Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

**Faculty of Veterinary Medicine and Animal Science**
Department of Clinical Sciences

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*Påverkan av sommareksem på hästens rörelseaktivitet och beteende*

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Degree project/Independent project • 30 credits
Husdjursagronom, Animal Science
Uppsala 2019

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**Supervisor:** Miia Riihimäki, SLU, Department of Clinical Sciences
**Assistant supervisor:** Pia Haubro Andersen, SLU, Department of Clinical Sciences
**Examiner:** Sara Ringmark, SLU, Department of Anatomy and Physiology

**Credits:** 30 credits
**Level:** Advanced, A2E
**Course title:** Degree project in Animal Science
**Course code:** EX0872
**Programme/education:** Husdjursagronom, Animal Science
**Course coordinating department:** Department of Animal Genetics

**Place of publication:** Uppsala
**Year of publication:** 2019
**Online publication:** https://stud.epsilon.slu.se

**Keywords:** pruritus, itching, IceTag, accelerometer

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## Abstract

It is well known that IBH in horses causes itching which in turn can lead to open wounds. The suffering and pain related to an open wound is relatively obvious, but less is known about how itching affect the welfare in the horse. The aim of this study was to investigate IceTag® accelerometers reliability to detect steps (n), lying (t) and lying bouts using two horses kept - and video filmed in boxes for one night (validation study). The aim was also to compare the movement activity of horses with IBH with non – affected horses using IceTags (IBH study). A higher movement activity, a lower lying time and a higher frequency of itching behaviours in horses with IBH compared to controls was hypothesised. Direct observations were performed to compare behaviours between horses with IBH and controls and to investigate the IceTags reliability to detect steps (n) and lying bouts. In total 11 horses of different breeds including six with known IBH and five controls were investigated. The horses were kept in paddocks and equipped with one IceTag on the hind leg and one at the neck for a study period of approximately seven days. Direct observations were performed in the morning and in the evening for 30 minutes and in total six times per horse. When comparing IceTag data with video recordings from the validation study, 43 – 99.7 % of the lying bouts recorded by IceTags were found to be false. Only small differences were found for steps (n) and lying (t) between IceTag data and video recordings. In the IBH study, IceTags placed at the hind leg recorded a mean lying time of 6.5±1.7 % of the study period (IBH – affected horses) versus 5.5±1.9 % (controls). IBH – affected horses performed in average and in total 15.5±3.9 lying bouts compared to 20.6±.6.4 in controls during the study period. For the approximately seven days study period, all horses included in the study had a mean motion index of 143 218, which is a measurement of the animal's overall activity measured from three different dimensions. For the same period, mean motion index of the head and neck when horses were standing still was 3.14 for IBH – affected horses and 1.425 for controls. No significant differences were found in motion index, steps (n), lying (t), number of lying bouts and head and neck activity between IBH – affected horses and controls. According to direct observations, both groups showed a similar time budget. IBH – affected horses were found to perform in average – and total 78 itching behaviours and behaviours that was considered to occur due to irritation for biting insects. The corresponding number for controls was 96.2 and was not significantly higher than the number for horses with IBH. In conclusion, no differences in movement activity and behaviour were found between IBH – affected horses and non – affected horses. IceTags placed at the hind leg of the horse were able to detect reliable data of steps (n) and lying (t) but overestimated number of lying bouts. In future studies, larger sample sizes and/or monitoring horses without protective clothing is suggested.


*Keywords:* pruritus, itching, IceTag, accelerometer

## Sammanfattning

Att sommareksem hos hästar bidrar till klåda vilket i sin tur kan leda till öppna sår är sedan tidigare välkänt. Lidandet och smärtan relaterat till ett öppet sår är relativt uppenbar, däremot är det mindre känt hur klåda, eller pruritus, påverkar hästens välfärd. Syftet med denna studie var att undersöka IceTag® accelerometerns pålitlighet att registrera antal steg, liggtid och antal liggtillfällen med hjälp av två hästar som filmades i box under en natt (valideringsstudie). Syftet var även att jämföra rörelseaktiveten hos hästar med sommareksem med friska hästar med hjälp av IceTags (eksemstudie). Hypoteserna var att eksemhästar har en högre rörelseaktivitet, en kortare liggtid och en högre frekvens av utförda kli-beteenden jämfört med kontroller. Direktobservationer utfördes för att jämföra beteenden mellan eksemhästar och kontroller samt för att undersöka sensorns pålitlighet att registrera antal steg och liggtillfällen. I studien ingick 11 hästar av olika raser där sex av dem visade kliniska symptom för sommareksem och resterande var kontroller. Hästarna hölls i hagar under en studieperiod på ungefär sju dagar utrustade med IceTags på ett av bakbenen samt en ytterligare på nacken. Direktobservationer utfördes på morgonen och på kvällen i 30 minuter och totalt sex gånger per häst. I valideringsstudien jämfördes data från IceTags med videofilmer vilket visade att 43 – 99,7 % av liggtillfällena registrerade av sensorerna var falska. Endast små skillnader i antal steg och liggtid mellan data från IceTags och videofilmer visades. I eksemstudien registrerade sensorerna som var placerade på hästarnas bakben en genomsnittlig liggtid på 6,5±1,7 % för eksemhästarna och 5,5±1,9 % för kontrollerna av studieperioden. För eksemhästarna registrerades i genomsnitt 15,5 liggtillfällen jämfört med 20,6 liggtillfällen för kontrollerna under den ungefär sju dagar långa studieperioden. Det genomsnittliga rörelseindexet, som är ett mått på hästens totala aktivitet registrerat från tre olika riktningar, uppmättes till 143 218 för alla hästar i studien för ungefär sju dagar. Under samma period var det genomsnittliga rörelseindexet av huvud och hals när hästarna stod stilla 3,14 för eksemhästar och 1,425 för kontroller. Inga signifikanta skillnader visades i rörelseindex, antal steg, liggtid, antal liggtillfällen och huvud – och halsrörelse mellan eksemhästar och kontroller. Enligt direktobservationerna visade båda grupperna även en liknande tidsbudget. Eksemhästar observerades utföra totalt 78 kli-beteenden eller beteenden som ansågs bero på irritation för knott. Den motsvarande siffran för kontroller var 96,2 och var inte signifikant högre än siffran för eksemhästar. Som slutsats visades inga skillnader i rörelseaktivitet och beteenden mellan eksemhästar och friska hästar. IceTags som var placerade på hästens bakben registrerade pålitliga data av antal steg och liggtid men överskattade antal liggtillfällen. I framtida studier föreslås att mätningarna utförs utan att skydd för knott (t.ex. flugtäcken och flughuvor) används och att antalet hästar inkluderade i studien är högre.

*Nyckelord:* pruritus, klåda, IceTag, accelerometer

# Populärvetenskaplig sammanfattning

Under sensommaren 2018 jämfördes rörelseaktivitet och beteende hos eksemhästar med friska hästar. För att registrera rörelseindex (ett mått på hästens totala aktivitet mätt från tre olika riktningar), antal steg, liggtid och antal liggtillfällen användes IceTag® accelerometrar som placerades på hästens bakben och nacke. Det vanligaste symptomet för sommareksem är klåda vilket leder till att de drabbade hästarna kliar sig. Svår klåda kan i värsta fall leda till öppna sår och infektioner som orsakar smärta och lidande hos hästen. Påverkan av klibeteende på hästens välfärd är däremot inte tidigare studerat. Hypotesen i studien var att eksemhästar har en högre rörelseaktivitet, kortare liggtid och mindre vila jämfört med friska hästar på grund av klåda och irritation för knott. Om rörelseaktiviteten påverkas av sommareksem kan denna metod användas för att till exempel övervaka mängden knott eller mäta effekten av en behandling. När data från IceTag® accelerometrarna utvärderades visades dock inga skillnader i varken rörelseindex, antal steg, liggtid eller antal liggtillfällen mellan eksemhästar och friska hästar. Direktobservationer med hjälp av ett etogram som beskriver olika beteenden utfördes totalt sex gånger per häst för att registrera utförda beteenden hos hästarna. Ingen skillnad i utförda beteenden visades mellan eksemhästar och friska hästar vilket var något oväntat eftersom klåda är ett vanligt symptom för sommareksem vilket bör ha lett till att eksemhästarna utförde fler klibeteenden än de friska hästarna. Svagheten med studien var att endast ett fåtal hästar användes (totalt 11 stycken) och de flesta var endast i liten grad påverkade av åkomman. Många av eksemhästarna hade även ett visst skydd mot knotten i form av flugtäcke och/eller flughuva, medan de flesta av de friska hästarna inte hade något skydd alls. För en rättvis jämförelse mellan dessa grupper bör hästarna observerats utan skydd, men detta betyder förmodligen att eksemhästarna hade fått ett större lidande vilket är ett etiskt problem. IceTag® accelerometrarna som användes i studien är tidigare främst använda på kor. För att undersöka huruvida accelerometern ger korrekt data av antal steg, liggtid och antal liggtillfällen genomfördes ytterligare en studie på två hästar uppstallade i box. När data från IceTags jämfördes med videofilmer kunde man se att accelerometrarna registrerade korrekt data av antal steg och liggtid, men överskattade antalet liggtillfällen. Fler studier om användning av IceTags på häst behöver göras för att minska risken för felaktiga data eftersom accelerometrarna kan vara till god nytta i framtida beteendestudier.

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# 1 Introduction

Insect bite hypersensitivity (IBH) is a common condition for horses throughout the world (Craig, 2011) and has been observed in several breeds (Hallamaa, 2009). In Finland and Norway, affected horses have shown clinical signs from May to October (Hallamaa, 2009; Halldórsdsóttir & Larsen, 1991), which is also the main grazing period in Sweden. It is well known that IBH causes itching which in turn can lead to open wounds (Broström & Larsson, 1987). In some cases, the condition can make the horse unsuitable for riding and reduce its commercial value. While the suffering and pain related to an open wound is relatively obvious, less is known about how itching contributes to decreased welfare in horses. In humans, itching, or pruritus, can cause enormous suffering (Brennan, 2016) and negatively affect the quality of life (Kini *et al.*, 2011). The itch signal is initiated by a complex interaction between skin cells and nerve fibres and transported to the central nervous system (Brennan, 2016). It has been shown that the scratching behaviour as a response of the pruritus is both highly relieving and rewarding (Yosipovitch *et al.*, 2008). Scratching the skin suppresses the itch temporarily, but this behaviour may lead to increased overall movement activity and development of stress. Increased movement activity may be associated with decreased rest, but this has never been studied in the IBH – affected horse. If movement activity is correlated with pruritus, this measure could be used in the monitoring of insect attacks or for example of the effect of treatment of IBH.

The study included two parts. The aim of the first part was to investigate if IceTag® accelerometers (IceRobotics Ltd, Edinburgh, UK) provide reliable detection of steps (n), lying (t) and lying bouts by comparing data from IceTags placed on horses in box with behaviours according to video recordings (validation study). The aim of the second part was to compare the movement activity and behaviour of horses with IBH with horses without clinical signs of the condition (controls). IceTag® accelerometers (IceRobotics Ltd, Edinburgh, UK) were used to measure motion index, number of steps, standing time, lying time and number of lying bouts for horses kept in paddocks. Direct observations were also performed to compare observed behaviours such as itching between IBH – affected horses and controls. Number of steps – and lying bouts were compared with IceTag data to investigate if IceTags could provide reliable detection of those behaviours.

The hypotheses were:

(i) IBH - affected horses have higher movement activity compared to controls
(ii) IBH – affected horses have higher movement activity of the head and the neck due to itching and shaking off insects compared to controls
(iii) IBH – affected horses have less lying time compared to controls
(iv) IBH - affected horses have less resting time compared to controls
(v) IBH – affected horses have a higher frequency of itching behaviours (rolling, scratching with teeth or head, scratching with hind leg, scratching against an object) compared to controls
(vi) IBH – affected horses have a higher frequency of the following behaviours; body shaking, head and neck shaking and leg lift, compared to controls

# 2 Literature

## 2.1 Insect bite hypersensitivity

IBH is a chronic and seasonal recurring condition (Broström & Larsson, 1987) that is caused by a hypersensitivity reaction of insect bites mainly of the species *Culicoides* (Quinn, Baker & Morrow, 1983). The reaction is either immediate (type 1) or delayed (type IV) (Quinn, Baker & Morrow, 1983), but the most common hypersensitivity reaction is immediate and occurs within six hours after the bite (Pilsworth & Knottenbelt, 2004). In Sweden, IBH is a well-known problem in especially Icelandic horses (Eriksson *et al.,* 2008; Björnsdóttir *et al.,* 2006; Broström & Larsson, 1987). Approximately 17 % of all Icelandic horses in Sweden suffer from IBH. Compared to Icelandic horses born in Sweden, horses imported from Iceland to Sweden have a higher risk to develop the condition. (Broström & Larsson, 1987).

Besides horses, sheep, cattle and donkeys can also be affected (Yeruham, Perl & Braverman, 2004; Yeruham, Braverman & Orgad, 1993). The protein in the saliva of the midgets is probably the main allergen that causes the allergic reaction in affected horses (Craig, 2011). Over 1000 different species of *Culicoides* are found in the world (Craig, 2011), but the number of species that causes allergic reactions in horses in Sweden is not clear (Eriksson *et al.,* 2008). The insects live in moisty and muddy grounds and are most active during early morning and early evening, and prefer a hot, humid and windless condition (Craig, 2011). The most common clinical sign for IBH is pruritus, mainly in mane and tail (Craig, 2011). Furthermore, in many areas in the world, the most common cause of equine pruritus is the hypersensitivity for insect bites (Fadok, 1995). Pruritus, or itching, often leads to self-trauma and may in turn lead to open wounds and secondary infections (Broström & Larsson, 1987). Besides itching, alopecia, excoriations, skin thickening, hyperpigmentation and lichenification are common clinical signs in more severe cases (Craig, 2011). The prevalence of IBH has not seen to be affected by gender (Hallamaa, 2009; Steinman, Peer & Klement, 2003; Halldórdsóttir & Larsen, 1991) or colour of the coat (Halldórdsóttir & Larsen, 1991). The condition presents normally when the horse is between two and three years old and the severity of the condition increases with age (Craig, 2011). The prevalence – and severity of IBH is also affected by the geographic location. Horses located in the south west coast area of Sweden is more vulnerable than horses located in the northern region of Sweden. IBH has a genetic connection and it has been shown that the offspring has higher risk to develop the condition if the dam is suffering from IBH. (Eriksson *et al.*, 2008). There is no cure for IBH, but the condition can be prevented by prophylactic methods. Protective clothing such as blankets can be used to reduce the area available for insect bites. Keeping the horse stabled during the night (from dusk to after dawn) protected from insects can also reduce the risk of clinical signs. Treatments such as anti-inflammatory, antihistamines, nicotinamide, immunotherapy and topical therapy (e.g. shampoos) can also be used but have varying effects. (Craig, 2011). Recently, a study where they developed a vaccine against IBH has been published with promising results (Fettelschoss-Gabriel *et al.*, 2018). The vaccine used in the study was a virus-like particle (VLP) – based therapeutic vaccine against IL-5 which is the master regulator of eosinophils. A total of 34 Icelandic horses were used in the study and 44 % of them were placebo-treated. Of the vaccinated horses, 47 % reduced the IBH lesions of 50 % or more. A reduction in IBH lesions of 75 % or more were seen in 21 % of the vaccinated horses. The difference between the reduction of IBH lesions in vaccinated horses and placebo-treated horses was significant and showed that vaccination against IBH in horses may be the future.

## 2.2 Time budget and activity of the horse

Adult free-living horses spend the majority of the time foraging (51 – 63 %), walking 7 – 10 %, resting while standing 14 – 22 % and lying 1 – 13 % (Duncan, 1980). In a study by Boyd, Carbonaro & Houpt (1988), younger horses (up to 4 year) were investigated on pasture and they had a feeding time of 46 %, standing 34 %, resting while standing 16 % and lying 5 % of a 24 hours period. Those results were similar to the time-budgets observed by Duncan (1980). According to Boyd, Carbonaro & Houpt (1988), feeding occurred at the highest percentage of the time during night (20.00 – 04.00). Similar results have been described by Duncan (1985) with a higher grazing time (61 %) during night than during day (50 %) for free – living mares in the summer. However, standing (both awake and resting) occurred at the highest percentage of the time during the daylight hours according to both Boyd, Carbonaro & Houpt (1988) and Duncan (1985). In the later study, resting while standing was observed 18 % of the time in the night and 22 % of the time in the day. Locomotion were found to be higher during the daylight hours than the night (Boyd, Carbonaro & Houpt, 1988; Duncan, 1985). For the free – living mares in the study by Duncan (1985), the time spent on walking was observed to 11 % in the day but only 3 % in the night. For those horses, lying occurred at a higher percentage of time during the night (6 %) compared to the day (0 %). Horses kept in paddocks 24 hours/day without pasture or in a yard 24 hours/day with pasture has shown a lying time of 3 – 4 % and a total of 0 – 4 lying bouts per day and night. The length of a lying bout was observed to last between 7.7 and 32.9 minutes and were found to occur most frequently during nights (01.00 – 09.00) (Chaplin & Gretgrix, 2010).

Przewalski horses kept in different sizes of enclosures with or without grass had an average feeding time of 54 % of the day when observed between 8 a.m. and 6 p.m. In average, resting while standing was measured to 10 % of the observation time, and was affected by the size of the enclosure. Horses kept in small enclosures spent less time (4 – 5 %) resting while standing compared to horses kept in large enclosures (9 – 18 %). (Boyd, 1988). The activity of the horse can also be affected by weather (Duncan, 1985) and gender (Boyd, 1988). During rainfall, horses have shown to increase the time resting while standing with 53 % and decrease the time lying with 55 % (Duncan, 1985). The effect of gender has been shown when comparing stallions and mares of the breed Przewalski kept in zoos (Boyd, 1988). In that case, stallions had a higher motion activity than mares.

## 2.3 Previous studies using IceTag® accelerometers

The IceTag® is a commercial limb – mounted accelerometer with the capacity to record motion index, number of steps, standing time, lying time and number of lying bouts. Data is collected multiple times per second with a sample rate of 16 Hz. The parameter motion index is a measurement of the animal's overall activity and is recorded from three different dimensions. (IceRobotics, 2017). The accelerometer is calibrated for – and mainly used in studies with dairy cows (IceRobotics, 2015) for different purposes such as detecting mastitis (Medrano-Galarza *et al.*, 2012), lameness (Kokin *et al.*, 2014; Blackie *et al.*, 2011) and oestrous (McGowan, Burke & Jago, 2007). However, IceTags has also been used on other animals such as sheep (Verbeek *et al.*, 2012), pigs (Parsons, Millman & Johnson, 2015), yaks (Barsila *et al.*, 2014), goats (Tsukahara *et al.*, 2014) and horses (Gulbrandsen & Herlin, 2015; Olson *et al.*, 2015; Lindberg, Herlin & Michanek, 2013). Gulbrandsen & Herlin (2015) recorded motion index and lying time of horses in different housing systems but did not mention whether they thought IceTags provided reliable data of the two recorded behavioural parameters (motion index and lying time). They found a motion index of about 12768 per day for horses loose housed in an Active-Stable system®, and a lying time of about 37 minutes per day (3 %). In a study by Olson *et al.* (2015), accelerometers were placed at the left hind leg on horses after castration. Compared to the study by Gulbrandsen & Herlin (2015) where motion index and lying time was measured, Olsen *et al.* (2015) measured only motion index. In another study, IceTags where evaluated for a total of 10 horses in two different housing systems (Lindberg, Herlin & Michanek, 2013). Data from IceTags where compared with behaviour evaluation from video recording. The authors concluded that the accelerometer gave a reliable detection of both locomotion and lying behaviour (lying time and lying bouts) for the horses included in the study.

Bachmann *et al.* (2014) studied mares in the prepartal period using IceQubes® accelerometers (IceRobotics Ltd, Edinburgh, UK) which provide the same outputs (motion index, steps (n), standing (t), lying (t) and lying bouts) as IceTags (IceRobotics, 2017). Compared to IceTags, IceQubes have a lower sample rate (4 Hz) and-granularity and are often used on a larger number of animals. When downloading data from IceQube, a separate file with lying bouts analysis is obtained. In addition to one accelerometer mounted on one of the front legs of the horse, another IceQube was placed at a neck collar. The authors claimed that the accelerometer placed at the front leg had a good ability to detect lying and standing events but did not mention how they could determine that. The accelerometer placed at a neck collar recognized a permanent lying position.

# 3 Materials and methods

## 3.1 Validation study

The study was performed at Ultuna, Swedish University of Agricultural Sciences including one seven years old mare (horse 1) and one 13 years old gelding (horse 2). Both were of the breed Swedish Warmblood. The horses were healthy and had no signs of IBH. For one night, the horses were kept in single boxes and equipped with an IceTag® (IceRobotics Ltd, Edinburgh, UK) accelerometer on each leg. During the study, a camera installed in the box was recording continuously. Number of steps, lying time and lying bouts were analysed for each horse with help of video recordings and compared with data from IceTags. Number of steps was measured from video recording for five minutes periods and in total 30 minutes for each horse. Statistical analysis was performed with Minitab® Statistical Software (Minitab Ltd, Coventry, United Kingdom). Number of steps according to video recordings were compared with IceTag data using a two – sample t – test. The test was considered significant if $P < 0.05$. Comparisons between data of lying time recorded by the four different IceTags and video were statistical analysed with a confidence interval with a confidence level of 95 %. Number of lying bouts recorded by IceTags and video were analysed with a Chi-Square Goodness-of-Fit Test. The test was considered significant if $P < 0.05$.

## 3.2 IBH study

### 3.2.1 Study design

The study was performed during August to September 2018 in Uppsala, located in the middle east of Sweden. A total of 11 horses were used in the study where six of them had known IBH and the rest had no clinical signs of IBH (controls). The horses were located on four different farms that were chosen by convenience sampling based on owner reports and full clinical examinations. Each IBH – affected horse was matched with one control from the same farm (except one farm that had two IBH - affected horses and one control). The movement activity of the horses was measured for approximately seven days using IceTag® accelerometers (IceRobotics Ltd, Edinburgh, UK), which are commercial limb-mounted accelerometers, developed for cows. Direct observations using an ethogram were performed in the morning and in the evening for 30 minutes per time and horse and in total six times per horse (table 1). Number of steps that was taken for each horse was calculated manually on the first observation to compare with IceTag data. The remaining observations were performed using an ethogram and with help of a software program for behavioural analysis (BORIS, Behavioural Observation Research Interactive Software). During the study period of approximately seven days per IBH horse and its matching control, the horses were kept on pasture day and night except the horses on farm number four which were kept in paddocks for approximately seven to 17 hours per day. The IBH – affected horses with the matching control from the same farm were kept together in the same enclosure during the whole study period, with or without other horses not included in the study. The enclosures had varying sizes and except for water throughs, two of them had no available objects in it, such as trees or buildings.

Table 1. *Ethogram used for direct observations for studying behaviours of the horse. References are given in the table*

| Behaviour | Explanation |
|---|---|
| Walking | Walking forward in a 4-beat rhythm with head up from the ground (walking with the muzzle at the ground counts as grazing) |
| Standing still | No movements and all hoofs on the ground without any leg resting |
| Resting | Standing still with head somewhat down and relaxed body. The horse has often the eyes a bit closed and one hind leg resting |
| Lying | Horse lying down on the ground in a sternal – or lateral recumbency (Pierard, McGreevy & Geers, 2018) |
| Trotting | Moves forward in trot in a 2-beat gait (Pierard, McGreevy & Geers, 2018) |
| Cantering/Galloping | Moves forward in canter (3-beat gait) or gallop (4-beat gait and a faster version of canter) (Pierard, McGreevy & Geers, 2018) |
| Grazing | Horse eating or has the muzzle on the ground searching for feed (Ransom & Cade, 2009) |
| Drinking | Horse drinking or has the muzzle in the water bowl |
| Body shaking | Movements in the whole body at the same time, e.g. when shaking off insects |
| Head and neck shaking | Rapidly shaking head and/or neck up and down or side to side e.g. when shaking off insects |
| Scratching with teeth or head | Horse scratch itself with teeth or head on any body part |
| Scratching with hind leg | Horse brings one hind leg to its head and scratches its head or neck with the hoof (Pierard, McGreevy & Geers, 2018), or scratches its leg with the teeth. |
| Scratching against an object | Horse scratch itself on any body part against an object e.g. a tree |
| Grooming | Scratching with teeth or licking on any body part on another horse |
| Groomed | Horse is being groomed |
| Allogrooming | Grooming between two horses (Pierard, McGreevy & Geers, 2018) |
| Leg lift | Leg moves forcefully up and down e.g. when shaking off insects |
| Aggressive behaviour | Horse biting or kicking against another horse |
| Rolling | Lying down, rolling from side to side and stands up afterwards |
| Defecating | Defecating (Pierard, McGreevy & Geers, 2018) |
| Urinating | Urinating (Pierard, McGreevy & Geers, 2018) |
| Out of sight | Horse cannot be seen by the observer |

### 3.2.2 Animals

Five mares and six geldings of different breeds were investigated (table 2). The ages of the horses ranged from three to 23 years. Ten of 11 horses were used for riding and the remaining horse was used for companion. The severity of the clinical signs of IBH varied between the horses. The majority (4/6) of the IBH – affected horses were wearing a fly mask and half of them were wearing blankets for protection from insect bites. One of the five controls were wearing a fly mask and blankets were not used in any of the controls. Some of the IBH – affected horses were treated for the condition with for example antihistamines, lotions, shampoos, insect sprays and cortisone. The included IBH – affected horses had clinical signs of the condition both in the current year (2018) and the previous year (2017) despite the use of prophylactic methods.

Table 2. *Information like age, breed and gender for the investigated animals*

| Horse | Farm | IBH/Control | Age (years) | Breed | Gender |
|---|---|---|---|---|---|
| A[1] | 1 | IBH | 16 | KWPN | Mare |
| A[2] | 1 | Control | 14 | Friesian | Gelding |
| B[1] | 2 | IBH | 10 | Tinker | Gelding |
| B[2] | 2 | Control | 18 | Knabstrupper | Gelding |
| C[1] | 3 | IBH | 6 | Lusitano | Mare |
| C[1] | 3 | IBH | 3 | Swedish Warmblood | Mare |
| C[2] | 3 | Control | 3 | Welsh pony | Mare |
| D[1] | 4 | IBH | 23 | Shetland pony | Mare |
| D[2] | 4 | Control | 20 | Welsh Mountain | Gelding |
| E[1] | 4 | IBH | 18 | Dutch Riding-Pony | Gelding |
| E[2] | 4 | Control | 16 | Welsh Mountain | Gelding |

[1] IBH – affected horses.
[2] Controls.

### 3.2.3 Accelerometers

The movement activity of the horses (motion index, lying (t), steps (n) and lying bouts) was recorded continuously for approximately seven days using IceTag® accelerometers (IceRobotics Ltd, Edinburgh, UK). IceTags were mounted at the lateral side of a boot and placed randomly at one of the hind legs of the horse. An additional IceTag was placed on the neck at a head collar. The horse owner was requested to take notes of the time if the horse was removed from the pasture during study time. Accelerometers were removed during exercise and time was noted.

### 3.2.4 Information sampling and clinical examination

At day one, IBH - affected horses were clinically examined and the severity of the IBH was graded using a protocol. The matching controls were also examined to ensure that they had no clinical signs of the condition. All horses were examined by the same person. Clinical signs evaluated were alopecia (*figure 1, 2 & 3*), excoriations (*figure 1*), seborrhoea (*figure 2*) and skin thickening (*figure 2*). Some of the horses had only minor skin changes such as alopecia and mild excoriations in the mane while some were more affected of the disease with all clinical signs evaluated in this study. Information of both IBH – affected horses and controls was collected, including age, gender, breed, usage of the horse, treatments, equipment (e.g. blankets and fly masks), abnormal behaviours and description of the paddock.

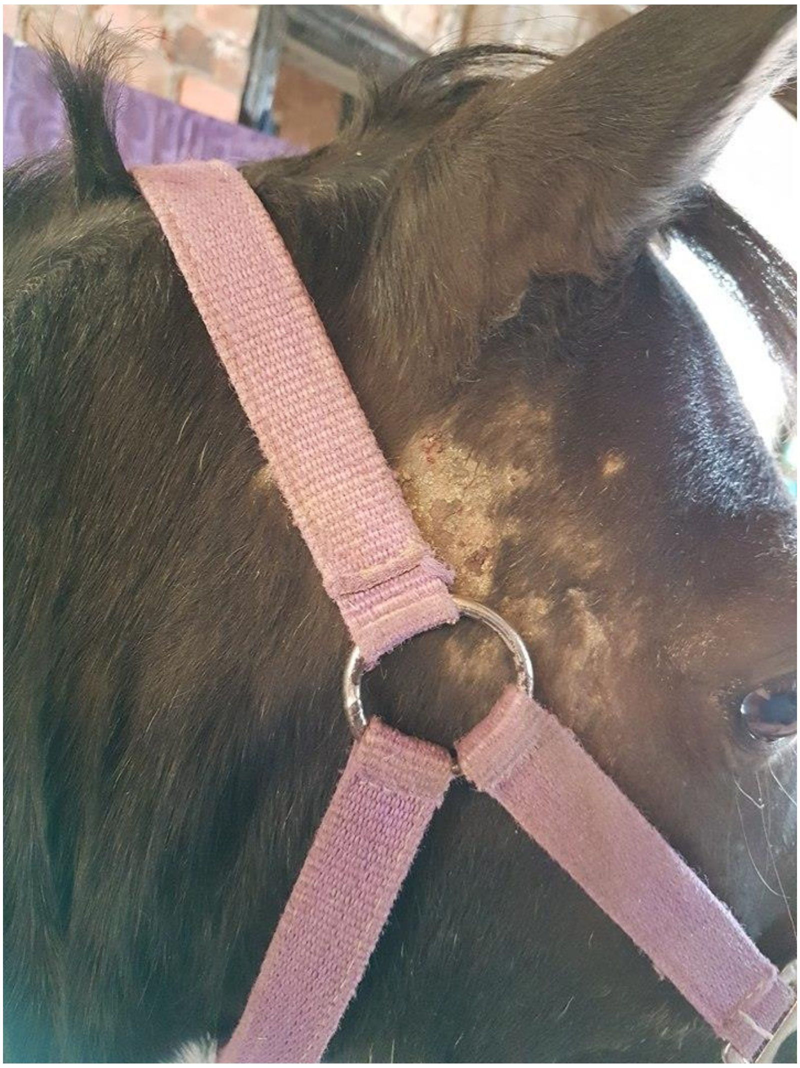

*Figure 1.* Excoriations and alopecia in the face of an 18 years old Dutch riding pony.

*Figure 2.* Skin thickening, seborrhoea and alopecia in the mane of a 23 years old Shetland pony.

*Figure 3.* Alopecia in the tail of a 23 years old Shetland pony.

### 3.2.5 Data management

Data from direct observations was exported from the behavioural software (BORIS, Behavioural Observation Research Interactive Software) to Microsoft Excel® sheets. IceTags were read in an IceReader (IceRobotics Ltd, Edinburgh, UK) and the data was processed in the IceManager (IceRobotics Ltd, Edinburgh, UK) software and then exported to Microsoft Excel®. Data from non-relevant time periods (e.g. when IceTags were removed from the horse) was removed. Data from accelerometers and direct observations was studied and compared with each other to evaluate the reliability of the accelerometers to detect steps (n) and lying bouts. Statistical analysis was performed with Minitab® Statistical Software (Minitab Ltd, Coventry, United Kingdom). IceTag data from IBH – affected horses was compared with data from controls using a two - sample t - test. The tests were considered significant if $P < 0.05$. The same statistical test was used when number of steps according to direct observations were compared with IceTag data. The frequency of itching behaviours (rolling, scratching with teeth or head, scratching with hind leg, scratching against an object) and behaviours that was considered to occur due to irritation for biting insects (body shaking, head and neck shaking and leg lift) was compared between IBH – affected horses and controls using a two – sample t – test. Motion index per hour was measured for periods in the mornings (06.00 – 09.59), days (10.00 – 14.59), evenings (15.00 – 23.59) and nights (24.00 – 05.59). Mean motion index for the different periods was compared using one – way ANOVA including a Tukey pairwise comparison. When evaluating the length of a lying bout and comparing head and neck activity when the horse was not moving, a sample size test with a power of 90 % was performed. Values are presented as mean ± standard error mean unless anything else is stated.

# 4 Results

## 4.1 Validation study

When validating IceTags placed on horses in boxes, only small differences (not significant) were found between number of steps recorded by video and IceTags (table 3 & 4) ($P > 0.05$).

Table 3. *Number of steps for horse 1 according to video recording and IceTags*

| Time | Video | IceTag left front leg | IceTag right front leg | IceTag left hind leg | IceTag right hind leg | *P* - value |
|---|---|---|---|---|---|---|
| 17.00 – 17.05 | 10[a] | 10 | -- | -- | -- | > 0.05 |
| 17.50 – 17.55 | 9[b] | -- | 7 | -- | -- | > 0.05 |
| 18.00 – 18.05 | 10[c] | -- | -- | 7 | -- | > 0.05 |
| 19.30 – 19.35 | 9[c] | -- | -- | 6 | -- | > 0.05 |
| 22.20 – 22.25 | 5[d] | -- | -- | -- | 7 | > 0.05 |
| 06.25 – 06.30 | 17[d] | -- | -- | -- | 16 | > 0.05 |

[a] Number of steps based on measurements of left front leg.
[b] Number of steps based on measurements of right front leg.
[c] Number of steps based on measurements of left hind leg.
[d] Number of steps based on measurements of right hind leg.

Table 4. *Number of steps for horse 2 according to video recording and IceTags*

| Time | Video | IceTag left front leg | IceTag right front leg | IceTag left hind leg | IceTag right hind leg | *P* - value |
|---|---|---|---|---|---|---|
| 18.35-18.40 | 6[a] | 5 | -- | -- | -- | > 0.05 |
| 19.50-19.55 | 7[b] | -- | 5 | -- | -- | > 0.05 |
| 20.00-20.05 | 10[c] | -- | -- | -- | 9 | > 0.05 |
| 21.55-22.00 | 8[c] | -- | -- | -- | 9 | > 0.05 |
| 02.35-02.40 | 6[d] | -- | -- | 4 | -- | > 0.05 |
| 04.05-04.10 | 10[d] | -- | -- | 4 | -- | > 0.05 |

[a] Number of steps based on measurements of left front leg.
[b] Number of steps based on measurements of right front leg.
[c] Number of steps based on measurements of right hind leg.
[d] Number of steps based on measurements of left hind leg.

According to video recording, horse 1 had a total lying time of one hour and 31 minutes which was not considered significant from the lying time recorded by the four different IceTags ($P > 0.05$). The video recorded four lying bouts and all four IceTags were able to detect those lying bouts. IceTags placed at the front legs of the horse detected several false lying bouts (approximately 97 % of the lying bouts were false) when compared to video recordings. IceTags placed at the hind legs of the horse were also detecting some false lying bouts (43 % of the lying bouts were false), however, those were fewer and not significant different from the number of lying bouts recorded by video ($P > 0.05$). Recorded data from IceTags and video for horse 1 is summarized in table 5.

Table 5. *Summary data of lying (t) and lying bouts from IceTags and video for horse 1 for one night (17.00 – 07.00)*

| Measurement | Lying (t) | Lying bouts |
|---|---|---|
| IceTag left front leg | 1:51:48[1] | 160[a] |
| IceTag right front leg | 1:54:48[1] | 132[a] |
| IceTag left hind leg | 1:40:34[1] | 7[b] |
| IceTag right hind leg | 1:36:34[1] | 7[b] |
| Video | 1:31:00[1] | 4[b] |

Values that do not share a number or a letter were statistically significant different ($P < 0.05$).

Horse 2 had a total lying time of 55 minutes and one lying bout according to video recording. All four IceTags were able to detect the lying bout observed at the video recording. The IceTag placed at the left front leg of the horse recorded a higher lying time compared to the other IceTags and video ($P < 0.05$). The IceTag placed at the right front leg and the IceTags placed at the hind legs of the horse did not recorded a statistically significant different lying time compared to video ($P > 0.05$). However, all four IceTags detected several false lying bouts (97.8 – 99.7 % of the lying bouts were false) (table 6).

Table 6. *Summary data of lying (t) and lying bouts from IceTags and video for horse 2 for one night (17.00 – 05.00)*

| Measurement | Lying (t) | Lying bouts |
| --- | --- | --- |
| IceTag left front leg | 2:13:12[a] | 357[a] |
| IceTag right front leg | 1:01:21[b] | 65[b] |
| IceTag left hind leg | 1:09:55[b] | 45[b] |
| IceTag right hind leg | 00:57:48[b] | 45[b] |
| Video | 00:55:00[b] | 1[c] |

Values that do not share a number or a letter were statistically significant different ($P < 0.05$).

## 4.2 IBH study

### 4.2.1 Lying time and lying bouts

Data of lying behaviours from IceTags placed at the hind leg of the horses are summarized in table 7. When comparing direct observations with IceTag data, several false lying bouts were found. A real lying bout was considered to result in a lying time of more than a minute, therefore, lying bouts that resulted in a lying time of less than a minute were removed. Before those lying bouts were removed, an average of 485.2 lying bouts were recorded for IBH – affected horses and 582 for controls for the approximately seven days study period. When lying bouts that resulted in a lying time of less than a minute were removed, a lower ($P = 0.001$) number of lying bouts were found (15.5±3.9 for horses with IBH and 20.6±6.4 for controls). For all horses included in the study, the average and total lying time for the study period was recorded to be 527.8 minutes before lying bouts less than a minute's duration were removed. After those lying bouts were removed, a lower, but not significantly different lying time was found (461.6±98.3 minutes) ($P = 0.629$). Lying bouts for all horses in the study occurred most frequent during nights (53.41±5.23 %) and less frequent during days (6.58±3.55 %), mornings (5.96±1.49 %) ($P = 0.000$) and evenings ($P = 0.028$) (*figure 4*).

A lying time of 3.6 – 12.2 % of the study period was recorded for horses kept on pasture day and night. The same horses had between 11 - 26 lying bouts during the entire study period. Horses with IBH spent in average 6.5±1.7 % of the approximately seven days observation time lying and the controls 5.5±1.9 % of the time. The mean minimum bout length was 5.9±4.8 minutes for horses with IBH and 1.8±0.49 minutes for controls. The mean maximum bout length was recorded to 83.4±17 minutes for IBH – affected horses and 82.6±26 minutes for controls. The average bout length in IBH - affected horses was measured to 34.5±9.9 minutes compared to 27.8±11 minutes in controls. The difference between IBH – affected horses and controls in lying (t), lying (%), lying bouts (n), bout length, minimum bout length and maximum bout length was not statistically significant ($P > 0.05$). Horse $E^1$ and $E^2$ performed only three and four lying bouts each during the entire study period. Due to the low number of lying bouts evaluated, data from those two horses was removed and lying bout lengths were calculated once again including nine horses. The mean maximum bout length for IBH – affected horses (excluding horse $E^1$) was lower (70.5±15 minutes), but not significant different than the mean maximum bout length for controls (excluding horse $E^2$) (99±26 minutes) ($P = 0.4$). According to a sample size calculation with a power of 90 %, the difference between the maximum bout lengths for IBH – affected horses and controls should have been statistically significant with 48 or more horses in each group.

Table 7. *Summary data of lying behaviours from IceTags placed at the hind leg of the horse*

| Horse | Lying time (min)* | Lying (%)* | Lying bouts (n)* | Minimum bout length (min)* | Maximum bout length (min)* | Average bout length (min)* |
|---|---|---|---|---|---|---|
| $A^1$ | 905 | 12.2 | 23 | 1 | 88 | 39 |
| $A^2$ | 585 | 7.9 | 9 | 3 | 110 | 65 |
| $B^1$ | 528 | 5.8 | 19 | 1 | 86 | 28 |
| $B^2$ | 330 | 3.6 | 18 | 1 | 116 | 18 |
| $C^1$ | 668 | 7.8 | 23 | 1 | 75 | 29 |
| $C^1$ | 522 | 6.1 | 20 | 2 | 91 | 26 |
| $C^2$ | 1029 | 12.0 | 26 | 1 | 146 | 40 |
| $D^1$ | 93 | 1.6 | 10 | 1 | 25 | 9 |
| $D^2$ | 166 | 2.9 | 41 | 1 | 24 | 4 |
| $E^1$ | 206 | 5.7 | 3 | 25 | 135 | 69 |
| $E^2$ | 46 | 1.3 | 4 | 3 | 17 | 12 |

* Lying bouts resulting in a lying time of less than a minute were removed.

[1] IBH – affected horses.

[2] Controls.

Horses with the same letter are the matching pair of one IBH – affected horse and one control.

The differences between IBH – affected horses and controls in lying time (min), lying (%), lying bouts (n), minimum bout length (min), maximum bout length (min) and mean bout length (min) were not statistically significant ($P > 0.05$).

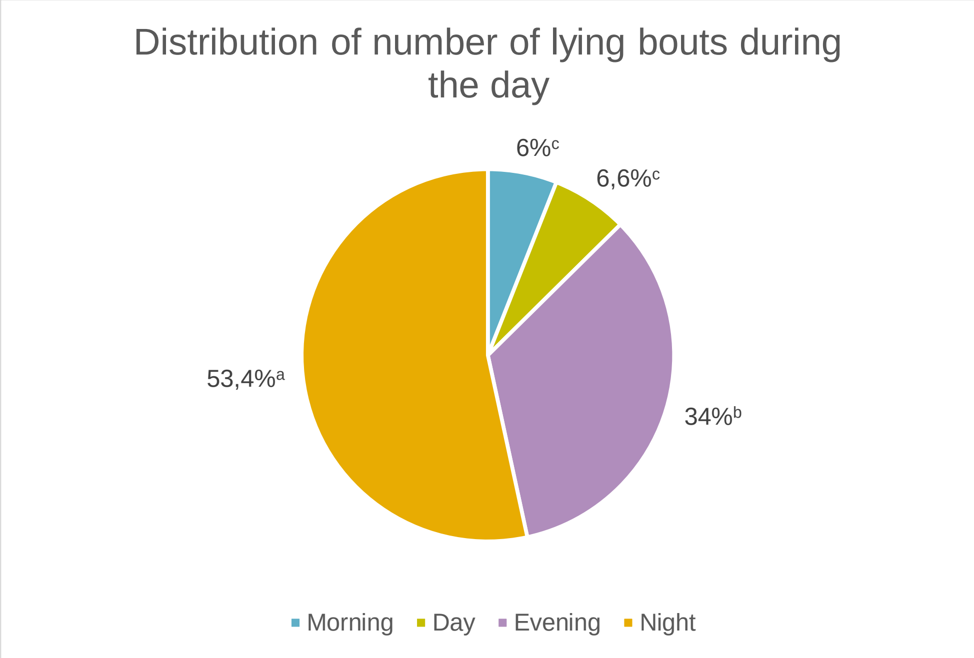


*Figure 4.* Distribution of number of lying bouts for all horses included in the study for mornings (06.00 – 09.59), days (10.00 – 14.59), evenings (15.00 – 23.59) and nights (24.00 – 05.59). Values that do not share a letter were statistically significant different ($P < 0.05$).

### 4.2.2 Motion index and step count

Horses kept on pasture day and night had a total mean motion index of 116 604 – 255 935 for the entire study period of approximately seven days (table 8). In average, they had a daily motion index of approximately 31 959±2993. For IBH – affected horses, the mean motion index for the entire study period was measured to 142 458±26 405 compared to 143 978±32 410 for controls. For the same period, number of steps was recorded to an average of 26 352±5209 for IBH – affected horses and 29 110±5544 for controls. No statistically significant differences were found between IBH – affected horses and controls in motion index and number of steps ($P = 0.972$ and $P = 0.728$). Including all horses in the study, a lower motion index during the night compared to the other time periods was found ($P \leq 0.001$) (*figure 5*). No statistically significant differences were found in motion index at different time periods between IBH – affected horses and controls ($P > 0.05$). When comparing number of steps according to direct observations with IceTag data, no statistically significant differences were found ($P > 0.05$).

Table 8. *Summary data from approximately seven days of motion index and step count from IceTags placed at the hind leg of the horse*

| Horse | Motion Index | Steps (n) |
|---|---|---|
| A[1] | 161 250 | 27 042 |
| A[2] | 116 604 | 28 458 |
| B[1] | 147 071 | 39 141 |
| B[2] | 174 547 | 45 078 |
| C[1] | 218 382 | 29 776 |
| C[1] | 233 783 | 40 634 |
| C[2] | 255 935 | 35 960 |
| D[1] | 109 547 | 20 232 |
| D[2] | 95 336 | 23 803 |
| E[1] | 68 339 | 10 139 |
| E[2] | 77 468 | 12 251 |

[1] IBH – affected horses.
[2] Controls.
Horses with the same letter are the matching pair of one IBH – affected horse and one control.
The differences between IBH – affected horses and controls in motion index and steps (n) were not statistically significant ($P > 0.05$).

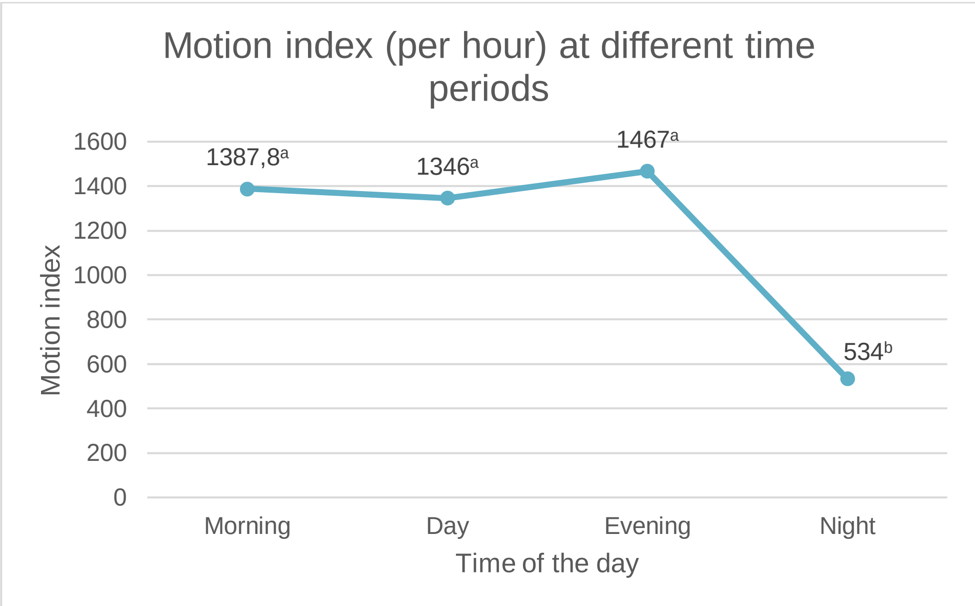


*Figure 5.* Mean motion index data (per hour) at different time periods from IceTags placed at the hind leg including all horses in the study. Values that do not share a letter were statistically significant different ($P \leq 0.001$).

### 4.2.3 Head and neck activity

Due to the position of the IceTags that was placed on a head collar, only motion index could provide reliable data and for that reason, number of steps, lying time and number of lying bouts from those IceTags were not considered in this study. Data from the entire study period from IceTags placed at a head collar was not available for three of the horses ($C^2$, $E^1$ and $E^2$) due to unpredictable circumstances. However, $C^2$ had available data the last four days of the study that could be compared with the two matched IBH – affected horses ($C^1$). The IceTag accelerometers measured a mean head and neck activity (motion index) of 45 302±15 246 for IBH – affected horses and 46 632±19 145 for controls for the entire study period (table 9), which was not considered significantly different ($P$ = 0.959). Motion index for IceTags placed at the neck were also compared between IBH – affected horses and controls when they were standing still (steps (n) = 0, according to IceTag placed at the hind leg). The mean motion index per minute in horses with IBH was 3.14±1.4 versus 1.425±0.17 in controls which was not significantly different ($P$ = 0.302). To get a significant difference, at least 31 horses in each group should have been included according to a sample size calculation with a power of 95 %.

Table 9. *Summary data from approximately seven days from IceTags placed at a head collar*

| Horse | Motion Index |
|---|---|
| $A^1$ | 45 569 |
| $A^2$ | 39 526 |
| $B^1$ | 16 019 |
| $B^2$ | 101 391 |
| $C^1$ | 38 932 |
| $C^1$ | 272 459 |
| $C^2$ | 33 139* |
| $D^1$ | 32 325 |
| $D^2$ | 12 470 |
| $E^1$ | No data |
| $E^2$ | No data |

* Data was only available from the last four days of the study.
[1] IBH – affected horses.
[2] Controls.
Horses with the same letter are the matching pair of one IBH – affected horse and one control.
The difference in motion index between IBH – affected horses and controls was not statistically significant ($P$ = 0.959).

Data recorded by IceTags placed at the neck of the horse showed a lower mean motion index per hour for all horses included in the study during the night compared to the day ($P$ = 0.011) (*figure 6*). The difference between motion index at night and morning – or evening was not statistically significant ($P > 0.05$). No statistical differences in motion index were found between IBH – affected horses and controls at the different time periods ($P > 0.05$).

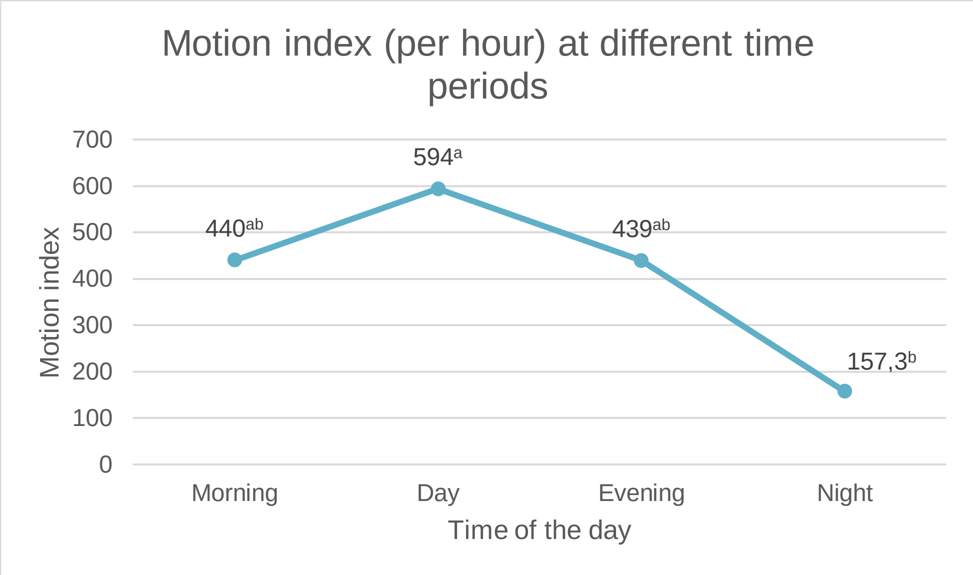


*Figure 6.* Motion index data (per hour) at different time periods from IceTags placed at the neck including all horses in the study. Values that do not share a letter were statistically significant different ($P = 0.011$).

### 4.2.4 Time budgets and behaviours observed from direct observations

According to direct observations, the 11 horses included in the study had a mean grazing time of 74.22±6.51 % of the day. Walking accounted for 4.06±1.06 % of the day, standing still 2.295±0.837 % and resting 7.24±3.36 % (table 10). No significant differences of the time budgets were found between horses with IBH and controls ($P > 0.05$).

Table 10. *Time budgets for IBH – affected horses and controls according to direct observations*

| Behaviour | IBH - affected horses | Controls | *P* - value | Total mean | SE Mean |
|---|---|---|---|---|---|
| Grazing | 78.51 % | 69.94 % | 0.545 | 74.22 % | 6.51 |
| Walking | 2.73 % | 5.4 % | 0.249 | 4.06 % | 1.06 |
| Standing still | 0.99 % | 3.6 % | 0.146 | 2.295 % | 0.837 |
| Resting | 5.28 % | 9.2 % | 0.593 | 7.24 % | 3.36 |
| Trotting | 0.11 % | 0.16 % | 0.804 | 0.135 % | 0.0919 |
| Galloping | 0 % | 0 % | N.A* | 0 % | 0.00 |
| Rolling | 0.1 % | 0.18 % | 0.552 | 0.14 % | 0.0618 |
| Lying | 0 % | 0 % | N.A* | 0 % | 0.00 |
| Drinking | 0 % | 0.1 % | N.A* | 0.05 % | 0.0342 |
| Allogrooming | 0.12 % | 0 % | N.A* | 0.06 % | 0.06 |

Differences between IBH – affected horses and controls were considered significant if $P < 0.05$.
* *P* – value was not available. Identical or near identical numbers for IBH – affected horses and controls.

IBH – affected horses were found to perform in average and- total 78±17 itching behaviours and behaviours that was considered to occur due to irritation for biting insects, according to direct observations (table 11). The corresponding number for controls was 96.2±38 and was not significantly higher than the number for horses with IBH ($P = 0.678$). Furthermore, no behaviour that was considered to be performed due to itching or irritation for biting insects differed significantly in the frequency between IBH – affected horses and controls ($P > 0.05$).

Table 11. *Total number of itching – and other relevant behaviours observed during direct observations*

| Horse | Head and neck shaking | Scratching with teeth or head | Scratching with hind leg | Scratching against an object | Rolling | Leg lift | Body shaking | **Sum** |
|---|---|---|---|---|---|---|---|---|
| A[1] | 37 | 41 | 2 | 1 | 0 | 14 | 0 | 95 |
| A[2] | 47 | 69 | 0 | 0 | 1 | 28 | 3 | 148 |
| B[1] | 3 | 22 | 0 | 0 | 0 | 102 | 0 | 127 |
| B[2] | 68 | 30 | 0 | 0 | 0 | 120 | 1 | 219 |
| C[1] | 33 | 11 | 0 | 4 | 0 | 1 | 0 | 49 |
| C[1] | 73 | 17 | 0 | 0 | 1 | 25 | 1 | 117 |
| C[2] | 26 | 14 | 1 | 3 | 1 | 8 | 0 | 53 |
| D[1] | 22 | 16 | 1 | 4 | 0 | 12 | 5 | 60 |
| D[2] | 10 | 9 | 0 | 0 | 5 | 9 | 1 | 34 |
| E[1] | 3 | 10 | 3 | 0 | 1 | 7 | 1 | 25 |
| E[2] | 5 | 16 | 0 | 1 | 0 | 5 | 0 | 27 |

[1] IBH – affected horses.
[2] Controls.
Horses with the same letter are the matching pair with one IBH – affected horse and one control.
No statistically significant differences in itching – and other relevant behaviours performed by IBH – affected horses and controls were found.

# 5 Discussion

## 5.1 Validation study

When comparing data from all four IceTags with video recording, no statistically significant differences were found in number of steps. Similar results were found for horses kept in paddocks which strengthen the theory that IceTags detect reliable data of number of steps. Thus, IceTags seems to be able to give correct data of step count when placed either on the front legs or the hind legs. Lying time were also similar when comparing recordings by IceTags and video, except for one IceTag placed at the front leg of one horse which recorded a significantly higher lying time compared to the other IceTags and video. IceTags placed at the front legs of the horse recorded several false lying bouts for both horse 1 and horse 2. For that reason, placement of that position is not recommended. IceTags placed at the hind legs were also detecting false lying bouts, but for horse 1, those were few. During the time when those IceTags recorded false lying bouts, scratching of the hind leg with the teeth was observed from the video recording in all cases for horse 1. Thus, IceTags placed at the hind legs does not seem to be able to distinguish between a lying bout and scratching. Accelerometers placed at the hind leg of horse 2 detected as many as 44 false lying bouts each. Compared to horse 1, no scratching of the hind leg was observed from the video recording. At the time of the false lying bouts, horse 2 was observed to rest with the hind leg or put the leg in a tilting position which may have put the IceTag in a horizontal position and thus explain all the false lying bouts. In the current study, accelerometers were fixed in a vertical position on a boot placed at the cannon bone. More studies are needed in this area to find a proper placement of IceTags on horses to reduce the false lying bouts recorded. Another possibility is to develop a new algorithm in the IceTag that is validated for horses. However, the false lying bouts recorded in this study were resulting in a lying time of less than a minute and therefore, a true lying bout could, as a suggestion, only be considered if the lying time last more than a minute.

## 5.2 IBH study

### 5.2.1 Lying time and lying bouts

The recorded lying time (3.6 – 12.2 % of the study period) for horses kept on pasture day and night according to IceTags placed at the hind leg of the horse was similar to previous studies (Chaplin & Gretgrix, 2010; Boyd, Carbonaro & Houpt, 1988; Duncan, 1980). Compared to previous studies (Chaplin & Gretgrix, 2010; Boyd, Carbonaro & Houpt, 1988; Duncan, 1980), lying time recorded by the IceTags placed at the hind leg of the horse seemed to detect reliable data of lying time. However, when comparing direct observations with data from IceTags, the accelerometers were found to provide several false lying bouts which was also seen in the validation study. When the accelerometer is put in a horizontally position, a lying bout is registered. That position may appear in other situations than an actual lying bout, for example when the horse brings the hind leg towards the head and scratch it with the teeth, which was seen in the validation study. In the current study, lying bouts that resulted in a lying time of less than a minute were considered false and were therefore removed. After the removal of those lying bouts, horses kept on pasture day and night performed approximately 1.7 – 4 lying bouts per day which is similar to the results found by Chaplin & Gretgrix (2010). When comparing direct observations with IceTag data, a lying bout followed by rolling was resulting in a lying time of less than a minute. Because all lying bouts that resulted in a lying time of less than a minute, rolling behaviour may have been removed and could not be considered in this study. A video camera recording continuously for the entire study time should have been preferred for a more correct result of number of lying bouts. Lindberg, Herlin & Michanek (2013) claimed that IceTags provided reliable data of number of lying bouts in their study. The reliability of the data provided by IceTags used on horses should for that reason be investigated further. However, no differences were found in lying time and number of lying bouts between IBH – affected horses and controls.

Lying bouts have earlier been shown to occur most frequent during nights (Chaplin & Gretgrix, 2010) which was also shown in the current study. Duncan (1985) have also reported that free-living mares spent the time lying at a higher percentage during the nights compared to the days. The average length of one lying bout including all horses was 30.82±6.41 minutes which was relatively similar to previous research (Chaplin & Gretgrix, 2010). However, in the previous study, the maximum bout length was found to be 32.9 minutes which was considerably lower than the average maximum bout length including all horses in this study (83±13.4 minutes). When observing data of lying bout lengths, IBH – affected horses seemed to have a slightly shorter maximum lying bout length compared to controls, except horse $E^1$ which had a considerably higher maximum lying bout length compared to its matching control ($E^2$). Therefore, a new calculation of the maximum lying bout lengths was performed excluding horse $E^1$ and $E^2$. Furthermore, those horses performed in total only three and four lying bouts each during the entire study period which was considerably less than the other horses. Horses on farm four ($D^1$, $D^2$, $E^1$ and $E^2$) were only kept in paddocks for approximately seven – 17 hours per day. At the remaining part of the day, IceTags were removed and the behaviours performed during that time was not known. Probably, lying bouts were performed during that time also. Due to different study time for the different horses included in the study, only IBH – affected horses should be compared to the matching control. Besides the different study time between horses, size of the enclosure may affect the result because horses in small enclosures spend less time resting while standing than horses in larger enclosures (Boyd, 1988). Other parameters such as number of horses that are kept together may also affect the results. For that reason, comparing horses between farms is not preferable.

### 5.2.2 Motion index and step count according to IceTag placed at the hind leg

In studies by Boyd, Carbonaro & Houpt (1988) and Duncan (1985), locomotion was found to be lower during the night compared to the morning, day and evening. Similar results were found in the current study. However, no differences in motion index between IBH – affected horses and controls were found at the different time periods. Due to a higher activity of *Culicoides* in the morning and the evening (Craig, 2011), a higher motion index for IBH – affected horses during those time periods was expected. The daily motion index for all horses kept on pasture day and night was approximately 31 959±2993 which was higher than horses kept in an Active-Stable system® (12 768) (Gulbrandsen & Herlin, 2015). Except that horses are kept day and night in both systems, it is still two different systems and cannot be compared to each other. Furthermore, no details of the Active-Stable system® (e.g. size of the enclosure, feeding routines and information about the horses) were mentioned in the paper.

During direct observations, especially the first days of the study, horse $C^1$ from farm four was observed to kick with the hind leg in a way that perceived to be due to irritation for the IceTag and not due to insects. Due to that, a lower motion index may have been expected if that behaviour was not performed. For a more realistic result, all horses included in the study should have been habituated to the equipment a time before the study. Unfortunately, that was not possible in this study due to lack of time. No significant differences were found in motion index and step count between IBH – affected horses and controls. In the hypothesis, a higher motion index was expected for IBH – affected horses. Either, the activity of the horse may be less affected of biting insects than expected, or, the number of biting insects during the study time was too low to observe any differences in the horse's activity. Insects that can cause hypersensitivity in horses prefer a hot, humid and windless climate (Craig, 2011). During the study time, the climate may not have been preferable for the biting insects. In the current study, only a total of 11 horses were included in the study. In future studies, a higher number of horses should be investigated to increase the possibility for significant differences between IBH – affected horses and controls.

Some of the IBH – affected horses included in the study had only mild clinical signs of the condition and may have not been sufficiently affected of the insect bites in a way that influenced the movement activity. In future studies, IBH – affected horses should have pronounced clinical signs of the condition that increase the difference between horses with IBH and controls. A high amount of biting insects in the studying area should also be ensured.

### 5.2.3 Head and neck activity

The activity of the head and neck was measured with help of IceTags placed on a head collar. Horse $C^1$ from farm four had the highest motion index from that IceTag compared to the other horses included in the study. During direct observations, horse $C^1$ was shaking the head and neck in a way that did not seem to be due to shaking off insects. The owner of $C^1$ mentioned that the horse was not used to wear a collar at pasture and the shaking could be due to irritation, which could explain the high motion index. However, IBH – affected horses did not have a higher head and neck activity compared to controls which is against the hypothesis. The majority (4/6) of the IBH – affected horses were wearing a fly mask and half of them were wearing blankets for protection from insect bites. Furthermore, only one of the controls was wearing a fly mask and blankets were not used in any of the controls. The fact that IBH – affected horses had a better protection against insect bites may had influence on the results. For an equivalent comparison between the two groups (IBH and controls), protection against insect bites such as blankets and fly masks should either been used for none of the horses or for both. In the current study, the investigated horses were managed in the same way as they were before the study (regarding e.g. type of enclosure, groups of horses kept together, treatments against IBH and usage of protective clothing). If any treatments or protection for insect bites should have been removed during the study time, a more correct comparison between IBH – affected horses and controls should have been possible. However, it should also have been an ethical question because horses with IBH should probably had more severe signs of the condition without any protection for the biting insects.

Another problem when analysing motion index from the IceTags placed at the neck is that the motion index increases when the horse moves. Consequently, a high motion index recorded by the IceTag is not always equal to a high head and neck activity but can be due to a high movement activity (e.g. walking, trotting or galloping). For that reason, periods when horses were not moving (steps (n) = 0) were analysed to eliminate a high motion index due to movement instead of head and neck activity when shaking off insects. Horses with IBH showed a slightly higher motion index (3.14±1.4) than controls (1.425±0.17), but the results were not significantly different. Motion index recorded when the horses were not moving was supposed to correlate to behaviours such as head and neck shaking, scratching with teeth or head and scratching against an object. Due to itching or irritation for biting insects, a higher head and neck activity in affected horses was expected. Because no data from IceTags placed at the neck was available for two of the horses, data from only eight horses was evaluated. According to a sample size calculation, the result should have been statistically significant with 31 or more horses in each group.

### 5.2.4 Time budgets and behaviours according to direct observations

According to direct observations performed in the morning and the evening, all horses included in the study had an average lying time of 0 % which corresponds to the results in the study by Duncan (1985). The direct observations were only performed in the morning and the evening, therefore, a 24 – hour time budget is not available for the horses in this study. In previous studies, a daily lying time of 1 – 13 % has been found (Gulbrandsen & Herlin, 2015; Chaplin & Gretgrix, 2010); Duncan, 1980). Because horses have shown a higher lying time during the night compared to the day (Duncan, 1985), a higher daily lying time should have been expected if direct observations were performed both during day and night. Furthermore, according to IceTags, average lying time for horses kept on pasture day and night was 3.6 – 12.2 % of the day, with a higher number of lying bouts during nights.

The average grazing time for all horses was higher compared to previous studies (Boyd, Carbonaro & Houpt, 1988; Boyd, 1988; Duncan, 1980). On the other hand, time budgets of resting while standing and walking were lower than the time budgets found by Duncan (1980). As mentioned earlier, direct observations were only performed in the mornings and in the night and cannot be compared to a 24 – hour time budget. In the study by Duncan (1980), only horses of the breed Camargue were observed. Also, the area available for the horses were much larger than for the horses in the current study which may have an effect of the time budgets.

Because itching is a common clinical sign for IBH in horses (Craig, 2011) a higher frequency of itching behaviours was expected in affected horses compared to controls. However, neither itching behaviours nor behaviours that was considered to occur due to irritation for biting insect were observed more frequent in IBH – affected horses. Direct observations were only performed in total three hours per horse during the study period and therefore, the frequency of itching behaviours performed during the remaining time of the study was not known. On two of the farms, horses were kept in enclosures without any objects available such as trees or buildings. That means that they basically had no ability to perform the behaviour "scratching against an object" which may have had an impact of the absence of difference between the frequency of itching behaviours in IBH – affected horses and controls. Because no significant difference was found in the frequency of itching behaviours between horses with IBH and controls, IBH – affected horses did not seem to perform itching behaviours as much as expected. If the IBH – affected horses included in the study should have had more severe clinical signs for the condition, a higher frequency of itching behaviours in those horses should most likely been observed.

# 6 Conclusion

IceTag accelerometers placed at the hind leg of the horse provided reliable detection of number of steps and lying time but overestimated number of lying bouts. More studies on the use of IceTags on horses should be done to reduce the number of false lying bouts. No statistically significant difference in movement activity and behaviour between IBH – affected horses and healthy horses kept in paddocks could be detected in this study. Larger sample sizes and/or monitoring horses without protective clothing would be needed to reveal this. If the method should be used for monitoring the effect of an anti-insect device, IBH – affected horses with more severe signs of the condition would probably be needed to show an effect.

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# Acknowledgements

I would specially like to thank my Supervisors Miia Riihimäki and Pia Haubro Andersen at the Department of Clinical Sciences, SLU, Uppsala, Sweden for your invaluable help. I am very grateful for the opportunity to be involved in this project and it has been a really good time. Also, a big thanks to Elin Hernlund for your truly kindness and willing to help. Catherine Malcolm, IceRobotics, thank you for discussing and answering my questions about the IceTags. Last but not at least, a big thanks to the horse owners that let me "borrow" their horses for this master thesis. You have all been very accommodating and helpful.

# Appendix 1

**Protocol for grading of clinical signs of IBH**

0 = normal, 1 = mild, 2 = moderate, 3 = severe
Skin thickness: 0 = <5mm, 1 = 5-10mm, 2 = 10-15mm, 3 = >15mm

| **Area** | **Alopecia** | **Excoria-tion** | **Sebor-rhoea** | **Skin thick-ness** | **Sum** |
|---|---|---|---|---|---|
| Face | | | | | |
| Ears | | | | | |
| Neck | | | | | |
| Base of mane | | | | | |
| Back | | | | | |
| Tail base | | | | | |
| Sides | | | | | |
| Croup | | | | | |
| Ventral mid-line | | | | | |
| Legs | | | | | |
| **Total** | | | | | |