

Increased Parasite Resistance and Recurrent Airway Obstruction in Horses of a High-Prevalence Family

S. Neuhaus, P. Bruendler, C.F. Frey, B. Gottstein, M.G. Doherr, and V. Gerber

Background: Equine recurrent airway obstruction (RAO) shares many characteristics with human asthma. In humans, an inverse relationship between susceptibility to asthma and resistance to parasites is suspected.

Hypothesis/Objectives: Members of a high-incidence RAO half-sibling family (F) shed fewer strongylid eggs compared with RAO-unaffected pasture mates (PM) and that RAO-affected horses shed fewer eggs than RAO-unaffected half-siblings.

Animals: Seventy-three F and 73 unrelated, age matched PM.

Methods: Cases and controls kept under the same management and deworming regime were examined. Each individual was classified as RAO affected or RAO unaffected and fecal samples were collected before and 1–3 weeks and 3 months after deworming. Samples were analyzed by combined sedimentation-flotation and modified McMaster methods and classified into 3 categories of 0 eggs per gram of feces (EpG), 1–100 EpG, and > 100 EpG, respectively.

Results: PM compared with RAO-affected F had a 16.7 (95% confidence interval [CI]: 2.0–136.3) times higher risk for shedding > 100 EpG compared with 0 EpG and a 5.3 (95% CI: 1.0–27.4) times higher risk for shedding > 100 EpG compared with 0 EpG. There was no significant effect when RAO-unaffected F were compared with their PM. RAO-unaffected compared with RAO-affected offspring had a 5.8 (95% CI: 0.0–1.0) times higher risk for shedding 1–100 EpG. Age, sex, breed, and sharing pastures with other species had no significant confounding effects.

Conclusion and Clinical Importance: RAO is associated with resistance against strongylid parasites in a high-prevalence family.

Key words: Equine; Genetic; Hygiene hypothesis.

Recurrent airway obstruction (RAO), also known as heaves, is the most common disorder of the lower respiratory tract of mature horses in the Northern Hemisphere. This chronic and potentially debilitating respiratory disease is also one of the rare natural models for human asthma^{1–3} with which it shares many characteristics: coughing, increased breathing effort because of cholinergic bronchospasm and bronchial hyperresponsiveness, neutrophilic (not eosinophilic) inflammation, and mucus accumulation of the airways.^{4,5} RAO is triggered in susceptible individuals by environmental factors, mainly allergens in hay dust. A strong genetic predisposition for this disease has been demonstrated,^{6,7} but the responsible genes and specific key mechanisms leading to the manifestation of RAO in susceptible horses are still under investigation. Because complete avoidance of exposure to environmental triggers is often difficult or impossible, many RAO-affected horses depend on symptomatic oral or inhaled corticosteroid, bronchodilator treatment, or both.

We have recently identified a half-sibling family (F) with a very high prevalence of RAO. Members of this family, all descendants of a stallion that was himself

Abbreviations:

EpG	egg per gram feces
F	members of a high-incidence RAO half-sibling family
PM	RAO-unaffected pasture mates
RAO	recurrent airway obstruction

RAO affected, have a markedly increased risk to develop RAO.⁷ Within this family, RAO follows a recessive mode of inheritance⁸ and there is linkage and association of the RAO phenotype with microsatellite markers near the gene for the interleukin 4 receptor alpha chain (*IL4R α*).⁹ Studies on the *IL4R α* gene in humans have revealed numerous polymorphisms, some of which are associated with phenotypes related to asthma and atopy.^{10,11} Moreover, *IL4R α* is associated with defense against parasites in humans and animals.¹² These findings, together with incidental observations that members of the high-prevalence RAO family show a low degree of intestinal parasitism, lead us to hypothesize that susceptibility to RAO and resistance to intestinal parasites are interrelated in this family.

Investigations on interactions between infections with different pathogens and development of allergies in humans have intensified because the hygiene hypothesis was postulated.¹³ Specifically, a reduction in the development of allergic disorders in individuals infected with intestinal parasites is reported in several studies.¹⁴ In horses, the regular use of modern anthelmintics and a decreased exposure to parasites may thus increase the risk for RAO and other allergic diseases. There is, however, another possible explanation for the inverse relationship between the intensity of certain parasitic diseases and measures of atopy: genetic determinants, which confer protection against worm burdens, may be the same determinants

From the Equine Clinic, Department of Clinical Veterinary Medicine (Neuhaus, Bruendler, Gerber), the Department of Paraclinics, Institute of Parasitology (Frey, Gottstein), and the Department of Clinical Research and Veterinary Public Health (Doherr), Vetsuisse Faculty, University of Berne, Berne, Switzerland.

Corresponding author: Vincent Gerber, Equine Clinic, Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Berne, Länggass-str. 124, CH-3012 Berne, Switzerland; e-mail: vinzenz.gerber@knp.unibe.ch.

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involved in atopic asthma.¹⁵ As a framework for exploring this alternative to the classical hygiene hypothesis, we hypothesized that RAO-affected half-siblings of the high-incidence RAO family shed fewer strongylid eggs compared with age-matched, RAO-unaffected pasture mates (PM), which are living under the same environmental conditions and are dewormed with the same compounds at the same time. In addition, we hypothesized that within the high-prevalence family, RAO-affected offspring would have lower strongylid egg counts than the RAO-unaffected half-siblings.

Materials and Methods

Horses and Study Design

Seventy-three direct descendants of a RAO-affected sire⁷ of Warmblood breed (family, F) were compared with 73 unrelated, healthy, age-matched PM. PM were kept at the same stable, were grazing on the same pasture, and were dewormed with the same anthelmintic agents at the same times as the corresponding F. Because of the reported relative lack of immunity to helminth parasites in younger horses,¹⁶ all horses in the study had to be adults, 3 years of age or older. On the basis of a standardized questionnaire focusing on the history of coughing, nasal discharge, poor performance, and abnormal breathing, which has been described in detail,⁷ all horses were classified as RAO unaffected or RAO affected. All horses (F and PM) had to have been exposed to hay as described before⁷ and all PM had to be RAO unaffected, again living under the same management conditions as the corresponding F. The study was approved by the animal use committee of the Canton of Berne.

Fecal Sample Collection

All fecal samples were obtained per rectum or from freshly voided feces and from each F and PM matched pair at the same time, before and 1–3 weeks and 3 months after the date of deworming. The 2nd sample was not an end-point measurement, but was used to exclude horses that had > 100 eggs per gram of feces (EpG), indicating resistance to the anthelmintic used or an otherwise ineffective deworming practice.^{17,18} The 3rd sample was used to monitor the agreement between EpG before and 3 months after the date of deworming, because helminth egg counts can vary considerably from day to day in horses. The 3 months time span between deworming and the 3rd sample collection was chosen based on the egg reappearance period, defined as the period when horses are expected to reach a maximum positive egg count after treatment.¹⁹

Copology

Each sample was examined without knowledge of group or health status at the Institute of Parasitology of the University of Berne. Samples were analyzed separately by a combined sedimentation-flotation method.²⁰ If strongylid eggs were found, a modified McMaster method was used for a quantitative determination (EpG) of egg expulsion.²⁰ All samples were also specifically scrutinized for occurrence of eggs from important potential confounders like *Dyctiocaulus arnfieldi* or *Parascaris equorum*.

Statistical Analyses

Analyses were performed with the statistical package NCSS 2007 (NCSS Statistical Software, <http://www.ncss.com>). Descriptive statistics are reported as frequencies and proportions (categorical variables) or means with 95% confidence intervals (CI) for continuous variables. After exploratory analysis, some variables were

categorized: 3 classes of EpGs (0 EpG, 1–100 EpG, and > 100 EpG); 4 age classes based on the quartiles (10.9; 11–13.3; 13.4–15; > 15 years of age); breeds of PM (eg, Warmblood, Pony, Haflinger, Freiburger) were grouped into 2 classes, Warmblood and other breeds. In a 1st step, univariable logistic regression analyses were performed. Effects on outcome (0, 1–100, > 100 EpG) of the independent variables group (F versus PM), age category, sex (female versus male), sharing of pasture with other species (yes versus no) were analyzed. If the univariable analysis (comparing the baseline outcome level to each of the two higher EpG groups) showed a Wald $P < .05$, the variable was included in a final multivariable logistic regression model. Furthermore, effects of group (F versus PM) were tested separately for RAO-affected and RAO-unaffected half-siblings and their PM; effects of breed (Warmblood versus others) within PM and of lung health status (RAO affected versus RAO unaffected) within the F were also analyzed univariablely. As outcomes, odds ratios (OR) and 95% CI are reported as OR; lower CI, higher CI. Significance level was set at $P < .05$.

Results

Horses

Mean ages of F (13.6 years; CI: 13.2–14.0) and of PM (12.3 years; CI: 11.2–13.4) were similar, but there was a modestly uneven distribution between F and PM when comparing the age classes (Table 1). Overall, 54% were male (all geldings) and 46% female horses, with slightly more males in F (Table 1). Of the 73 F, 26 (36%) were classified as RAO unaffected and 47 (64%) as RAO affected. PM consisted of 85% Warmblood horses and 15% other breeds (Pony, Haflinger, Freiburger) (Table 1). Eighteen matched pairs (25%) in total were sharing their pasture with other species (ie cattle and/or sheep) either at the same time or in rotation.

Coprological Findings

Before deworming, 146 fecal samples (73 from F and 73 from PM) were examined. Three months after deworming, only 78 valid samples, 40 F and 38 PM, could be obtained, because of logistic and/or technical reasons: Only 1 horse was excluded because it had shown > 100 EpG in the 2nd sample, all the others were lost to follow up either because of various forms of breaches in protocol—such as exceeding one of the predefined time intervals—or because the owners simply lost interest in the study. No horses had to be excluded because of signs of infection with *D. arnfieldi* or *P. equorum*. The decrease in the number of 3rd samples being entirely adventitious, the proportions of EpG classes were very similar before and 3 months after deworming, both in F and PM (Fig. 1A and B) as well as in RAO affected and RAO unaffected F (Fig. 2A and B). More than a 5th of the PM showed > 100 EpG before (1st sample, 22%) and 3 months after deworming (3rd sample, 21%). In comparison, only 4 and 3% of F were shedding > 100 EpG before and 3 months after deworming, respectively. Within F, RAO-affected offspring had fewer positive samples (> 0 EpG; 6% before and 7% 3 months after deworming) than the RAO-unaffected ones (27 and 17%, respectively).

Table 1. Results of descriptive statistics.

Variable	Groups			
	Before Deworming		3 Months after Deworming	
	F	PM	F	PM
Total number of horses	73	73	40	38
Female (n, %)	32, 44	35, 48	16, 40	19, 50
Age class 1 ^a (< 11 years, %)	12	40	5	47
Age class 2 ^a (11–13.3 years, %)	31	16	40	11
Age class 3 ^a (13.4–15 years, %)	40	11	45	10
Age class 4 ^a (> 15 years, %)	17	33	10	32
Warmblood breed (n/%)	73, 100	62, 85	40, 100	30, 79

Sex, age, and breed of direct descendants of a RAO-affected sire (F) and unrelated, healthy, age-matched pasture mates (PM) in a study on the shedding of stronglylid eggs.

^aNumerical code that was used in statistical analyses.

RAO, recurrent airway obstruction; F, members of a high-incidence RAO half-sibling family.

Logistic Regression Analyses

On the data before deworming (1st samples), complete regression analyses could be performed. Compared with F, PM had a 7.3 times higher OR (CI: 2.0–26.5, $P = .002$) for shedding > 100 EpG compared with 0 EpG (Table 2). Age showed a significant effect indicating that 13.4–15 year old horses were shedding fewer eggs than those < 10.9 years of age (1–100 EpG compared with 0 EpG: OR, 0.1; CI, 0.0–0.8; $P = .031$; > 100 EpG compared with 0 EpG: OR, 0.1; CI, 0.0–0.7; $P = .019$). Sex and

pasture management (ie, mixed with other species versus horses only) showed no significant effects on egg shedding. Multivariable logistic regression analysis confirmed an increased (5.4 times higher) OR (CI: 1.4–21.6, $P = .016$) for shedding > 100 EpG compared with 0 EpG without a significant effects of age classes (Table 3). When the effect of group (F versus PM) was analyzed univariably in separate datasets for RAO-affected and for RAO-unaffected half-siblings (Table 2), PM compared with RAO-affected F had a 16.7 times higher OR (CI: 2.0–136.3, $P = .008$) for shedding > 100 EpG

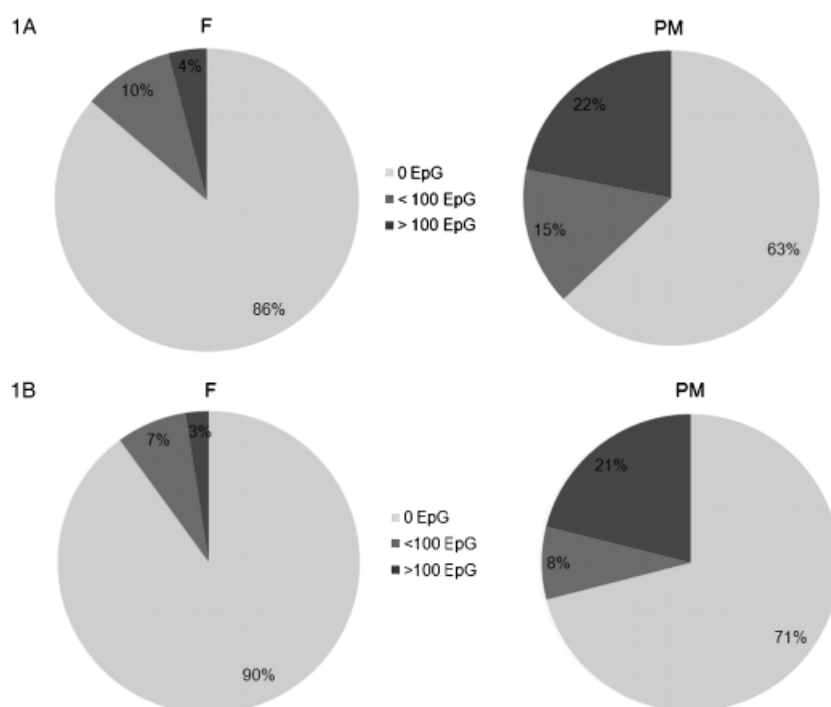


Fig 1. (A–B) Distribution of EpG in half-siblings and pasture mates (PM) before and 3 months after deworming. Percentages (%) of eggs per gram feces (EpG in 3 categories: 0 EpG; 1–100 EpG; > 100 EpG) in members of a high-prevalence RAO family (F; half-siblings descending from a sire affected with recurrent airway obstruction) compared with matched unrelated PM, before (A; 73 F and 73 PM) and 3 months after deworming (B; 40 F and 38 PM).

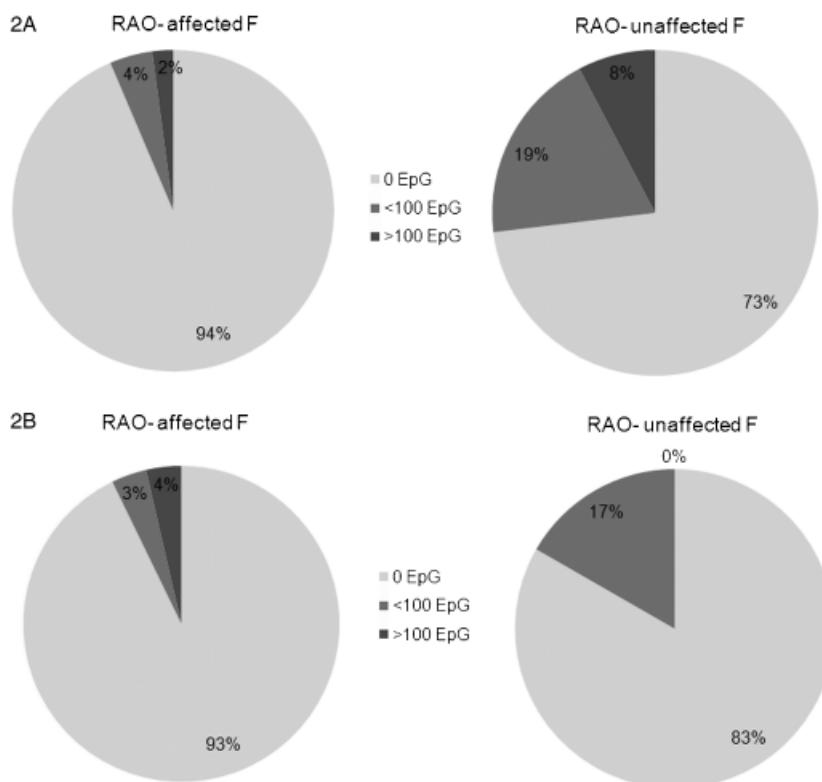


Fig 2. (A–B) Distribution of EpG in RAO-affected and RAO-unaffected half-siblings before and 3 months after deworming. Percentages (%) of eggs per gram feces (EpG in 3 categories: 0 EpG; 1–100 EpG; > 100 EpG) in RAO-affected compared with RAO-unaffected members of a high-prevalence RAO family (F; half-siblings descending from a sire affected with recurrent airway obstruction), before (A; 26 RAO-unaffected compared with 47 RAO-affected half-siblings) and 3 months after deworming (B; 13 RAO-unaffected compared with 27 RAO-affected half-siblings).

compared with 0 EpG and a 5.3 times higher OR (CI: 1.0–27.4, $P = .046$) for shedding >100 EpG compared with 0 EpG. There was no significant effect when RAO-unaffected F were separately compared with their PM. Within F, RAO-affected horses had a 5.8 times

lower chance (OR: 0.2, CI: 0.0–1.0, $P = .046$) for shedding 1–100 EpG compared with 0 EpG in contrast to RAO-unaffected horses. Within PM, breed (Warmblood versus other breeds) had no effect on egg shedding (Table 2).

Table 2. Results of univariable logistic regression before deworming.

Groups Analyzed	Variables Analyzed	Comparisons	Classes ^a	Odds Ratio	95% CI	<i>P</i> Value
F and PM	Group	F versus PM	1–100 EpG	2.2	0.9–6.0	.141
			> 100 EpG	7.3	2.0–26.5	.002
	Sex	Female versus male	1–100 EpG	1.3	0.5–3.6	.586
			> 100 EpG	1.8	0.7–4.9	.236
	Age	Age 2 versus age 1	1–100 EpG	0.7	0.2–2.5	.608
			> 100 EpG	0.3	0.1–1.2	.081
		Age 3 versus age 1	1–100 EpG	0.1	0.0–0.8	.031
			> 100 EpG	0.1	0.0–0.7	.019
		Age 4 versus age 1	1–100 EpG	0.5	0.1–1.8	.267
			> 100 EpG	0.5	0.1–1.6	.206
F	Sharing of pasture	Yes versus no	1–100 EpG	1.0	0.3–3.2	.946
			> 100 EpG	2.0	0.7–5.5	.201
	Health	Affected versus unaffected	1–100 EpG	0.2	0.0–1.0	.046
			> 100 EpG	0.2	0.0–2.5	.221
PM	Breed	Other breeds versus Warmblood breed	1–100 EpG	1.1	0.2–5.8	.951
			> 100 EpG	0.3	0.0–2.8	.297

^aBaseline is 0 EpG; F, descendants of a RAO-affected sire; PM, unrelated pasture mates; CI, confidence interval. Italics denote statistical significant value ($P < .05$).

Table 3. Results of multivariable logistic regression before deworming.

Groups Analyzed	Variables Analyzed	Comparisons	Classes ^a	Odds Ratio	95% CI	P Value
F and PM	Group	F versus PM	1–100 EpG	1.6	0.5–4.94	.417
			> 100 EpG	5.4	1.4–21.6	.016
	Age	Age 2 versus age 1	1–100 EpG	0.9	0.2–3.2	.839
			> 100 EpG	0.5	0.1–2.3	.367
		Age 3 versus age 1	1–100 EpG	0.1	0.0–1.1	.059
			> 100 EpG	0.3	0.1–1.8	.190
		Age 4 versus age 1	1–100 EpG	0.5	0.1–1.9	.290
			> 100 EpG	0.5	0.1–1.7	.271

^aBaseline is 0 EpG; F, descendants of a RAO-affected sire; PM, unrelated pasture mates; CI, confidence interval. Italics denote statistical significant value ($P < .05$).

Three months after deworming (3rd samples), PM compared with F still had a 10.7 times higher OR (CI: 1.3–90.5, $P = .030$) for shedding > 100 EpG compared with 0 EpG, confirming the results before deworming. There were no significant effects of either sex or pasture management. Because of the diminished number of samples, the complete regression analyses as above could not be performed. Specifically, effects of group (F versus PM) separately by RAO-affected and RAO-unaffected F as well as effects of age classes, health status (RAO affected versus RAO unaffected) within F and of breed (Warmblood versus other breeds) within PM could not be further analyzed due to the low frequency of observations in some classes.

Discussion

The present results support our hypothesis that members of a high-incidence RAO family shed fewer strongylid eggs when compared with RAO-unaffected PM. Specifically, the descendants of the RAO-affected stallion had a several-fold decreased chance for shedding > 100 EpG compared with 0 EpG. The odds decreased only slightly, when the only other univariably significant factor, age class, was included in the multivariable analysis. This “family-effect” was caused by the RAO-affected half-siblings: the OR more than doubled when only the RAO-affected F were compared with their PM and there was no significant effect when RAO-unaffected F were separately compared with their PM. The 2nd part of our hypothesis, that RAO-affected offspring within the high-prevalence family would have lower strongylid egg counts than the RAO-unaffected descendants, is also supported by our findings. RAO-affected individuals exhibited a 5.8 times smaller chance than their RAO-unaffected half-siblings for shedding 1–100 EpG compared with 0 EpG. Even though the number of samples after deworming was too small for a complete statistical analysis, the good agreement before and after deworming strengthens the findings.

There is a considerable wealth of evidence supporting the classic concept of the hygiene hypothesis in the context of parasitism.²¹ The data are contradictory where helminthes are concerned, however, with helminth infections being associated with protection from, but also with promotion of allergy and asthma.²² Acute infections seem to promote allergic manifestations, while chronic

infections rather decrease them.²³ For asthma specifically, a recent meta-analysis on 33 earlier studies showed that parasitism is not in general protective, but that *Ascaris lumbricoides* infections are actually associated with increased odds for asthma, while hookworms, with increasing infection intensity measured by fecal egg counts, had a protective effect.²⁴ Horses in Switzerland are mostly infected with strongylids belonging to the family *Cyathostominae*, ie, small strongyles²⁵ and important confounders like *P. equorum* and *D. arnfieldii* were not revealed in our study.

Interestingly, the authors of the meta-analysis²⁴ also noted that because of the cross-sectional nature of the studies on asthma and parasitism, the inverse causal explanation, ie, that asthma protects against hookworm infection, cannot be ruled out. The classical hygiene hypothesis postulates that infections and unhygienic contact confer protection from allergic disease. An underinvestigated alternative explanation for this association is that genetic determinants that confer protection against detrimental worm burdens are the same determinants involved in allergic disease.¹⁵ The results of the present study support the latter concept, because the half-siblings were completely matched to their PM for environmental effects and RAO affection was clearly associated with markedly decreased egg counts.

There are very few epidemiological studies that specifically investigate this inverse relationship between measures of atopy and resistance to parasitic diseases in humans. To our knowledge, no investigations exist on this phenomenon in animal populations. Moreover, some of the results observed in humans are conflicting. An early report found evidence that the atopic state confers resistance to helminth infection in an endemically hookworm-contaminated human population in New Guinea.²⁶ A later study performed in the same population did not support these findings, however.²⁷ Again conversely, children with a strong atopic background had lower fecal helminth egg counts associated with an enhanced protective IgE response.²⁸ The limitation of this latter study was that the authors compared atopic children from a closed population on a Venezuelan island to nonatopic children living under similar hygienic conditions, but coming from the Venezuelan mainland. The fact that the environment there was approximately similar but not identical leaves room for unrecognized confounding environmental

factors to potentially influence the results. In contrast, the present study compared adult horses of a high-prevalence RAO family to very closely matched PM, avoiding major environmental confounders.

The results thus provide important evidence supporting the concept that an allergic state may have conferred a selective advantage in the form of an enhanced protection against intestinal strongylid parasites in the evolution of the horse. However, the present study is limited because only 1 high-prevalence RAO family was investigated. Similar to findings in human asthma, the genetic background for RAO in this family differs from that in another high-prevalence family we have investigated.^{8,9} Extrapolation from limited numbers and relatively closed populations, such as the family investigated here, would be treacherous. Further studies will have to show if this concept is valid in other high-prevalence RAO families and ultimately in the general equine population and will also need to focus on the mechanisms that might orchestrate these events. An important aspect in this respect is the association and linkage of the RAO phenotype with genetic markers near *IL4R α* in this high-prevalence RAO family.⁹ *IL4R α* is associated with human asthma and atopy^{10,11} as well as with defense against parasites in humans and animals.¹² IL-4 and IL-13 operate through the IL-4 receptor, both cytokines are reported to be upregulated in RAO.^{1,29} IL-4 promotes immunoglobulin isotype switching from IgM to IgE.³⁰ In fact, half-siblings of the high prevalence family show increased allergen-specific, but not total serum IgE levels (Gerber, unpublished results). Thus, the Th2-type immune reaction in general, and *IL4R α* , IL-4, IL-13, and IgE more specifically, are candidates for the common mechanisms and pathways by which the susceptibility for RAO may be genetically linked to an enhanced protection against helminth parasites.

In comparison to man, domesticated animals usually are closed populations derived from a relatively small number of founders. This increases the chance of identifying genes involved in RAO and resistance against parasites. Such investigations should not focus solely on genes involved in Th2-type immune reactions, because the evidence for a clear Th2-type bias in equine RAO, as in human asthma, is far from unequivocal.³¹ In fact, in another high-prevalence RAO family we have not found an association of *IL4R α* with RAO, which indicates genetic heterogeneity of this disease. In their review on potential common genetic determinants for asthma and resistance to parasitic disease, Eysker et al¹⁶ not only point out IL-4, IL-13, *STAT6*, or IFN- γ , but also for instance the gene encoding the β 2-adrenoceptor. Genetic variants of this latter gene have been associated with both helminthiasis and asthma,³² and β 2-adrenoceptor dysfunction is associated with the reversible bronchospasm in equine RAO.³² Besides uncovering the underlying genes in this family, it is important to also investigate if this relationship between RAO and helminth resistance also exists in other high-prevalence families and in the general horse population. It may furthermore be worthwhile to determine whether a similar association can be found between parasite resistance and susceptibility to insect bite

hypersensitivity, a disease that in contrast to RAO clearly shows characteristics of atopy and that also has a genetic basis.³¹

The present findings show that RAO-affected half-siblings of a high-prevalence family are more resistant against intestinal strongylid nematodes when compared with unrelated PM and that within the family, the RAO-affected offspring are more resistant than their RAO-unaffected half-siblings. These associations are unlikely confounded by environmental factors, since the environment was completely matched for each case and control pair. The results of this study thus offer an alternative explanation to the classical hygiene hypothesis for the inverse relationship between the intensity of certain parasitic infections and measures of allergic diseases: Genes that offered an evolutionary advantage by providing increased protection against intestinal helminth parasites may confer an increased risk for RAO when these horses are fed hay.

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