robinson, N.E., Olszewski, M.A., Boehler, D., Berney, C., Hakala, J., Matson, C. and Derksen, F.J. (2000) Relationship between clinical signs and lung function in horses with recurrent airway obstruction (heaves) during a bronchodilator trial. *Equine Vet. J.* **32**, 393-400.
11. Ward, M.P. and Couetil, L.L. (2005) Climatic and aeroallergen risk factors for chronic obstructive pulmonary disease in horses. *Am. J. Vet. Res.* **66**, 818-824.
12. Lowell, F.C. (1964) Observations on heaves. An asthma-like syndrome in the horse. *J. Allergy Clin. Immunol.* **35**, 322-330.
13. Hodgson, D.R., Davis, R.E. and McConaghy, F.F. (1994) Thermoregulation in the horse in response to exercise. *Br. Vet. J.* **150**, 219-235.
14. Marlin, D.J., Schroter, R.C., White, S.L., Maykuth, P., Matthesen, G., Mills, P.C., Waran, N. and Harris, P. (2001) Recovery from transport and acclimatisation of competition horses in a hot humid environment. *Equine Vet. J.* **33**, 371-379.
15. McConaghy, F.F., Hodgson, D.R., Rose, R.J. and Hales, J.R. (1996) Redistribution of cardiac output in response to heat exposure in the pony. *Equine Vet. J.* **28**, *Suppl.* **22**, 42-46.
16. McKeever, K.H., Eaton, T.L., Geiser, S., Kearns, C.F. and Lehnhard, R.A. (2010) Age related decreases in thermoregulation and cardiovascular function in horses. *Equine Vet. J.* **42**, *Suppl.* **38**, 220-227.
17. Hsu, C.C., Lin, R.L., Lin, Y.S. and Lee, L.Y. (2013) Bronchoconstriction induced by increasing airway temperature in ovalbumin-sensitized rats: role of tachykinins. *J. Appl. Physiol. (1985)*, **115**: 688-696.
18. Ruan, T., Gu, Q., Kou, Y.R. and Lee, L.Y. (2005) Hyperthermia increases sensitivity of pulmonary C-fibre afferents in rats. *J. Physiol.* **565**, 295-308.
19. Lavoie, J.P., Thompson, D., Hamilton, E., Debrue, M., David, F. and Hickey, G. (2008) Effects of a MAPK p38 inhibitor on lung function and airway inflammation in equine recurrent airway obstruction. *Equine Vet. J.* **40**, 577-583.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

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.