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

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

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12

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16

17

18 **Ethical considerations:** All the procedures described have been approved by the Animal Ethics
19 Committee of the Université de Montréal (Rech-1324).

20

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27

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30 approval of the manuscript. JPL contributed to study design, data interpretation, preparation and
31 approval of the manuscript.

32

33 **Competing interests:** None.

34

35

36 **Summary**

37 Reason for performing the study – Clinical exacerbations of severe equine asthma are more
38 frequently reported during winter, when horses are exposed to airborne dusts during stabling.

39 However, we have also observed a worsening of clinical signs on days of heatwave.

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

42 Study design – Retrospective longitudinal study.

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

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

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

59 **Introduction**

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

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

80

81 **Methods**

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

85 519±64 kg were studied. There were 5 Quarter Horse, 4 Standardbred, 2 Canadian, 2 Paints and 1
86 Arab mixed breed, of which 4 were geldings and 10 females. Study design is summarized in **Fig 1**.
87 All horses had been kept at pasture for at least 4 months before the beginning of the experimental
88 phase of the study. Antigen exposure started on the 15th of April 2014 and was protracted for 6
89 weeks. During this period, horses were stabled and fed hay. Stabling conditions (bedding,
90 ventilation, number of animals kept within the facility, hay type/batch and quantities administered)
91 remained the same for the duration of the study. Horses were turned out in a paddock 2 to 6
92 hours/day in the afternoon. An 8-point clinical score previously validated in horses and ranging
93 from 1 (normal) to 4 (severe effort) for both nasal and abdominal effort during breathing [10] was
94 performed between 8:00 and 10:00 a.m. during the first 5 weeks of antigen exposure. Scoring was
95 made by one of 3 trained operators in optimal agreement (interclass correlation coefficient>0.8), as
96 this is part of the antigen challenge monitoring protocol of our laboratory. During the 6th week,
97 pulmonary mechanics were performed in the stable where horses were housed between 8:00 and
98 10:00 a.m., on Monday (retrospectively considered a “hot” day based on the average temperature
99 for this time of the year in our geographical area: 25°C, 60% relative humidity, RH, 55.27 kJ/kg
100 enthalpy (h), indoor values at 8:00 a.m.; versus 19°C, 71% RH, 43.65 kJ/kg h outdoor) and on
101 Friday (retrospectively considered a “warm” day: 18°C, 61% RH, 37.82 kJ/kg h indoor at 8:00 a.m.,
102 versus 15.5°C, 77% RH, 36.82 kJ/kg h outdoor). Briefly, transpulmonary pressure was measured
103 with an esophageal balloon catheter connected to a pressure transducer, and breathing flow signals
104 obtained from a heated pneumotachograph connected to a mask. Pulmonary resistance and
105 elastance values were derived using the flexiWare 7.6 software^a.

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

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

117 Statistical analyses were performed with SAS/STAT software^c and Prism 5^d. A regression model
118 was used to identify data to be included in the analysis. In order to avoid biases due to the
119 concomitant effects of antigen exposure and season-related increase in temperature on the horses'
120 clinical scores, a piecewise regression model was employed for differentiating the initial raising
121 phase of the clinical score curve, where barn antigen exposure is likely to exert a predominant
122 effect, from the following plateau, where the effect of antigen exposure has reached a stable phase.
123 We fitted a model including two different slopes and an inflexion point. The equation for the first
124 segment before the inflexion time is $score = a + b*time$ and the equation for the second segment is
125 $score = a + b*time + c*(time - inflexion\ time)$. Only data obtained during the second segment of the
126 curve (stable phase) were studied. The effect of the environmental variables on the mean daily
127 clinical score obtained from the horses was analyzed using Pearson or Spearman correlation test,
128 depending on data distribution. Indoor and outdoor meteorological variables were compared with
129 Pearson correlation tests. The effect of hot vs warm environment on lung function was assessed
130 with paired t-tests. Pearson correlation coefficient was also calculated to determine whether the
131 pairing was effective (that is, whether the direction and magnitude of the variation induced by the
132 warm vs the hot conditions were similar in all horses). Normal distribution of data was assessed
133 with the Kolmogorov-Smirnov test. P-values <0.05 were considered significant.

134

135 **Results**

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

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

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

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

163 Lung function significantly worsened on the hot compared to the warm day, as demonstrated by the
164 reduction of transpulmonary pressure ($p=0.005$), pulmonary resistance ($p=0.008$) and elastance
165 values ($p=0.005$, **Fig 4**). On average, a 32%, 27%, and 36% decrease was detected for
166 transpulmonary pressure, pulmonary resistance, and pulmonary elastance, respectively. The
167 statistical pairing was effective for all 3 parameters ($r=0.56$, $p=0.03$ for transpulmonary pressure;
168 $r=0.69$, $p=0.007$ for resistance; and $r=0.75$, $p=0.002$ for pulmonary elastance), indicating that a
169 similar improvement in lung function occurred proportionally in all subjects when environmental
170 heat was reduced. Respiratory rate ($p=0.48$) and tidal volume ($p=0.12$) were not significantly
171 affected by temperature and RH variations. The pairing was effective for tidal volume ($r=0.6$,
172 $p=0.02$) but not for respiratory rate ($r=0.3$, $p=0.18$). As environmental conditions on the days
173 preceding the lung function test could have exerted a carryover effect, their description is provided
174 in **Table 1**.

175

176 **Discussion**

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

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

190

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

207

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

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

223

224 Within the range of environmental conditions studied, heat dissipation is prevented to a greater
225 extent by increases in temperature than in RH (i.e. RH should increase of 7-8% in order to produce
226 the same effect on enthalpy as a 1°C-increase in temperature), which could explain why a more
227 severe airway obstruction was detected on the hot day compared to the warm in presence of similar
228 RH but different temperature values. Furthermore, the correlation between RH and clinical scores
229 did not reach significance at the 5% level but there was some weak evidence of a relationship, and
230 this in spite of a significant correlation of the scores with temperature and enthalpy, which further
231 highlight the great effect of temperature on heat dissipation. The study power was, however, only
232 0.54 for RH, and doubling the time points studied would have been necessary in order to raise the
233 power to 0.8 with the same alpha level (0.05). However, as enthalpy is determined by the
234 integration of temperature and RH, both of them can be considered as causal factors associated to
235 environmental heat.

236

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

239 but evidence directly linking severe equine asthma exacerbations to these antigens is lacking.
240 Nevertheless, they could act as non-specific irritants for the reactive airways of affected horses, and
241 it has been estimated that up to approximately 30% of the variance in equine asthma prevalence in
242 veterinary hospitals could be explained by the sum of climatic factors and their effect on
243 aeroallergen concentrations in ambient air [11]. As the horses studied spent a few hours per day at
244 pasture, we investigated whether airborne concentrations of pollens and spores could have affected
245 disease severity. Our findings confirm and even strengthen the evidence for a correlation existing
246 between daily outdoor temperature and RH values and air airborne pollen and spore levels.
247 Airborne pollen but not spore concentrations were correlated with the horses' clinical scores,
248 suggesting that they could play a role in disease severity. It is interesting to notice that the
249 correlation was significant between the clinical scores and the pollen concentration of the previous
250 day, as horses spent their afternoon outside and the scores were performed early in the morning.
251 Also, outdoor concentration of pollens were increased on average 3-fold on the hot compared to the
252 warm day during which pulmonary function tests were performed. In particular, increases in birch
253 (*Betulla*, 5.4-fold increase on the hot day), ash (*Fraxinus*, 12.6-fold), mulberry (*Morus*, 5.5-fold),
254 and oak (*Quercus*, 12-fold) pollens were most marked. The same trend was observed on the 3 days
255 preceding the hot and the warm days. *Alternaria* and *Aspergillus/Penicillium* spore concentrations
256 were also higher (4-fold and 6-fold, respectively) on the 3 days preceding the hot compared to the 3
257 days preceding the warm day. An association between monthly prevalence referrals for equine
258 asthma exacerbations in veterinary hospitals and pollen counts measured 3 months before was
259 observed for *Quercus*, *Fraxinus*, and *Morus spp* in a previous study, as well as with *Alternaria*
260 spore counts measured during the same month [11]. Although these data would support an
261 association between the increase in airborne pollens and equine asthma pathobiology, it is not
262 possible to separate the specific role of environmental temperature/humidity and inhalable allergens
263 based on our observations. However, the same is true in clinical practice. With this study we have
264 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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339

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

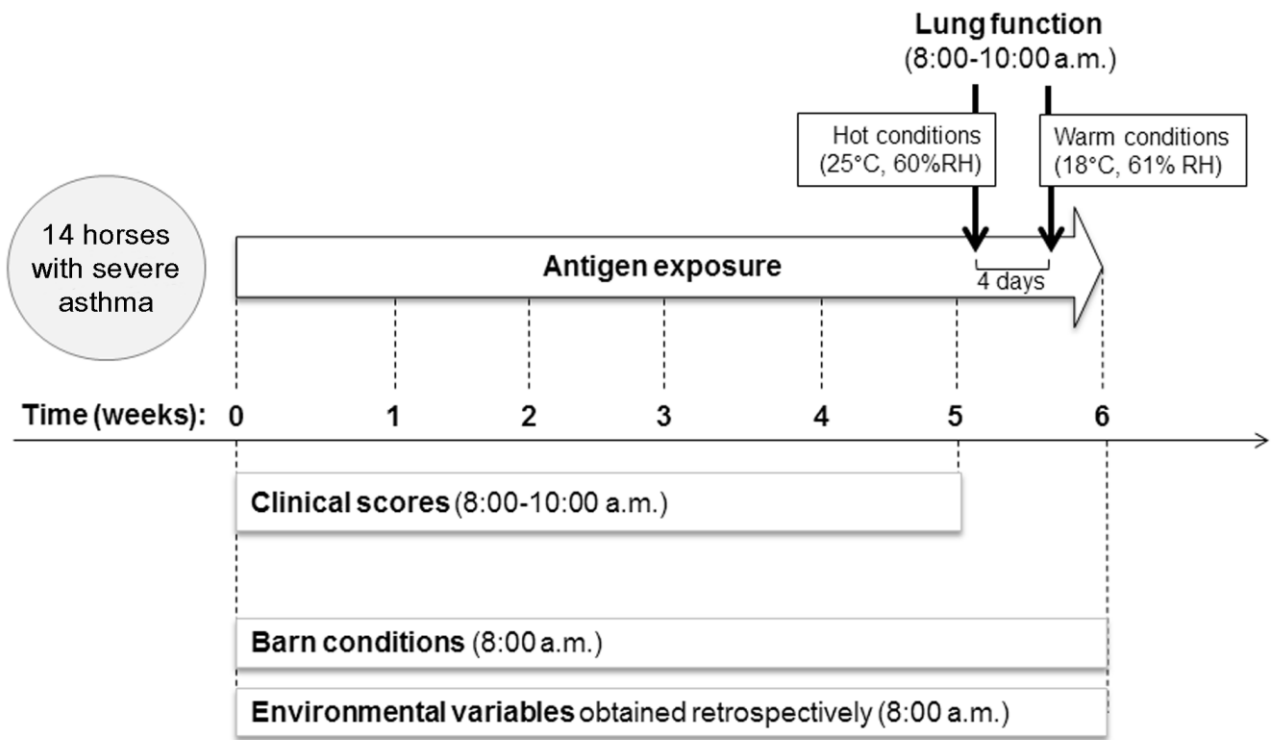
	Hot day	Warm day	<i>P</i> (paired <i>t</i>-test)
Breathing frequency* [Hz]	0.344 (± 0.084)	0.304 (± 0.123)	0.48
Tidal volume* [L]	5.6 (± 0.9)	6.2 (± 1.5)	0.12
Indoor temperature (barn) 8h a.m.	25°C	18°C	-
Indoor RH (barn) 8h a.m.	60%	61%	-
Outdoor temperature 8h a.m.	19°C	15.5°C	-
Outdoor RH 8h a.m.	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/m³: particles per cubic meter of air. *: daily mean \pm SD of individual values

343 observed in horses.

344 **Figures**

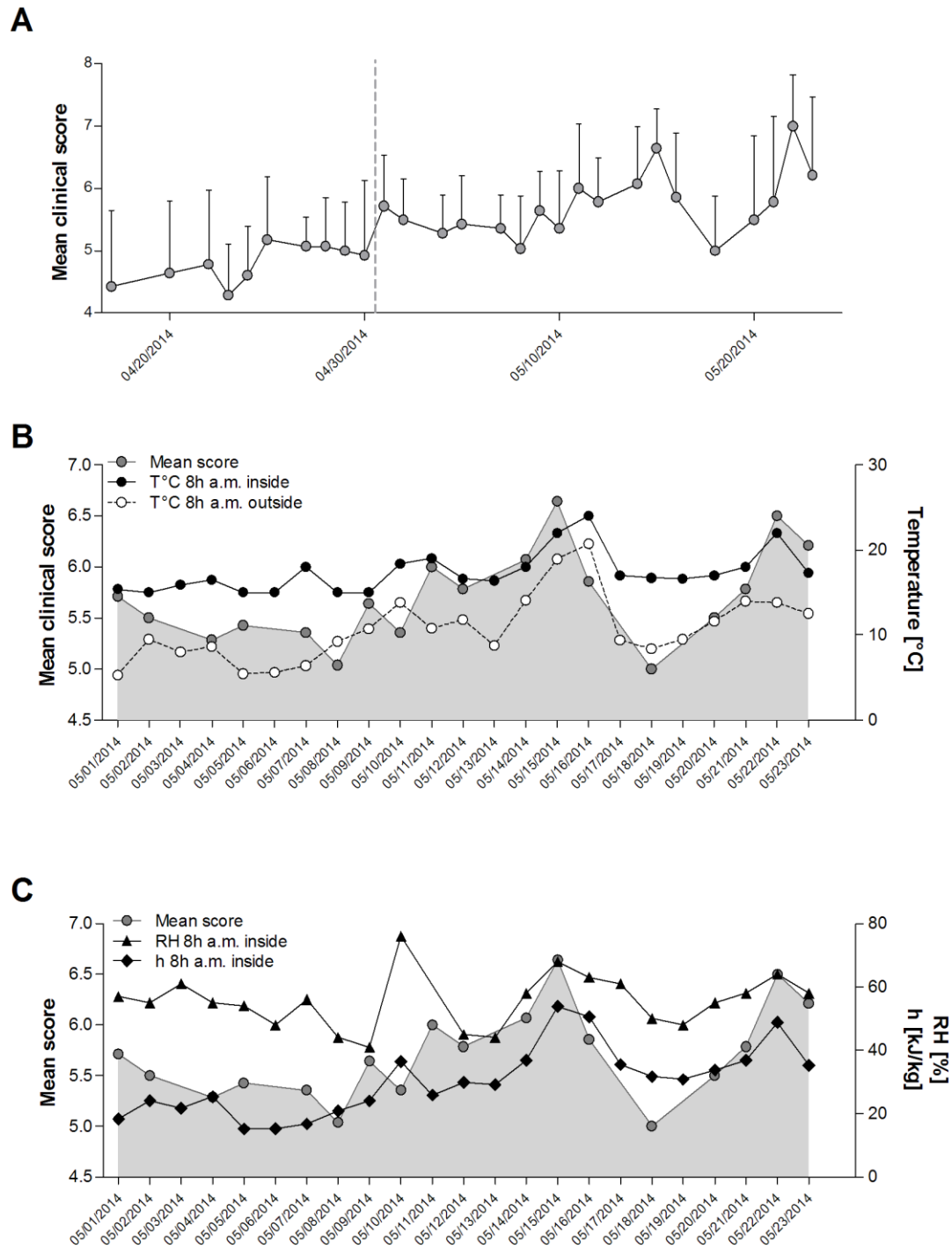
345



346

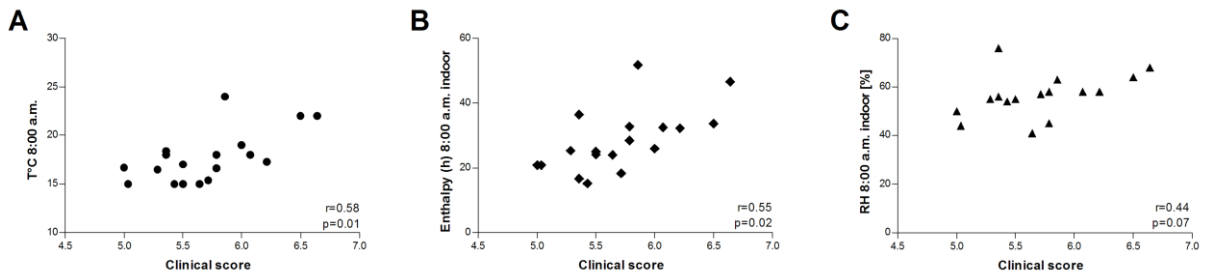
347 **Figure 1.** Experimental design. RH: relative humidity.

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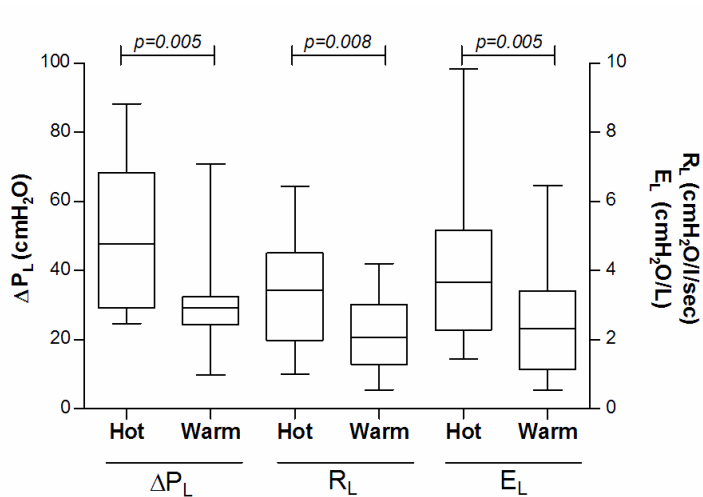
350 **Figure 2.** (A) Time trend of daily mean clinical score of the group of horses studied (n=14, error
 351 bars correspond to S.D.) for the whole period of antigen exposure. Data on the left of the dashed
 352 line were not considered for statistical analysis. (B, C) Time trend of daily mean clinical score,
 353 indoor and outdoor temperature, indoor relative humidity (RH) and enthalpy measured at 8:00 a.m.
 354 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.

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

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369 **Supporting information**

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

373 Supplementary item 2: Details for enthalpy calculation.

374

375 **Word count: 3894**