Insect bite hypersensitivity in Belgian warmblood horses: prevalence and risk factors

Zomereczeem bij Belgische warmbloedpaarden: prevalentie en risicofactoren

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ABSTRACT

Insect bite hypersensitivity (IBH) is an allergic reaction to the bites of certain *Culicoides* spp. or other insects. In this study, risk factors for IBH in Belgian warmblood horses stabled or grazing in Flanders (Belgium) were investigated. IBH records (n=3409) were collected in 2009 and 2011 using a questionnaire and face-to-face interviews. The classification of IBH-affected versus unaffected horses was based on the owner's statement, and the reported IBH lifetime prevalence was 10%. Thirty eight percent of IBH affected horses had no clinical symptoms at the time of questioning. When only the presence or absence of clinical symptoms at the time of questioning was taken into account, the prevalence of IBH symptoms was 6.2%. Seventy percent of IBH-affected horses were treated with IBH measures to reduce clinical symptoms. Model selection was based on backwards elimination in a logistic regression framework starting with 17 factors. The age of the horse, vegetation of surrounding pasture and stud size were found to be significantly associated with the self-reported IBH status.

SAMENVATTING

Zomereczeem is een allergische reactie op specifieke antigenen in het speeksel van *Culicoides*-muggen of andere insecten. In deze studie werden omgevingsfactoren geïdentificeerd die geassocieerd zijn met het voorkomen van zomereczeem bij Belgische warmbloedpaarden (BWP) in Vlaanderen (België). Daarvoor werden met behulp van een enquête 3409 gegevens over zomereczeem verzameld tijdens sportwedstrijden, BWP-prijskampen en via persoonlijk contact met grote fokkers (stoeterijbezoeken en telefoongesprekken). De paarden werden ingedeeld in "zomereczeempositief" of -negatief op basis van de informatie die door de eigenaars aangereikt werd. De gemiddelde prevalentie van zomereczeem bij BWP bedroeg 10% in Vlaanderen. Zeventig procent van de zomerczeempositieve paarden werd behandeld met preventiemaatregelen om de symptomen te onderdrukken en 38% van de positieve paarden vertoonde geen symptomen op het moment dat de enquête werd afgenomen. Hierdoor was de gemiddelde prevalentie van merkbare symptomen slechts 6,2%. De volgende risicofactoren voor het optreden van zomereczeem bij BWP werden geïdentificeerd: leeftijd, de methode van datacollectie, de periode waarop de enquête werd afgenomen, de trainingsfrequentie en de vegetatie van de omliggende weiden. Daarenboven beïnvloedde het aantal paarden per eigenaar eveneens de (gerapporteerde) prevalentie van zomereczeem.

INTRODUCTION

Insect bite hypersensitivity (IBH) is a chronic, recurrent, seasonal dermatitis. Worldwide, it is the most common allergic skin disease in horses (Gortel, 1998). IBH represents a hypersensitivity reaction to salivary antigens from *Culicoides* species and possibly from other insects. It is characterized by numerous papules and tufted hair, which is followed by intense pruritus and self-excoriation (Anderson et al., 1988). The welfare as well as the commercial value of affected horses are seriously reduced (Fadok and Greiner, 1990). Apart from the genetic susceptibility (Lange, 2004, Eriksson et al., 2008, Schurink et al., 2011), multiple factors are considered to play a role in the occurrence and severity of symptoms associated with IBH, such as geographical location, habitat, management, nutrition, age of the horse and the age of first exposure to allergens (Halldórsdóttir and Larsen, 1991; O'Neill et al., 2002; Steinman et al., 2003; Pilsworth and Knottenbelt, 2004; van Grevenhof et al., 2007). Although many studies have been performed on IBH, most were performed on a small number of individuals, involving more than one breed and gender (Braverman et al. 1983; Broström et al., 1987, Halldórsdóttir and Larsen 1991, Steinman et al. 2003).

Recently, risk factors that might affect IBH prevalence in Dutch Shetland ponies and Dutch Friesian horses have been identified (Schurink et al., 2012) using a large number of IBH records, i.e. 7074 and 3453, respectively. These records were collected by foal inspectors (Schurink et al., 2012), but this approach seems unfit for warmblood populations. Warmblood horses are most commonly used in competitions (dressage, jumping, etc.) and because of their economic value, the overall management might differ from that of ponies. Risk factors for IBH were first studied in Belgian warmblood horses based on a survey held in 2009 (Peeters et al., 2010). Results pointed at effects of vegetation (surrounding the pasture), body condition and age on IBH. However, the yearly variation and the effect and the role of owners in IBH were not investigated, although this is crucial information in the framework of breeding, for which the IBH status of many individual horses is required.

MATERIALS AND METHODS

Collection of data and population

A questionnaire was designed to collect data on the IBH status (current and lifetime) of individual Belgian warmblood horses, together with information on management, environment and any preventive or curative measures taken by the owner. The questionnaire was pre-tested on ten horse owners. The duration for completing the final questionnaire was about three minutes per horse. In order to obtain a representative sample of the breeding population of Belgian warmblood horses, events were sampled from the list of events within each of the years 2009 and 2011, and approximately reflect the number of BWP horses in each province. In order to collect information simultaneously at events, five investigators were trained for the study by the principal investigator (Table 1). Data were collected using face-to-face interviews with horse owners attending breeding days (n=6) and sport competitions (n=35) in 2009 and 2011 in Flanders (Belgium). Every owner attending an event was invited by one of the interviewers to participate in the survey. Owners with more than thirty horses were visited at home due to time constraints at the events.

The questionnaire started with individual animal information (name, id-number, age), IBH status of the horse and time of onset. Twelve questions were devoted to background information, such as surrounding vegetation and soil humidity of pastures, body condition of the horse, housing system and horse management (Table 1). Another eight questions concerning the severity and history of IBH-clinical symptoms were answered in case the respondent classified his horse as IBH-affected. The questionnaire was completed for all horses owned by each respondent.

Sire and dam of each horse, age, coat color and sex were retrieved from the BWP studbook database based on the stud book number (Table 1). If the stud book number was missing, a custom made program implementing pattern matching techniques was used to search in the database, based on the name of the horse (Van Geystelen, 2011).

Trait definition of IBH

The IBH status was based on the owners' observations of typical symptoms of sweet itch, e.g. itching, hair loss at tail and manes. If IBH was observed during life, the horse was considered affected (status=1). Alternatively, if clinical symptoms had never been observed by the owner, the horse was classified as IBH-unaffected (status=0). The status of the horse at the time of data collection was also recorded and referred to as "Clin_status" (1=currently affected or 0=currently unaffected)

For affected horses, the earliest age of symptoms, any IBH measures taken by the owner to suppress the symptoms and their effectiveness were recorded. The term IBH measure is used for every measure taken by horse owners to reduce clinical symptoms, i.e. preventive and curative measures. All immunosuppressive treatments, e.g. corticosteroids, were grouped as medication.

Data handling and statistical analysis

Data were coded and stored in a database (MS Access) and procedures in SAS (SAS Institute, Cary, NC, version 9.2) were used for descriptive and statistical analyses. The age of the horses and the number of horses per owner were transformed in categories. Two criteria for categorizing continuous variables were a) the low number of data in some parts of the range of the continuous variable and b) a non-linear association with the dependent variable, which would require several higher order terms in the model. Age was categorized based on biological criteria (development of immune response) and a non-linear association. Stud size was categorized in two categories (up to ten horses and > ten horses) so that at least 25% of the horses were in one category. A model was built using logistic regression and aiming for robustness rather than causality of associations of factors with IBH status and with IBH clin_status. Therefore, predictor variables were first screened individually

Table 1. Description of factors in the study on insect bite hypersensitivity in Belgian warmblood horses. The information refers to individual horses and was collected using a questionnaire and studbook data.

Factor	Information collected	Categories used for analysis
Year of questioning	Year, in which the record was collected	1= 2009 2= 2011
Period of questioning	Period of the year, in which the questionnaire was taken	1= Pre summer (April, May, June) 2= July 3= August 4= Post summer (September and October)
Method of data collection	Type of competition where the record was collected, or if records were collected by visiting or calling breeders (personal contact)	1= Sport competitions 2= BWP breeding days 3= Breeder visits and calls
Investigator	The person who questioned the horse owner. Three investigators collected less than 100 records and were grouped (investigators)	1= Investigator 1 2= Investigator 2 3= Investigator 3 4= Investigators (4+5+6)
Vegetation around pasture	Question: “Does the horse graze in ‘open land’, close to a ‘hedgerow’ or in a ‘woody’ pasture?	1= Open land 2= Hedgerow 3= Woody
Humidity of soil of pasture	Question: “Would you describe the pasture of the horse as dry, soggy or very wet?”	1= Dry 2= Wet (soggy + very wet)
Body condition	Question: “Assign a linear score for the body condition (BC-score) going from 1 (very skinny), 5 (normal) to 10 (heavily obese)”	1= Skinny or overweight (<5 or > 5) 2= Normal (score 5)
Fitness	Question: “Assign a linear score for the fitness going from 1 (very unfit) to 10 (very fit)”	1= Fit (score >=7) 2= Unfit (score <7)
Housing	Question: “Is the horse stabled? And if so, is it stabled in a box inside a building (‘inside-box’), in a box with direct outdoor access (‘outside-box’) or in a ‘barn’?”	1= Inside-box 2= Outside-box 3= Barn 4= No stable
Management during summer	Question: “Is the horse stabled the whole time or does it mainly graze during the day, during the night, during sunset and sunrise or just a few hours a day?”	1= Stabled (stabled the whole time) 2= Partially Stabled (stabled during night or during sunset and sunrise or stabled a few hours a day) 3= Not Stabled
Frequency of training	Question: “How many times do you train with this horse during summer”	1= Rarely (less than 1 time a week) 2= Often (1 time a week or more)
Frequency of deworming	Question: “How many times a year is the horse dewormed?”	1= Rarely (less than 3 times a year) 3= Often (3 times a year or more)
Age of horse	Derived from birthdate in pedigree file	1= 0-1-2 years 2= 3-4-5-6 years 3= older than 6 years
Coat color	Derived from pedigree file	1= Gray 2= Chestnut 3= Bay 4= Black 5= Non-standard (e.g. tobiano, …)
Sex	Derived from pedigree file	1= Mare 2= Gelding+Stallions
Province	Based on address of the owner of the horse	1= West-Flanders 2= East-Flanders 3= Antwerp 4= Brabant 5= Limburg
Stud size	The number of records of the same owner in the database	1= Small (<=10 horses) 2= Large (>10 horses)

(univariable models). Secondly, all pairwise associations were computed to identify multicollinearity. Thirdly, a backward model selection was performed starting from a full model (main effects only) by removing factors with P-values >0.20 for the likelihood ratio test and by decreasing corrected Akaike information criterion (AICc, Akaike, 1974). A final multivariable model was chosen based on 1) the significance of a factor in a univariable model (P-values<0.20, chi squared test), 2) the significance in multivariable model and 3) considerations of the survey structure and relevance of the factor for the study (Dohoo et al., 2003). The method of data collection and the investigator were forced into the model based on the latter criterion. Two-way interactions were tested between factors in the final models.

RESULTS

Questionnaire

The respondents were always willing to cooperate in the survey and no non-response was observed. The data collected by three interviewers were considered too few (<100 horses/interviewer) to draw inferences and were therefore grouped.

From 4295 questionnaires, 3409 complete records, pertaining to registered Belgian Warmbloods, were used for further analysis. A number of other records were removed because of missing or incorrect identification or because the horse was not registered as BWP.

The average (standard deviation [sd]) age of the horses was 7.4 years (± 5.7) and ranged from 1 to 31 years. The stud size ranged from 1 to 72 horses per owner (on average (sd) 10.3 (± 15.5 horses per owner).

IBH clinical symptoms and history

Based on information provided by the horse owners, 342 of 3409 horses were classified as IBH affected (10%) (Table 2). Two hundred and forty-one of those 342 IBH-affected horses were treated with at least one IBH measure to reduce clinical symptoms. One hundred and twenty-nine of the 342 IBH-affected horses did not show clinical symptoms at questioning (38% of IBH-affected horses). These horses did not show clinical symptoms at questioning because 1) they were treated with IBH measures (98 of 129 horses) or because 2) they had shown clinical symptoms in the past and the IBH symptoms disappeared when growing older (28 horses of 129 horses) or because 3) IBH symptoms had occurred earlier that year but were healed by the time of questioning (3 of 129 horses).

Most IBH-affected horses showed IBH-clinical symptoms for the first time at the age of two or three years, 32% and 20% respectively (Figure 1). The average age (sd) of IBH onset was 3.6 (±3.0). Most IBH-affected horses showed their first IBH symptoms before the age of 7 (88% of IBH-affected horses), but IBH symptoms may occur at every age. Four percent of the IBH-affected horses showed IBH symptoms as a foal (<1 year old).

IBH-clinical symptoms were recurrent (more than one summer) in 276 of the 342 IBH-affected horses. Thirty eight IBH-affected horses showed their first clinical symptoms during the summer, in which the horse owner was questioned, and 28 of the IBH-affected horses had shown IBH symptoms during previous summers, but spontaneously stopped showing these symptoms when ageing. The most common symptoms were pruritis (97%), hair loss (90%) and change of hair structure (88%). IBH symptoms were mainly located at the tail (86%) and manes (76%) (Figure 2).

Table 2. Overview of the number of horses classified as IBH-affected and unaffected based on owner statement (status), the number of horses showing clinical symptoms at the time of questioning (symptoms at questioning), if preventive measures were performed to reduce clinical IBH symptoms and if IBH symptoms were recurrent for at least two summers or if it was the first summer the horse had shown clinical symptoms of IBH.

Total	Status	Symptoms at questioning	Preventive measures	Recurrent
			Yes / No	Yes / No / First year
		342 Total	241/ 101	276 / 28 / 38
	342 affected	213 Symptoms	143 / 70	187 / 0 / 26
3409		129 No Symptoms	98 / 31	89 / 28 / 12
	3067 unaffected	/	/	/

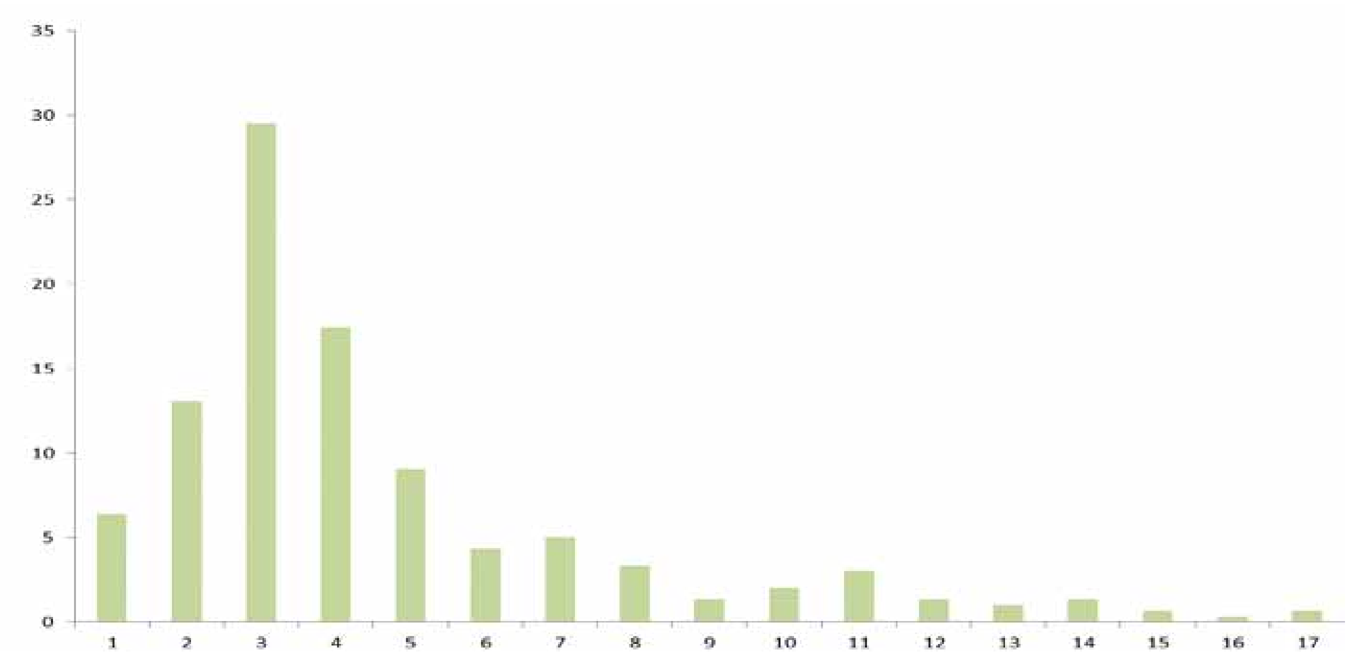

Figure 1. Percentage of IBH-affected BWP that showed IBH-clinical symptoms for the first time, at a certain age (0-20).

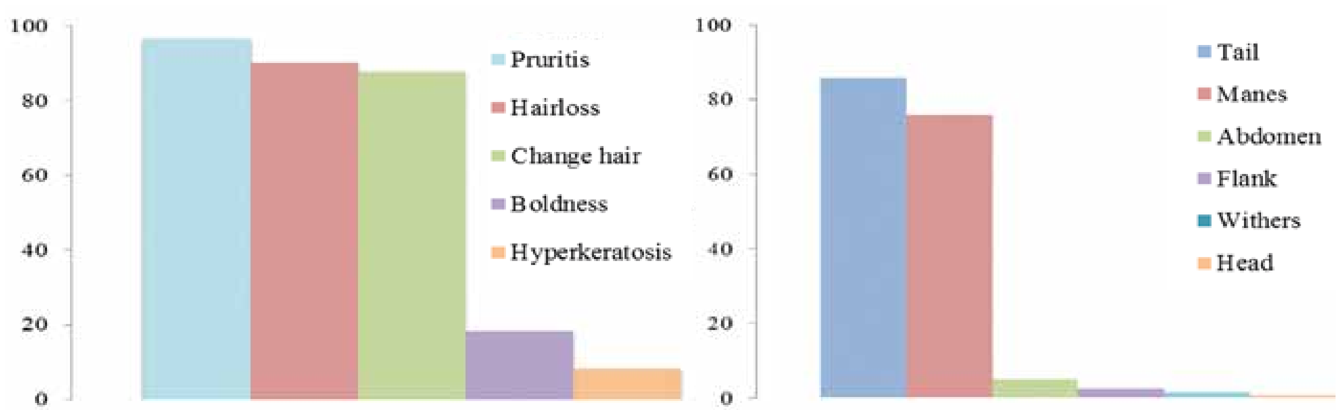

Figure 2. The prevalence of IBH symptoms (left) and their locations on the body (right) in affected BWP.

Use and effectiveness of IBH measures

As mentioned before, 241 of 342 IBH-affected horses (70%) were treated for IBH by applying one or more IBH measures to reduce clinical symptoms (= treated IBH-affected horses). The use and the effictiveness of the most commonly used IBH measures are summarized in Table 3. All immunosuppressive treatments, e.g. corticosteroids, were referred to as the IBH measure medication. The top three were itching reducing products (48%), insect repellants (37%) or blankets (32%). Other measures were stabling (12%), desensibilization (6%), medication (5%) or move the horse to another location (3%). The application of all IBH measures reduced IBH symptoms in treated IBH-affected horses (55%-67%). Stabling and the use of blankets suppressed all IBH-clinical symptoms in respectively 59% and 49% of the treated horses (Table 3).

Risk factors associated with IBH: lifetime and current status

Associations between single factors and lifetime IBH status or current IBH status are given in Table 4. The lifetime IBH status was associated with the period of questioning”(P=0.04), method of data collection (P=0.013), surrounding vegetation (P<0.01), training frequency (P<0.01), age (P<0.01) and stud size (P<0.01). When considering the current IBH status, the investigator (P=0.03), surrounding vegetation (P<0.01), fitness (P=0.02), age (P<0.01) and stud size (P<0.01) were associated (Table 4).

The associations between lifetime IBH or current IBH with single factors were often similar except for the period of sampling and the investigator, where odds ratios differed.

The lifetime IBH prevalence was higher (approximately 11%) when the questioning of horse owners

Table 3. Use and effectiveness of preventive measures ranked by their proportional usage.

	Measure or treatment	Number	(%)	Effectivity (%)		
1	Itch reducing products	115	(48)	Symptoms suppressed	3	(26)
				Symptoms reduced	**74**	**(64)**
				No effect	11	(10)
2	Insect repellents	90	(37)	Symptoms suppressed	20	(22)
				Symptoms reduced	**62**	**(69)**
				No effect	8	(9)
3	Blankets	77	(32)	**Symptoms suppressed**	**38**	**(49)**
				Symptoms reduced	38	(49)
				No effect	1	(2)
4	Stabling	29	(12)	**Symptoms suppressed**	**17**	**(59)**
				Symptoms reduced	11	38)
				No effect	1	(3)
5	Desensibilization	29	(6)	Symptoms suppressed	8	(29)
				Symptoms reduced	**18**	**(61)**
				No effect	2	(7)
6	Medication	12	(5)	Symptoms suppressed	3	(27)
				Symptoms reduced	**7**	**(55)**
				No effect	2	(18)
7	Move to another location	7	(3)	Symptoms suppressed	2	(33)
				Symptoms reduced	**5**	**(67)**
				No effect	0	(0)

The number of horses that were treated with a specific preventive measure (Usage) is shown together with their corresponding percentages of IBH-affected, treated horses (241 in total). 'Effectivity' shows the number of horses (percentage) that were reported with no clinical symptoms (symptoms suppressed), milder symptoms (symptoms reduced) or the same severity of IBH symptoms (no effect) after applying a measure or treatment.

was done from April up until July than when the questioning was done from August to October (lifetime IBH prevalence was around 8%). This was different for the current IBH status with the highest prevalence in July. In horses that grazed in a pasture close to a hedgerow, the IBH prevalence (both lifetime (12.5%) and current (8.5%)) was higher than in horses that grazed in a woody pasture (7.2% and 5.4%, respectively) or in open land (9.6% and 4.8%, respectively). When horse owners were questioned at home or by telephone contact, the lifetime IBH prevalence was lower (7%) than when horse owners were questioned at sport competitions (11%) or breeding days (10%).

Furthermore, for the lifetime IBH status, a higher IBH prevalence was observed in horses that were trained at least once a week (12.1%) than in horses that were not or rarely trained (7.0%). When the stud size increased, the IBH prevalence was lower. The IBH prevalence was 6% when the stud size was above ten horses relative to a prevalence of 11% when stud size was below ten. Young horses, i.e. younger than three years old, had a lower IBH risk (3.1%) than older horses (11-12%). Analogous results were obtained when considering the current IBH status but with lower risks.

No significant associations was found for the factors year of questioning, body condition, housing, frequency of deworming, coat color, sex and province (Table 4).

Associations between independent factors

The factors training frequency and age were highly associated (P<0.01). Only 3% of the horses younger than three years old were trained regularly. Age was also associated with management during summer (P<0.0001). Young horses (younger than three) were most of the time not stabled (73%) or partially stabled (26.5%) during summer. Only 0.5% of the young horses were stabled all summer. The percentage of stabled older horses however was 8%.

The factors stud size, investigator and method of data collection were most often involved in significant pairwise associations. Investigators that recorded a lower IBH prevalence (investigators 1 and 2) had collected respectively 15% and 30% of their data by breeder visits and calls, whereas the other investigators (investigators 3 and 4) only collected 8% or 0% of their data this way. In addition, an association was found between the factors method of data collection, period and stud size (all P-values <0.01). Seventy percent of the data collected by breeder visits and phone calls were data on horses of large studs, whereas this was only 8% and 12% of the data collected on sport competitions and BWP breeding days, respectively. During the periods August and post summer, 33% and 29% of the data were from horses of a large stud, whereas this was only 16% and 19% for the periods pre summer and July, respectively. Twenty-

Table 4. Relative risk factors (Logistic regression) for IBH in Belgian warmblood horses. 'Answering%' represents the percentage of 3409 IBH records that included information about a specific factor. The number of records per category (N° records), the percentage of IBH affected horses (%IBH), the odds ratio (95% confidence interval) and the P-value of the likelihood ratio statistic for the factor is included.

		Lifetime IBH				IBH at questioning		
Factor	**Answering %**	**N° records**	**% IBH**	**Odds ratio**	**P-value**	**% IBH**	**Odds ratio**	**P-value**
Year of sampling	100%				0.74			0.88
2009		1234	9.81	0.96 (0.76 – 1.21)		6.08	0.98 (0.73 –1.31)	
2011		2175	10.16	1		6.21	1	
Period of questioning	100%				0.04			0.17
Pre summer		644	11.3	1		4.66	1	
July		1493	11	0.97 (0.73 - 1.30)		6.97	1.50 (1.01 - 2.32)	
August		922	8.2	0.70 (0.50 - 0.99)		5.64	1.22 (0.77 - 1.94)	
Post summer		350	8.0	0.68 (0.43 - 1.07)		6.86	1.51 (0.86 - 2.62)	
Method of data collection	100%				0.013			0.20
Sport competitions		2346	10.9	1		6.56	1	
BWP breeding days		390	10.0	0.91 (0.64 - 1.30)		6.15	0.93 (059 – 1.45)	
Breeder visits and calls		673	7.1	0.63 (0.46 – 0.87)		4.75	0.71 (0.48 –1.05)	
Investigator	100%				0.09			0.03
1		484	8.1	1		6.61	1	
2		1751	9.5	1.20 (0.83 - 1.72)		5.14	0.76 (0.50 –1.16)	
3		828	11.4	0.46 (0.99 - 2.16)		6.88	1.04 (0.66-1.63)	
4		346	12.4	1.46 (1.03 – 2.56)		8.96	1.39 (0.83-2.33)	
Vegetation of pasture	99%				0.002			0.002
Open land		1757	9.6	1		5.35	1	
Hedgerow		1041	12.5	1.34 (1.05 - 1.71)		8.45	1.63 (1.21 –2.20)	
Woody		566	7.2	0.73 (0.51 - 1.05)		4.77	0.88 (0.57 –1.37)	
Humidity of pasture	99%				0.07			0.05
Dry		2586	9.6	0.79 (0.62 - 1.02)		5.76	0.73 (0.53 - 0.99)	
Wet		775	11.9	1		7.74	1	
Body condition	100%				0.44			0.27
Under- or overweight		50	10.98	1.16 (0.83 – 1.52)		7.25	1.23 (0.85-1.78)	
Normal		2899	9.87	1		5.97	1	
Fitness	100%			1	0.10			0.02
Fit		3176	9.8	0.71 (0.48 - 1.05)		5.89	0.57(0.36 – 0.90)	
Unfit		233	13.3	1		9.87	1	
Housing	100%				0.13			0.14
Inside box		1344	10.2	1.16 (0.87 – 1.54)		5.88	0.93 (0.66 –1.32)	
Outside box		761	12.0	1.38 (1.02 – 1.89)		7.49	1.21 (0.83 –1.76)	
No stable		387	8.3	0.92 (0.59 – 1.44)		3.98	0.62 (0.33 - 1.14)	
Barn		976	8.9	1		6.25	1	
Management during summer	100%				0.09			0.26
Stabled		211	7.6	0.66 (0.39 - 1.12)		3.79	0.56 (0.27 –1.18)	
Partially stabled		1751	11.1	1		6.09	0.93 (0.69 –1.24)	
Not stabled		1446	9.13	0.81 (0.64 - 1.02)		6.51	1	
Training frequency	100%				<0.0001			0.12
Rarely		1396	7.0	1		5.37	1	
Often		2009	12.1	1.82 (1.43 - 2.33)		6.67	1.26 (0.94 – 168)	
Deworming frequency	100%				0.47			0.33
>= 3 Times /year		2041	10.29	1.12 (0.86 – 1.37)		5.78	0.86 (0.65 –1.37)	
< 3 Times/year		1364	9.53	1		6.60	1	
Age	100%				<0.0001			<0.0001
0-2 years		623	3.1	0.29 (0.15 – 0.40)		2.41	0.34 (0.20-0.59)	
3-6 years		1258	12.1	1.09 (0.86 – 1.38)		7.31	1.09 (0.81-1.46)	
older than 6 years		1528	11.2	1		6.74	1	
Coat color	98%				0.14			0.08
Brown		2157	10.11	1.82 (0.78 – 4.2)		6.21	3.35 (0.82-13.7)	
Gray		382	12.8	2.38 (0.99 – 5.72)		8.12	4.46 (.05-18.9)	
Chestnut		695	9.6	1.72 (0.72-4.08)		5.90	3.16 (0.75-13.3)	
Black		103	5.8	1		1.94	1	
Sex	100%				0.48			0.74
Male		1295	10.5	1.09 (0.86 1.366)		6.33	1.0 (0.79-1.39)	
Female		2114	9.7	1		6.05		
Province	98%				0.47			0.26
Antwerp		1115	9.6	0.90 (0.57 – 1.41)		5.20	0.88 (0.49-1.58)	
Limburg		600	9.33	0.87 (0.54 -1.417)		6.17	1.06 (0.57-1.96)	
East-Flanders		683	11.86	1.14 (0.72-1.81)		7.91	1.38 (0.76-2.49)	
Flemish Brabant		684	9.21	0.86 (0.53 – 1.38)		5.99	1.02 (0.56-1.88)	
West-Flanders		256	10.55	1		5.86	1	
Studsize	98%				<0.0001			0.001
Small (<=10 horses)		2595	11.25	2 (1.45 – 2.77)		6.92	2.1(1.40-3.12)	
Large (>10 horses)		756	5.95	1		3.43	1	

Table 5. Significance of factors in initial and final multivariable logistic regression models for IBH status and lifetime IBH in Belgian warmblood horses following backward selection.

Factors	Lifetime IBH status		IBH status at questioning	
	Full model	Final model*	Full model	Final model*
Year of questioning	0.477	-	0.309	-
Period of questioning	0.150	0.12	0.070	-
Method of collection	0.545	0.636	0.236	0.510
Investigator	0.397	0.447	0.208	0.0458
Vegetation	0.001	0.001	0.001	<0.001
Humidity	0.095	0.072	0.057	0.081
Body condition	0.952	-	0.929	-
Fitness	0.298	-	0.162	0.084
Housing	0.519	-	0.043	0.152
Management	0.670	-	0.498	-
Training frequency	0.164	-	0.371	-
Deworming frequency	0.939	-	0.282	-
Age in categories	< 0.001	<0.001	<0.001	<0.001
Coat color	0.240	-	0.184	0.189
Sex	0.88	-	0.849	-
Province	0.269	-	0.211	-
Stud size	<0.001	<0.001	0.001	<0.001
AICc	2084.87	2063.78	1479.7	1466.3

*Obtained through backward selection (P-value <0.20) but retaining investigator and method of collection always in the model.
(AICc= corrected Akaike information criterion)

three percent of horses classified as fit came from a large stud, whereas only 10% of the unfit horses came from a large stud.

Model selection

The backward model selection starting with 17 factors resulted in slightly different models for current and lifetime IBH status (Table 5). For both definitions of IBH (lifetime versus current) and in the full and final models, the factors vegetation, age and stud size were always significant and the investigator was significant in the final model for current IBH status. The factors period of questioning, fitness, humidity, coat color and type of stable, had P-values between 0.05 and 0.20 in some models only. Two-way interactions between main effects in the final models were tested but resulted in non-estimable least-squares means. Hence, these interactions were not considered.

DISCUSSION

The data in this study was collected using a questionnaire in face-to-face interviews during sport competitions, breeding days, breeder visits and by telephone calls. Self-reporting of IBH by horse owners is known to work well, but previous studies had considerable non-response rates as they were either internet-based (Eriksson et al., 2008, van den Boom et al., 2008), or because surface mail was used, i.e. a questionnaire was sent to the horse owners, (Braverman et al., 1983; Broström et al., 1987). In comparable studies, only a low number of records were obtained (Steinman et al., 2003; Lange, 2004; Björnsdóttir et al., 2006). The authors of the present study collected more than 3,400 records on individual horses using face-to-face interviews with the horse owners. Schurink et al. (2012) collected data through professional foal inspectors who can be considered more objective than self-reports of horse owners. However, the owners assess the horse's sensitivity over a longer period of time, and may therefore provide accurate information on each individual with respect to IBH (Eriksson et al., 2008).

In addition, 70 % of the IBH-affected BWP were treated to reduce clinical symptoms at the time of questioning, and 38% of the IBH-affected BWP did not show any clinical symptoms according to the owner at the time of questioning. When the IBH prevalence would have been based on the screening of IBH symptoms, the IBH prevalence would have been only 6% compared to 10% in case the classification was based on the owners' statements. This illustrates the importance of lifetime IBH as a parameter when studying the population of warmblood horses.

IBH symptoms were mainly located at the tail and manes, and the most common symptoms were pruritis, hairloss and change of hair structure. This is in agreement with earlier reports (Lange et al., 2004; Björnsdóttir et al., 2006; Eriksson et al., 2008; van de Boom et al., 2008). Stabling and the use of blankets were considered by the horse owners as the most effective IBH measures to suppress IBH symptoms. Many researchers advise housing of IBH sensitive horses as the best method of controlling the disease (Bancroft, 1891; Riek, 1953; Anderson et al., 1988; Björnsdóttir et al., 2006). In the present study, the authors could not demonstrate effects of housing or management on both the lifetime and current IBH status.

The association between a high IBH prevalence and certain geographical areas has been described in numerous reports (Braverman et al., 1983; Broström et al., 1987; Björnsdóttir et al., 2006; Grandison et al., 2006; van Grevenhof et al., 2007). In the present study, no association could be found between different provinces and their IBH prevalences. This might be explained by the fact that data collection was limited to the Flemish part of Belgium (approximately 13,000 km^2). However, the type of vegetation surrounding the pasture seemed to affect the IBH risk. A higher prevalence was observed when the pasture was close to a hedgerow ($p<0.01$). This might be explained by the fact that biting midges are weak fliers and prefer areas with little or no wind (van Grevenhof et al., 2000). A nearly significant effect of the humidity of the pasture was also found ($P=0.07$), with dry soils having lower IBH prevalence. This might be explained by the fact that midges need water to lay eggs.

The IBH prevalence was lower in horses younger than three years than in older horses. The age, at which horses became affected, varied, but the average age of onset was 3.6 ± 3.0 years. In addition, there seemed to be no fixed threshold age, after which the development of clinical symptoms was no longer possible. These findings are supported by other studies (Anderson et al., 1988; Reiher and Björnsdóttir, 2004; Steinman et al., 2003; Schurink et al., 2012). A certain period of sensitization to an allergen is necessary for allergy development. Initial sensitization occurs at an early age and subsequent exposure to the otherwise harmless allergen rouses the allergic reaction (Baker and Quinn, 1978; Holgate and Polosa, 2008). In the present study, age was associated with the frequency of training, which most likely explains the effect of training frequency when investigated as univariable factor ($P<0.01$). In younger horses, there was a lower IBH prevalence. These horses were rarely or had never been trained. The effect of training frequency was thus no longer significant in a multivariable model.

In some studies, stallions were found to be more susceptible to IBH than mares, and dark horses were to be found more susceptible than pale horses (Braverman et al., 1983, Broström et al., 1987, Eriksson et al., 2008, Lange et al., 2004). Lange et al. (2004) described that mares are more often affected than geldings and stallions. However, most studies support the findings of the present study that gender and color bear no significance in susceptibility to IBH (Hesselholt and Agger, 1977; Broström et al., 1987; Anderson et al., 1988; Björnsdóttir et al., 2006; van Grevenhof et al., 2007).

In the present study, stud size was associated with IBH prevalence. When the stud size increased, the IBH prevalence was lower. Some explanations for this finding are suggested: 1) horse owners with more than ten horses can be assumed to be breeders and might select against IBH in their studs, 2) management of horses in large studs is performed by grooms and the owner might hence be unaware of the true IBH status of his horses, 3) the owner is less honest in reporting IBH-affected horses since these horses are his livelihood and since IBH decreases the economic value of a horse. Stud size might also reflect the personal involvement of the owner with each individual horse. This might explain why some questions were less accurately answered for individual horses. For example, horses in a large stud were more frequently classified as fit, hereby possibly explaining the lower IBH prevalence for fit horses ($P=0.10$). However, due to the structure of the data, the effect of the period of questioning, the method of data collection and the investigator may also interfere at this point. More training and standardization are needed. An alternative analysis using a logistic mixed model with owner as random effect failed to converge, probably due to the large number of small owners (results not shown).

Importantly, no effect of year of recording on the IBH-status (lifetime and current) was detected in the present study. Hence, yearly recording of BWP might not be necessary to assess individual horse sensitivity to *Culicoides* bites. For a successful breeding program, a large scale recording of the individual lifetime IBH status at regular intervals seems cost effective.

CONCLUSIONS

The results of the present study, based on self-reporting by horse owners, confirm that insect bite hypersensitivity is a problem in the BWP population with a lifetime prevalence of 10%. Because of the common use of IBH measures to reduce clinical symptoms in IBH-affected BWP (70%), the percentage of horses actually showing IBH symptoms at the time of questioning was only 6%.

The most important risk factors for IBH in BWP were age and vegetation surrounding the pasture. Stud size was identified as another risk factor but this finding requires confirmation and clarification.

CONFLICT OF INTEREST STATEMENT

None of the authors has any financial or personal relationship that could inappropriately influence or bias the content of the paper.

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