**Introduction**

The increasing knowledge of mammalian genetics has shown that genetic factors not only influence physical traits such as conformation, coat colour, etc., but also susceptibility to diseases and performance. In the horse, single gene diseases are relatively rare compared to other domestic species such as the dog. During the last decade, the genes responsible for a few of the single gene diseases of the horse have been identified [1-3]. However, other diseases of the horse probably have a genetic basis as well, but the occurrence of these diseases is also influenced by other factors such as the environment. This renders the recognition of the genetic component difficult. To our knowledge, genetic studies on equine respiratory diseases have only been carried out for recurrent airway obstruction and idiopathic laryngeal hemiplegia so far. We can speculate that other diseases, as for example exercise-induced pulmonary haemorrhage, may also have a genetic basis. Studies confirming or refuting this hypothesis still need to be performed. Additionally, susceptibility to infectious diseases, as well as the ability to mount an immune response after immunisation [4], are probably also influenced by genetic factors.

**Recurrent Airway Obstruction (RAO)**

Recurrent airway obstruction (RAO) or heaves [5], which is sometimes also called chronic obstructive pulmonary disease (COPD) or chronic bronchitis/bronchiolitis (CB), is a hypersensitivity reaction to mould spores present in hay and straw dust and/or to other allergens (see Recurrent Airway Obstruction (Heaves)). This disease shares a number of similarities with the allergic bronchitis-asthma syndrome in man. The presence of a genetic predisposition for heaves was already suggested by Schaeper in 1939 [6]. He showed that 14 of 27 offspring from the heavey stallion "Egmont" were also suffering from heaves. His study, however, did not include a control group, i.e., offspring, from a stallion that was not affected with RAO. Similar multiple-case families were also reported later by different authors [7,8].

A more extensive study was performed in 1991 [9] to see whether a genetic predisposition for RAO could be demonstrated. This investigation was carried out in two different studs (90 German warmblood and 42 Lipizzan horses, age ≥ 8 years, with known clinical history of their parents). Additionally, a third group was included in the study, consisting of 153 half-siblings (age ≥ 5 years) from different farms and regions in Switzerland, sired by three healthy and three RAO-affected stallions. The 153 dams of this group could not be evaluated for a history of RAO. Within the two studs, the environmental conditions were quite similar for all horses, i.e., they were fed the same hay, etc. The mares were kept loose in group housing stables, while the stallions were housed in individual loose boxes or stalls. Conversely, the 153 Swiss half-siblings were kept in many different farms. In this group, the hygene of the stables was classified by air circulation, dust and humidity (poor, satisfactory, good).

In both studs the clinical examinations were carried out by the studs’ veterinarians, and the presence or absence of RAO was based on prolonged (several years) observations and multiple examinations. The lung status of the sires of the 153 Swiss half-siblings was also based on prolonged observation and clinical examination, including bronchoscopy, cytology of tracheobronchial secretions and arterial blood gas measurements. These stallions were between 17 and 20 years old. The clinical examination of the half-siblings included history, heart rate, rate and character of respiration, rectal temperature, observation of mucous membranes, palpation of the lymph nodes, palpation of larynx and trachea. Auscultation of heart and lung were performed at rest and after exercise or forced respiration. A complete blood count was also performed. Circumstances on the various farms did not allow the application of bronchoscopy or other more objective methods. Horses in which the examination revealed ambiguous symptoms or histories were not included in the analysis (n = 8). Horses were divided into RAO-affected or RAO-unaffected. They were considered as RAO-affected when a chronic cough with or without dyspnea was present in horses kept indoors and fed hay. These horses showed biphasic double expiratory effort and increased breathing sounds during the auscultation. In more severe cases wheezing could be heard. Horses that had...
previously shown clinical signs of RAO but were in remission after being removed from hay and straw were included in the RAO-affected, i.e., RAO-susceptible group.

Horses from both studs were grouped according to the clinical history of their parents (three categories: none, one or both parents affected). Figure 1 shows that in both studs the percentage of offspring suffering from RAO increases with increasing number of affected parents.

In the German warmblood stud 17%, 48% and 69% of the offspring, respectively, were affected with RAO when none, one or both parents were affected with this disease (P <0.005). Similar results were obtained in the Lipizzan stud (6%, 35% and 44%, respectively, P = 0.062). Interestingly, these findings were quite similar to the results from a study on the inheritance of atopy in humans (Fig. 1, [10]). In both horse groups, the gender of the affected parent did not influence the susceptibility to RAO in the offspring. There were fewer RAO-affected offspring in the Lipizzan stud than in the German warmblood stud. However, this difference between the two studs, which might be due to environmental or genetic factors, was statistically not significant.

The overall results of the 153 Swiss half-siblings showed that stallions with RAO sired significantly more affected offspring (31 out of 79) than healthy stallions (9 affected offspring out of 74).

Additionally, the 153 Swiss half-siblings were grouped according to their age (four age groups: 5 - 7, 8 - 10, 11 - 13, ≥14 years) and according to the clinical status of their sires (Fig. 2).

In each halfsibling group the frequency of affected horses increased with increasing age. However, in each age group, the percentage of affected horses was higher in the group of offspring sired by RAO-affected stallions than in those produced by healthy sires. An analysis of variance showed that sire, age and environment (stable) all exerted a significant effect on the disorder (Table 1).

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degree of Freedom</th>
<th>Chi Square</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sire</td>
<td>5</td>
<td>15.33</td>
<td>0.009</td>
</tr>
<tr>
<td>Age Group</td>
<td>3</td>
<td>11.11</td>
<td>0.011</td>
</tr>
<tr>
<td>Environment</td>
<td>2</td>
<td>6.05</td>
<td>0.049</td>
</tr>
</tbody>
</table>

The results from both studs were pooled to calculate the relative risk according to Mantel-Haenzel that a particular horse would develop RAO based on the clinical condition of his parents (Table 2). When both parents were free of RAO, the prevalence of the disease among the offspring was low (6 of 45 affected horses, 13%). If either of the parents was affected, the chance of developing RAO increased 3.2 times (p <0.05); when both parents were affected the risk was 4.6 times greater (p <0.05).

This study demonstrates that genetic factors play an important role in the pathogenesis of RAO. Interestingly, these findings display striking similarities with some studies on the genetics of asthma in humans [11]: Affected individuals have a higher percentage of positive family histories, the predisposition and not the disease is transmitted, and the mode of inheritance is not clear. Although the genes influencing the genetic susceptibility for RAO have not been identified, we can speculate that,
like for asthma in humans, a number of different genes could be involved. The equine major histocompatibility complex does not seem to influence the genetic predisposition for RAO, as no association between the equine leukocyte antigens and RAO could be demonstrated [9].

Although the pathogenesis of RAO is still not fully understood, a number of studies suggest that immunoglobulin E (IgE) against moulds or other allergens may be involved in the pathogenesis of this disease [12,13]. In man, it has been known for many years that IgE levels are elevated in atopic individuals and different studies have shown that the ability to produce high or low IgE responses is inherited [14] and linked to a number of different genes (see [15] for a review).

In a recent study [16], IgE levels against moulds were determined in sera from 450 Lipizzan horses from six different studs. Analysis of the results showed that not only the stud, i.e., the environment, but also genetic factors influence the specific IgE response. Heritability estimates of 0.33 (standard error = 0.09) were found for IgE levels against moulds. This means that 33% of the variation of the IgE levels were due to genetic factors. Further studies are now needed to see whether genes influencing the IgE response account for the genetic predisposition to RAO.

### Hemiplegia Laryngis

Hemiplegia laryngis is a disease occurring world-wide, which is of special importance in high-performance sport horses. In the literature there are numerous references to the possible causes of this disease [17]. It is undisputed that the visible characteristics of a neurogenic muscle atrophy can be determined endoscopically in the cases of hemiplegia laryngis. The larynx of affected horse shows an atrophy of the left-sided laryngeal muscles which are supplied by the left recurrent laryngeal nerve (Fig. 3). This muscle atrophy was first described by Cole in 1946 [18].

<table>
<thead>
<tr>
<th>Number of Affected Parents</th>
<th>% Affected Offspring</th>
<th>Relative Risk (Mantel-Haenzel)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>13%</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>One</td>
<td>44%</td>
<td>3.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Both</td>
<td>60%</td>
<td>4.6</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

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Further histological investigations [19-22] showed that signs of neurogenic muscle atrophy correlated with the degree of laryngeal hemiplegia. It was also shown that the adductor muscles are more often and more severely affected than the abductor muscles [20-22], which are important for the laryngeal opening. In horses with a low-grade hemiplegia there is already an increase of connective tissue between the muscle fibres compared with unaffected muscles (Fig. 4). Further investigations, in particular electron microscopic studies of the distal part of the recurrent laryngeal nerve show a clear loss of myelinated fibres compared to the unaffected side [20,21,23-26].

Laryngeal paresis refers strictly to reduced laryngeal movements, whereas the term laryngeal plegia applies to the complete absence of laryngeal movements. However, the expression of "laryngeal hemiplegia" is commonly used both for the hemiparesis and for the hemiplegia. In over 99% of the cases, this disease occurs on the left-side, while the right-sided form occurs very rarely and the both-sided form is very uncommon. This disease can occur in an idiopathic form, almost exclusively on the left-side. For the idiopathic laryngeal hemiplegia no etiological causes can be determined which is in contrast to left- right- or both-sided secondary laryngeal hemiplegia with known causes [17], for example:
irritant perivascular/perineural injections
accidents to the neck
guttural pouch mycosis
neoplasia
organophosphate intoxication
lead and plant poisoning
bacterial and viral infections
4th branchial arch defect

Two theories about a possible mechanical etiology of idiopathic laryngeal hemiplegia (ILH) have been proposed. On one hand, ILH could be caused by a stretch to the left recurrent laryngeal nerve, on the other hand, a compression of the left recurrent laryngeal nerve in the area of the aortic arch could have an influence. Thiamine deficiency has also been suggested as a possible cause of ILH [27].

A genetic predisposition for ILH has been suspected for a long time. However, other pathogenic factors have also been discussed. It has been suggested that the occurrence of the disease is influenced by the breed, age, sex, size and conformation of the horse as well as by management, climate and geographical conditions. While ILH is known to occur more frequently in larger breeds and in larger, heavier and taller horses [28,29], the influence of the sex is unclear. Furthermore, the conformation of the horse, in particular long light necks, flat sides, a narrow chest and a narrow intermandibular space seem to be predisposing factors for ILH. It is not known yet whether the occurrence of ILH is influenced by genetic factors specific for the function of the larynx or whether a genetic predisposition for ILH is just seen because the occurrence of ILH is associated to the size and conformation of the horse. Physical traits usually display a rather high heritability. The effect of the age of the horse also needs further investigations, although the current opinion is that ILH is a disease of young horses.

The presence of hereditary factors contributing to the pathogenesis of ILH has already been postulated in 1577 by Max Fugger and by various authors in the 19th and 20th century (Table 3, [30,31]). As a further sign of the heredity of ILH, the occurrence of this condition in foals and yearlings has been analysed and described. Goebbelk [32] saw a left-sided laryngeal paralysis in a 9 days old foal. Hillidge, Lane and Sloet-Oosterboan [33-35] described the disease in foals and yearlings as well. However, one study did not demonstrate a genetic basis for ILH: Kuhn [36] could not observe an increase in frequency of ILH in 200 offspring from an affected stallion.

<table>
<thead>
<tr>
<th>Table 3. Studies suggesting a genetic predisposition for idiopathic laryngeal hemiplegia. (* cited in [30], # cited in [31]).</th>
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</thead>
<tbody>
<tr>
<td><strong>Fugger * M. (1577)</strong></td>
</tr>
<tr>
<td><strong>Günther * K. (1843)</strong></td>
</tr>
<tr>
<td><strong>Möller H. (1888) [31]</strong></td>
</tr>
<tr>
<td><strong>Dupuy * (1827)</strong></td>
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<tr>
<td><strong>Markham # (1833)</strong></td>
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<td><strong>Youtt # (1833)</strong></td>
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<tr>
<td><strong>Delafondin # (1844)</strong></td>
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<tr>
<td><strong>Goux # (1868)</strong></td>
</tr>
<tr>
<td><strong>Charon * (1886)</strong></td>
</tr>
<tr>
<td><strong>v. Öttingen * (1918)</strong></td>
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</tbody>
</table>
To estimate the heritability of ILH and to analyse which other factors influence the occurrence of this disease, Ohnesorge [39,40] and Miesner [41] examined a total of 240 foals and 862 adult horses between 1989 and 1990. The laryngoscopical investigation took place in sedation with Domosedan® (0.015 mg/kg BW, IV or 0.0130 mg/kg BW, IM). The arytenoid movements were evaluated at rest and after forced respiration to determine the degree of ILH. Additionally, 120 of the adult horses could be exercised to determine whether an inspiratory noise was present. For the genetic analysis 421 offspring (205 foals and 216 adults) of 20 sires and 308 dams were included in the study. All parents and offspring had been examined endoscopically. Also, 35 foals and 259 adults descending from the same 20 stallions but whose dams had not been examined were used (Fig. 5).

In healthy horses with a normal arytenoid movement there was a synchron and symmetrical opening of the larynx during the inspiration. In contrast, an asymmetrical opening of the larynx during the inspiration was considered as a sign of ILH. An asymmetry up to one-third was classified as low-grade ILH, an asymmetry between one and two-thirds as moderate, and an asymmetry of more than two-thirds as severe ILH (Fig. 6). Using these gradings, 8 stallions showed no ILH, 5 stallions were mildly affected, while 7 stallions showed signs of moderate to severe ILH.

Many clinicians recommend performing laryngoscopy without sedation because sedation may have an effect on laryngeal function. However, Ohnesorge and Miesner performed the laryngoscopic examination on sedated horses for the following reasons: Sedative drugs like detomidine (alpha-2-adrenoceptor-agonists) affect both the right and the left recurrent nerve in the synaptic-area [42] and should thus not influence the symmetry of arytenoid movements. Furthermore, use of detomidine allowed a standardized examination and permitted the recognition of low degrees of ILH. Low-grade ILH may not be detected during laryngoscopy of unsedated animals because only a small proportion of the muscle fibres are affected at this stage of the disease and the remaining, still unaffected muscle fibres may be sufficient for a complete opening of the larynx. A recent study [22] suggests that asymmetry of the arytenoid movements in sedated horses is a sign of low-grade ILH, as it is correlated with signs of muscle atrophy. Finally, all investigated horses were examined under the same conditions (all sedated) and this is the most relevant aspect for the genetic study presented here.

To investigate whether ILH was influenced by genetic factors, Ohnesorge et al. [39,40] grouped the offspring according to their own diagnosis (ILH present or absent) and according to the diagnosis of their parents. This analysis was performed in the foals and in the adult horses separately. Foals from healthy parents were significantly less often affected with ILH than foals descending from parents both suffering from ILH (8.9% and 41.1%, respectively, P < 0.01, Fig. 7). This difference was also significant (P < 0.001) in adult offspring: Only 39.6% of the offspring from healthy parents were affected with ILH, while 60.6% of the offspring from two affected parents suffered from ILH (Fig. 8). Additionally, in 250 offspring from the stallions mentioned above the clinical status (healthy or ILH) of the mares was not known. Also in this group, affected stallions sired significantly more offspring with ILH than healthy sires (57.7% and 44.8%, respectively; P < 0.05).

Analysis of the relationship between ILH and age shows that the disease develops particularly during the first five years of
The incidence of moderate to severe ILH was low in foals (4 foals from 240; Fig. 9 and Fig. 10). However, 24.7% of these 240 foals had already low-grade to moderate ILH. The frequency of ILH affected animals was significantly higher in adult horses than in foals, as 49.7% of the examined adults showed low-grade to severe ILH. Additionally, two to three year olds were compared with four to five year old horses (Fig. 9). The frequency of ILH was slightly higher in the older groups; this difference, however, was not significant (46.9% versus 53.3%, P >0.05).

This investigation also showed that the only determination of the presence or absence of an inspiratory noise is not sufficient to identify horses affected with ILH, and in particular horses with low-grade ILH. Only 54.8% of 70 horses with ILH showed an inspiratory noise during exercise. However, as expected, the frequency of ILH was high in horses with a detectable inspiratory noise (80.9% of 47 horses).

Using the same horses but with an index to quantify the degree of ILH and complex statistical models, Miesner [41] found a high heritability of 0.61 for ILH (standard error = 0.17). This study confirms that genetic factors strongly influence the occurrence of ILH, as had been postulated by different authors previously. Additionally, he showed that the age of the horse exerts a significant effect on the occurrence of ILH. Furthermore, his study demonstrated a significant positive correlation between ILH and the length of the neck and between ILH and narrow intermandibular spaces. However, no significant effects of sex, breed, use of the horse, stabling, anti-parasite treatment, strangles, heights at withers and weight could be demonstrated.

The studies of Ohnesorge et al., [39,40] and Miesner [41] demonstrated that genetic factors play a significant role in the pathogenesis of ILH. However, they could not show a simple recessive or dominant mode of inheritance and suggest that ILH is a polygenic trait. Future investigations will now involve the search for the genes responsible for this disease. For this purpose better knowledge about the pathogenesis of ILH could help to identify candidate genes. As already stated by Cahill and Goulden [17] the distal axonopathy, causal for ILH, can be due to different mechanisms, of which genetic defects have to be considered: first, an enzyme- or cofactor inactivation could lead to energy dependent disorders. Second, a defective synthesis or supply of antioxidant compounds could result in a reduced protection of the cell membranes. A third hypothesis involving a structural change in neurofilament configuration leading to an obstruction of the axonal transport should also be considered. Investigating such patho-mechanisms may allow the identification of the molecular genetic basis of ILH in the future.

**Conclusion**

As a consequence of the studies described above, RAO and ILH should be taken into account when selecting horses for breeding. The identification of genetic markers for these diseases could help to recognize horses genetically predisposed to these conditions. However, a prerequisite for finding genetic markers is the availability of a genome map of the horse showing the chromosomal location of genes and markers. This is an essential tool for finding genes or markers for genetic traits such as susceptibility genes for diseases, genes for conformation, coat colour, performance, etc. Although genes for some traits can be identified without a gene map through the candidate gene approach, this strategy is nearly always only applicable for single gene diseases, when genes can be rationally selected based on the pathogenesis of a disease or from comparative studies in other species. RAO and ILH are most probably diseases influenced by multiple genes. The horse genome map has been constructed through an international collaboration over the last 6 years (http://locus.jouy.inra.fr; http://www.uky.edu/ag/horsemapping) and has now reached the stage where it can start being used for finding genetic markers for different traits in the horse. The identification of genetic markers for RAO and ILH could facilitate the breeding of animals less susceptible for these diseases by allowing the identification of genetically predisposed individuals early in life. However, we have to keep in mind that both RAO and ILH are diseases occurring with a relatively high frequency in the riding-horse population, rendering selection against these traits quite difficult. Furthermore, some of these susceptible horses may have other positive traits which breeders want to select for. Breeders will have to weigh the risk of having a horse predisposed to
RAO or ILH against other valuable traits this individual exhibits. Selection against these diseases may even be more difficult if the condition is associated to traits which are desired in riding-horses, e.g., the length of the neck which is also significantly associated with ILH. However, adequate breeding programmes including disease resistance as well as physical and performance traits should allow with time, the breeding of healthier but still high-performance horses.

References

41. Miesner K. Untersuchung über die abduktorische Funktionsstörung des Kehlkopfes beim Pferd: Genetische und umweltbedingte Einflussfaktoren sowie mögliche züchterische Massnahmen. Thesis of the Agricultural Faculty of the Friedrich-Wilhelms University, Bonn, Germany, 1996.

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