ASSESSING WELFARE OF VEAL CALVES ON FARMS

Measures of behaviour and respiratory disorders and potential ways for welfare improvement

Hélène Leruste

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ABSTRACT

In Europe, minimal standards for the protection of veal calves are defined in EU directives but do not necessarily guarantee a sufficient level of animal welfare at all farms. The European Union supported a research program called Welfare Quality® from 2004 to 2009, which aimed at “developing a standardized monitoring system of animal welfare” and “identifying practical solutions to improve animal welfare”. The objective of the present thesis was to develop and validate a number of measures on veal farms including measures related to the assessment of human-animal relationship, abnormal oral behaviours, and respiratory disorders. In that perspective, a number of measures were tested for their validity, reliability and feasibility and specific risk factors for impaired welfare at farm level were identified. Data were collected on 174 farms in the three main veal producing countries in Europe (France, the Netherlands and Italy). Farms were visited at several time point during fattening and observations were performed at slaughter. Several behavioural tests for the assessment of human-animal relationship were validated as they showed good test-retest and inter-observer repeatability and were feasible on farms. Total number of calves on the farm, number of calves per stockperson, space allowance, type of milk distribution environmental enrichment, stockperson’s experience, breed and season of observation influenced the outcome of the human-animal relationship tests. Veal calves raised under intensive conditions may express abnormal oral behaviours as signs of mental suffering and reduced welfare. The observation of these abnormal oral behaviour of calves could be performed by direct observation which prooved to be a more suitable observation method than observations from video recordings for these behaviours in veal calves even though the presence of an observer had a short term effect on abnormal oral behaviours of calves. Again, factors such as space allowance, farmer’s experience, breed of calves and season but also type of solid feed, amount of milk, number of calves per pen and babybox use influenced the expression of abnormal oral behaviour in veal calves. The presence and severity of lung lesions recorded post-mortem is commonly used as an indicator to assess the prevalence of respiratory problems in batches of bovines. In the context of welfare assessment based on on-farm measures, the recording of clinical signs on calves at the farm would be more convenient than the recording of lung lesions at slaughter. Regardless of the stage of fattening, the prevalence of in vivo signs of respiratory disorders in calves was low However, at postmortem inspection, a significant proportion of lungs evaluated showed mild/moderate or and severe signs of pneumonia. Clinical signs of respiratory disorders were not enough predictive of lung lesions and therefore both measures are to be kept in the monitoring system tools. Different risk factors for respiratory disorders were involved at different stages of the fattening period. Among all potential risk factors considered, those concerning the characteristics of the batch were predominant but factors related to housing, management and feeding equipment were also relevant. This thesis showed that animal-based measures on human animal relationship, abnormal oral behaviours and respiratory disorders are valid and contribute reliably to assess the welfare of veal calves. In addition, some conditions were identified that should be considered carefully in welfare assessment. The study allowed assessing multiple factors across farms that influenced the outcome of welfare measures assessed in veal calves. These factors imply a potential negative or positive impact on welfare. Knowing these factors can help in advising farmers in their effort to improve welfare of veal calves although the biological causality between some factors and the welfare measures is not always known.

CONTENT

CHAPTER 1

General introduction

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VEAL PRODUCTION IN EUROPE

Veal calves are young bovines slaughtered before 8 months of age (Council regulation 700/2007). In Europe, veal calves are surplus calves (generally males) mainly originating from the dairy sector. The predominant breeds are dairy breeds (e.g. Holstein-Friesian) but dual-purpose breeds, cross breeds, and, less frequently, meat breeds can also be found.
Veal production aims to produce tender, pale-coloured meat. To achieve this, calves receive a diet controlled for iron content consisting mainly of milk replacer with additional solid feed (e.g. concentrates, maize silage and straw). Calves are fed milk replacer via a bucket, trough, or automated milk delivery device (AMD). Veal calves are raised indoors in specialised fattening units and are generally kept on wooden or concrete slatted floors. Approximately 5.8 million veal calves are slaughtered each year in Europe at an age of between 18 to 32 weeks. The three main veal producing countries are France (230 000 tons carcass-weight equivalent (tcwe)), the Netherlands (220 000 tcwe) and Italy (190 000 tcwe) (Claxton and Brown 2011).

WELFARE OF VEAL CALVES

Until the late 1990s, the main concerns for the welfare of veal calves were social isolation, limited space allowance and an all-milk diet low in iron content (Broom 1991) which can lead to stress and mental suffering, locomotion problems, digestive disorders, and anaemia. In Europe, the first minimal standards were defined in the 1990s in EU directives (Council directives 91/629/EEC and 97/2/EC) and additional regulations were established in 2008 (Council directive 2008/119/EC). Since 2007, all calves are housed in groups with a minimum space allowance according to their live weight (e.g., 1.8 m^2/calf for animals with a live weight above 220 kg). No calf may be individually housed after the age of 8 weeks unless its condition (health or behaviour) requires separating it from the group members. Calves should also be fed with feed containing sufficient iron to ensure an average blood haemoglobin level in the batch of at least 4.5 mmol/L. Calves over 2 weeks old should receive a minimum daily ration of fibrous feed from 50 g to 250 g per day for calves from 2 to 20 weeks old (Council directive 2008/119/EC). These regulations are minimum standards for the protection of calves. Minimum standards, however, do not necessarily guarantee a sufficient level of animal welfare. Each farm has specific conditions regarding housing, management, etc., that may cause welfare issues although the farm itself does comply with the regulations. Moreover, health state of the animals may have an important impact on welfare as well. Therefore, on-farm welfare assessment of animals will allow a better determination of the actual welfare state as compared to setting minimum standards. With regard to health, calves are susceptible to a number of diseases, especially at a young age when their immune system is not yet mature (Autio et al. 2007). One important risk factor for health problems is that calves from different origins (farms, regions, countries) are mixed upon arrival at a veal farm. Additionally, stocking densities, group housing, microclimatic conditions and feeding strategies can affect health problems such as digestive and respiratory disorders in calves (EFSA 2006; Cozzi et al. 2009).

The possibility to express a large range of natural behaviours such as social and play behaviours may be an important determinant of the welfare of captive animals (Jensen et al. 1998; Babu et al. 2004). Veal calves housed in an environment and under a management system that does not fulfil their needs may experience stress and frustration which might develop into malfunction of the animal. This is expressed, for example, through abnormal behaviours. These behaviours can be a serious sign of mental suffering and reduced welfare (Broom and Fraser 2007). While housing may restrict calves in the freedom to perform natural behaviour, animal management of stockpersons also has an important impact on the welfare of calves (Lensink et al. 2000a). This impact can be through the quality of care given to calves which may affect health and behaviour, and through the quality of contact and handling which may affect fear and stress (Boivin et al. 2003). The level of fear (of humans) in calves can be assessed by the behavioural response of calves to humans, e.g. in so-called human approach or human avoidance tests (Lensink et al. 2000b).

Welfare in a social context

The welfare of farm animals is increasingly gaining societal interest. European citizens consider animal welfare as an important issue as 58% of the citizens wish to be more informed on the conditions under which animals are farmed and nearly 80% of them express that there is a need for further improvement of welfare and protection of farm animals (European Commission 2007). Consumers spontaneously consider outdoor access, space, “natural” feed, humane slaughtering, absence of mutilation, and limited transport as important issues for welfare of animals (Miele and Evans 2005). There is a demand from part of the consumers to be informed on the level of welfare of farm animals and therefore a need for a scientifically approved on-farm assessment tool of the welfare state of the animals. The European Union, therefore, supported a research project called Welfare Quality® from 2004 to 2009, which aimed, amongst others, to “develop a standardized monitoring system of animal welfare” and to “identify practical solutions to improve animal welfare” (Veissier et al. 2005).

Principles of welfare monitoring systems

Before a monitoring system (intended for the follow up of the farm over a period of time) can be implemented, assessment tools (intended for the evaluation at a given time point) need to be developed. The development of an assessment tool for animal welfare requires defining a set of criteria (that should cover as many aspects of welfare as possible) and testing these criteria under farm conditions. In the case of Welfare Quality®, four welfare principles were defined: good housing, good feeding, good health and appropriate behaviour (Botreau et al. 2007). Within each welfare principle, several welfare criteria were distinguished (Table 1). The aim of this general framework was to help developing assessment tools that can be used in different species to evaluate all European farms for the main meat and dairy production systems in a similar way.

Table 1
The definition of animal welfare in terms of principles and criteria within the Welfare Quality® project (Welfare Quality® 2009).

Welfare principles	*Welfare criteria*
Good feeding	1 Absence of prolonged hunger
	2 Absence of prolonged thirst
Good housing	3 Comfort around resting
	4 Thermal comfort
	5 Ease of movement
Good health	6 Absence of injuries
	7 Absence of disease
	8 Absence of pain induced by management procedures
Appropriate behaviour	9 Expression of social behaviours
	10 Expression of other behaviours
	11 Good human-animal relationship
	12 Absence of general fear

Within each species, a set of quantifiable measures was chosen to evaluate the different welfare criteria. The chosen measures needed to be of good quality. First, they had to be valid (measuring what they are supposed to be measuring). Second, measures should reach sufficient reliability: different assessors should achieve the same results (inter-observer reliability) and repeated tests with the same subjects should give similar results (test-retest reliability). Additionally, measures must be applicable at a large scale on commercial farms, with the lowest time and money costs and without disturbing daily farm routine (Engel et al. 2003; Knierim and Winckler 2009). Finally, the evaluation of the welfare of farm animals should be based mainly on measures on the animal rather than on measures of the environment (Veissier et al. 2005), since environmental-based measures are often poorly linked with aspects of animal welfare (Welfare Quality® 2009). Animal-based measures of welfare can be separated into four main categories: neurological, physiological, physical and behavioural measures. Neurological and physiological measures are scarcely included in on-farm assessment tools of welfare because they are time consuming, sometimes invasive and some may induce pain, discomfort or stress for the animal. For instance, blood cortisol levels can be examined as an indication of stress. To examine blood cortisol levels, however, animals need to be handled, restrained and samples need to be taken which can induce stress itself and is not applicable at a large scale on farms. Measures based on observation of clinical signs of disease or disorders and animal behaviour were therefore preferred for the evaluation of most of the 12 welfare criteria.

Improvement of veal calves welfare

The on-farm welfare assessment tool developed in the Welfare Quality® project had three main objectives: it should produce a scientifically approved measurement tool, it should inform the farmer about the welfare status on their farm and allow for welfare improvements on farms (Figure 1). The improvement of welfare of veal calves can be reached by first indicating for each individual farm, which welfare criteria could be improved and then provide the farmer with advices for potential remedial actions to apply for the specific welfare related measures that are considered to be improved. The development of potential remedial actions requires evaluating the impact of environment or management features on the different welfare measures. This can be done through a risk factor analysis.

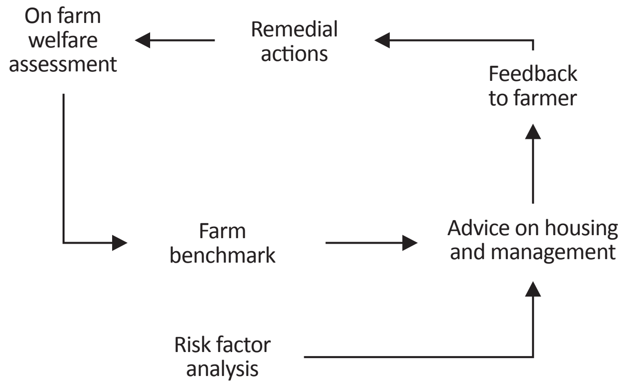

Figure 1
Diagrammatic representation of welfare monitoring for improvement of welfare on farms.

Objectives of the thesis

In veal calves, a number of measures were not validated on farms at the start of the Welfare Quality® project. Human-animal relationship tests were developed in a number of species in experimental setups (Hemsworth et al. 1996; Lensink et al. 2003; Tallet et al. 2005) but were never adapted to commercial farms in veal calves and therefore needed to be developed for the criterion "good human-animal relationship". In order to assess the criterion "expression of other behaviours" of veal calves, the expression of abnormal oral behaviours was considered. Including this measure in a welfare assessment tool required the development of standardized observation protocols of behaviour of calves on commercial farms. The criterion "absence of diseases" is assessed through a number of measures related to respiratory disorders, digestive disorders, anaemia and general health state. There was a lack of knowledge on how to assess respiratory diseases and to what extent clinical signs can be predictive for respiratory pathologies.

In order to contribute to the development of a reliable on-farm welfare assessment tool for veal calves, the specific objectives of this thesis were (i) to study the quality in terms of reliability and feasibility of measures assessing three different aspects of veal calf welfare on farm, and (ii) to identify specific risk factors for impaired welfare at farm level.

The three welfare aspects discussed in this thesis reflect different fundamental and operational features: a behavioural test (human-animal relationship), behavioural observations (with special emphasis on abnormal oral behaviour) and clinical health observations (respiratory disorders). In this thesis potential critical points were analysed about the measures related to the three welfare aspects. The risk factor analysis identified factors influencing the measures of welfare and could be used by farmers to take remedial actions to improve the welfare status of the calves on their farm.

Thesis outline

In order to select which tests could be used at a large scale for on-farm assessment of fearfulness of humans in calves the feasibility and repeatability between and within observers of several human-animal relationship tests were evaluated on a sample of farms (Chapter 2). In Chapter 3, the response of veal calves to humans was investigated using two tests selected from Chapter 2 and the factors influencing the response of calves to human approach tests were determined. In Chapter 4, direct observations of the behaviour of calves were compared to observations from video recordings to estimate the potential impact of the presence of the observer during on-farm assessments on abnormal oral behaviour of calves. In Chapter 5, the prevalence of abnormal oral behaviours in veal calves on a large sample of commercial farms was estimated and potential influencing factors associated with high levels of abnormal oral behaviours were determined. In Chapter 6, the relationship between three respiratory disorders recorded on farm and pathological findings on calves' lungs at slaughter were studied to determine which measure(s) would give the best indication of the respiratory status of batches of calves through their fattening. In Chapter 7, potential influencing factors associated with the occurrence of respiratory problems in veal calves, were investigated in a large sample of farms. The thesis ends with a general discussion on the quality of the measures developed, the use of risk factor analysis for welfare improvement and some practical issues related to the on-farm use of the assessment tool in veal calves fattening units.

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CHAPTER 2

Inter-observer and test-retest reliability of on-farm behavioural observations in veal calves

CHAPTER 2

Inter-observer and test-retest reliability of on-farm behavioural observations in veal calves

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Abstract

The objective of this study was to investigate inter-observer and test-retest reliability of different behavioural observations to be used in an on-farm, animal welfare monitoring system for veal calves. Twenty-three veal calf farms, varying in size, housing system, feeding regime and age of the calves were visited twice with two observers, simultaneously. Behavioural tests were conducted in eight pens per farm, measuring the response of calves to: a human entering the barn; a novel object; a passive, unfamiliar person; disturbance in the pen and an active approach by an unfamiliar and a familiar person. Furthermore, behaviour was recorded 20 min before and 20 min after feeding in eight other pens per farm. For all behavioural tests, inter-observer reliability was very high. Farm effects and test-retest reliabilities were high and significant for all behavioural tests, except for the test measuring response to disturbance in the pen. Although the active approach test with the familiar person was reliable, it was not feasible in practice due to the availability of the farmer. Since the active approach test with the unfamiliar person gave similar results, this test was recommended for an on-farm animal welfare monitoring system. For most behavioural elements recorded around feeding, farms differed significantly and inter-observer and test-retest reliabilities were high as well as being significant. The behavioural tests with entering the barn, novel object and unfamiliar person, and the behavioural observations before and after feeding were feasible and distinctive and reliable enough to be performed on-farm. These methods are promising tools to use as a monitor of animal welfare in veal calves.

Introduction

It is generally accepted that an animal welfare monitoring system should be built upon animal-based parameters (EFSA 2006; Smulders et al. 2006). An animal welfare monitoring system will be developed for veal calves as part of the Welfare Quality® EU programme. A veal calf tends to be a bull calf coming from a dairy farm. The diet for veal calves ranges from largely milk replacer and some solid feed (e.g. maize silage, grain, pellets) to milk replacer and solid feed during the first 12 weeks and thereafter only solid feed. Calves are at least two weeks old on arrival at the farm and the fattening period takes 21–35 weeks. Group housing is compulsory in the European Union after eight weeks of age. Group size may vary from 4 to 90 individuals per pen. Veal calves have a minimum space of 1.8 m2 per calf and are generally kept on a (hardwood) slatted floor.

Fear in farm animals is recognised as an important indicator for welfare (Boissy and Bouissou 1995). Fear, however, cannot be assessed by a single measurement (Forkman et al. 2007). Different measurements that gain insight into the level of fear should, therefore, be included in an animal welfare monitoring system. Several behavioural tests and observation methods in, e.g. pigs, dairy cattle, laying hens, and also in (veal) calves have been developed and validated to study the level of fear and its effect on animal welfare (Rushen et al. 1999). Examples of such fear tests are measurement of the behavioural response of a calf to an active approach by a familiar and an unfamiliar person, and measurement of the latency to touch when a calf can voluntarily approach a person (Lensink et al. 2000; 2001; 2003). Another example of investigating fear in calves is looking at the behavioural response to a novel object. Although a number of studies in (veal) calves and cattle suggest that fear of a human may be dissociated from fear of a novel object, novel object tests are widely used to study fear in farm animals (Hemsworth et al. 1996; Jago et al. 1999; Waiblinger et al. 2003; van Reenen et al. 2004; 2005; Graml et al. 2008).

Next to fear, abnormal behaviour is widely accepted as an indicator of poor welfare (Fraser and Broom 1997; Anonymous 2001) while play behaviour is an indicator of good welfare (Fagen 1981; Newberry et al. 1988). Play behaviour of calves has been studied extensively (e.g. Jensen et al. 1998; Jensen and Kyhn 2000) as well as abnormal behaviour. Veal calves typically develop abnormal oral behaviour, comprising the following four behavioural elements: tongue playing, tongue rolling, sham ruminating and persistent biting/sucking on substrates such as bars and troughs (Bokkers and Koene 2001). Although housing system may affect the frequency of abnormal oral behaviour in veal calves (Bokkers and Koene 2001), it has been clearly demonstrated that abnormal oral behaviour evolves to a large extent as a result of a lack of appropriate roughage in the diet (Heeres et al. 2000; van Vuuren et al. 2004). Other abnormal (oral) behaviours in veal calves include cross- sucking and excessive self-licking. Cross sucking, defined as one calf sucking the ear, mouth, scrotum, prepuce, tail, udder area or navel of another calf (Lidfors 1993), is seen most often in young calves that have been separated from their mother. Persistent preputial sucking may adversely affect the prepuce (swelling, irritation, inflammation) of the calf being sucked and the calves that suck may risk poor health and reduced growth due to drinking urine (de Wilt 1985). Self-licking is normal behaviour for a calf, but this can develop to an abnormal, excessive level, especially when a calf is kept in social isolation (Terosky et al. 1997; Bokkers and Koene 2001).

Until now, relatively simple and feasible behavioural measures in veal calves have been sufficiently validated under experimental conditions. Before behavioural tests and observation methods, can be considered for inclusion into an animal welfare monitoring system, however, they have to be studied for feasibility and reliability under commercial conditions. The aim of this study was to investigate the feasibility of different behavioural responsiveness tests and of observation methods to study spontaneous behaviour in veal calves kept under commercial conditions, and to analyse inter-observer reliability and test-retest reliability for different variables. Variables that appear feasible and reliable may be suitable for an on-farm animal welfare monitoring system.

Materials and methods

Twenty-three veal farms were included in this study. These farms varied, intentionally, according to type and origin of calves, group size, size of farm, diet (amount of milk replacer and amount and type of solid feed), climate control, light intensity, and management. They were assumed to represent a cross-section of veal farms in The Netherlands.

Farm visits

Each farm was visited twice to collect data, with a 1–3 day interval between two visits in order to be able to study the test-retest reliability of the observations. Farms were visited either when the calves had been at the farm for 13–15 weeks (11 farms) or two weeks prior to slaughter (12 farms) to be able to study feasibility at different ages. These two age categories were chosen to observe calves at an age whereby abnormal behaviour might have been developed, but also to observe calves that vary in terms of ease of handling. During all visits, data were collected by two observers simultaneously in order to study inter-observer reliability. Four observers (two men and two women) visited the farms in different combinations, but always the same combination of observers within a farm. The observers wore the same overalls as the farmer. Although observers were experienced in behavioural research, they completed a training assessment with videos and photographs of calf behaviour and practised together at a farm beforehand.

Behavioural tests

With the exception of the Unit Entry Test (see below), eight pens per farm were selected for the behavioural responsive- ness tests in such a way that observations could be done without mutual disturbance.

In the Unit Entry Test (UET), the first reaction of calves to the appearance of an unfamiliar person was measured. The test was performed approximately two hours after morning feeding. No one was allowed in the barn one hour prior to testing. A unit of the barn (defined as a part of the farm complex where groups of calves are kept in pens that are oriented to a central feeding corridor; units within a farm building are separated by a solid wall) was entered quietly and the first two pens on the left and right side were observed. The number of calves standing in each pen was recorded. The artificial light was then switched on and the observers entered the unit with a few steps inwards. After one minute, the number of calves standing per pen was registered again. In addition, the total number of calves per pen was recorded. Depending on the number of units, the UET was conducted 8–20 times at a farm.

In the Novel Object Test (NOT), the reaction to an unknown object was measured. The novel object (NO) was a plastic football covered with a grey, plastic bag that was tied up to a stand. The stand was placed in front of a pen. Observers remained outside the pen. The NO was turned into position, which meant dangling just above calf head level and, following this, a three-minute period

of observation began. Latency of every first touch of the NO was recorded for each individual calf. A maximum time of three minutes was recorded for those calves not touching the NO during the test. In the Human Approach Test (HAT), the reaction to a passive, unfamiliar person was tested. One observer leant against the front of the pen, allowing calves to approach voluntarily and touch the observer. Eye contact was avoided. The other observer was standing in front of the pen but could not be touched. The test began when the first observer with a clear but normal voice said “hello, you are looking good today” to catch the attention of the calves. Latency of every first touch was recorded for each individual calf. The maximum time of three minutes was recorded for calves that did not touch the observer.
The Rest Recovery Test (RRT) was conducted to study the effect of a deliberate disturbance at a moment (around 13:00h) when calves are normally resting (Bokkers and Koene 2001). First, the number of calves standing was recorded per pen. Then, the observers forced the calves to stand by entering the pen. The observers left the unit and returned after 30 min to record the number of calves standing in each pen again.

In the Calf Escape Test with an Unfamiliar person (CET_U), the behavioural response of a calf to an active approach by an unfamiliar person was measured. One of the observers entered the pen and waited for one minute to allow the calves to habituate. Next, the observer in the pen chose a calf standing with its head oriented towards the observer at a distance of approximately 1.5 m. In the four- stage test the person: i) made eye contact; ii) took one step towards the calf with one arm outstretched and stood still with two feet next to each other for one second; iii) took a second step and stood still again for one second and iv) touched the calf’s snout. The test was ended whenever the calf moved one of its forelegs backwards. For each successful stage, one point was awarded (0- to 4-point scale), with 0 points for calves unable to make eye contact (maximally three attempts per calf). In large groups, (> 20 calves, three farms) a maximum of 10 randomly selected calves per pen were included in this test.

The Calf Escape Test with a Familiar person (CET_F) was similar to the CET_U except that the test was conducted by a familiar person (the farmer).

Observations of spontaneous behaviour

Behaviour in the home pen was observed in eight randomly selected pens, 20 min before and 20 min after evening feeding. Choosing a fixed moment of the day to conduct these observations meant that results between farms and days could be compared. These observations were conducted only in calves two weeks prior to slaughter (12 farms). Every two minutes, posture and activity of each calf were recorded (instantaneous scan sampling). The ethogram is shown in Table 1.

Table 1
Ethogram for the behavioural observations around feeding (based on de Wilt 1985; Jensen et al. 1998).

Posture	***Definition***
Standing	Standing on three or four straightened legs, without locomotion.
Lying	Lying on brisket with either forelegs or hind legs bent or stretched.
Activity	***Definition***
Walking	The left foreleg and right hind leg are placed forward simultaneously, followed by the right foreleg and left hind leg and so forth.
Running	The left foreleg and right hind leg are placed forward simultaneously, followed by the right foreleg and left hind leg and so forth. This is performed with great vigour and velocity (trotting). Sometimes both fore- and hind legs are placed forward alternately (galloping).
Jumping	Pushing the forequarters upwards with a sudden movement of the fore legs and head, often followed by the kicking of both hind legs backwards. The tail may be lifted and the ears set close against the neck.
Grooming (self-licking)	After extending the tongue, the upper side is shifted along the body while retracting it into the mouth and at the same time moving the head upwards. This sequence is usually repeated.
Nose licking	Moving the tongue over the muzzle, inserting the tip of the tongue in one or alternately in both nostrils and back into the mouth again.
Scratching	After bending the head and neck sideways the claw of the hind leg on the same side is rubbed over head, neck or shoulder repeatedly.
Mounting	Laying the head on the loins, back or withers of a pen mate, jumping upwards with both forelegs, putting them on either side of the head which is simultaneously raised.
Butting	Lowering the head with the muzzle pointing downwards, the forehead is pushed against the front of the head, neck, shoulder or other parts of a pen mate and twisted or moved up and down.
Stretching	Bending the back upwards and then downwards (back stretch) with the tail slightly lifted or bent sideways to the seat bones (tail stretch), the hind legs placed backwards and sometimes lifted separately (leg stretch). The neck may be lifted whiled the muzzle is moved downwards (neck stretch). These stretches may occur simultaneously.
(Sham)Ruminating	After eructation, visible from the stretching of the head forwards and the widening of the throat, the bolus is chewed in a relatively slow and regular fashion and then swallowed. When chewing movements are performed in a relatively fast, irregular fashion and with only a limited range it is assumed to be sham ruminating.
Idle	No visible activity, calf is just looking ahead.
Tongue playing/rolling	Extending the tongue and swaying it sideways, turning and partly rolling and unrolling it. The tongue may also be repeatedly rolled and unrolled inside the open mouth.
Oral manipulation of prepuce or testicles	Sucking at the prepuce or testicles of a pen mate, sometimes resulting in drinking urine. Urine drinking may also occur spontaneously when a pen mate urinates.
Oral manipulation fence/wall	Licking, nibbling, sucking, or biting a fence or a wall
Oral manipulation bucket/trough	Licking, nibbling, sucking, or biting the bucket or the feeding trough
Oral manipulation pen mate	Licking, nibbling, sucking, or biting a pen mate
Oral manipulation floor	Licking, nibbling, sucking, or biting the floor
Other activity	Not performing one of the activities described in this ethogram.
Not visible	Activity is not visible for the observer.

In cases of small groups (< 20 calves per pen), four pens were observed simultaneously. The observers stood next to each other in the feeding corridor. Prior to starting the observation, a 5 min adaptation period was maintained. The observers had 30 s of observation time per pen and they switched to the next pen at the same moment (clockwise). In total, ten scans per pen per observation were recorded. After 20 min, the observers moved to the next four pens to repeat this procedure. After the calves had been fed, behavioural observations began, once again, at the first location. On farms with large groups, two pens were observed (2 × 20 min for each pen at approximately the same time as for small groups).

Testing schedule

The tests were all performed in the same order on each farm to a tight time schedule. A day would start with the UET, followed by the NOT and the HAT, before a one-hour break to allow the calves to lie down. After the break, observations resumed with the RRT followed by the CET_U and the CET_F. At the 12 appropriate farms, the observation day ended with the behavioural observations around feeding.

Statistical analysis

Data were analysed at pen level with the statistical software package, Genstat (2005). Spearman's rank correlations were calculated as a measure for reliability between days and between observers. P-values are given when correlations differed significantly from 0. Correlation coefficients were considered low when below 0.4, moderate when 0.4 to 0.7, high when 0.7 to 0.9, and very high when 0.9 and above (Martin and Bateson 1993). Inter-observer reliabilities were analysed for the three pairs of observers. Overall, inter- observer reliabilities were analysed with Kendall's tau. Test-retest reliability was analysed at pen level and at farm level.

In large groups, not all the calves could reach the novel object or the human within the three-minute duration of the test. As a result of this, a relatively large number of calves showed a maximum latency to touch of three minutes. In order to gain a robust variable for the NOT and the HAT, which is not affected by the unwanted effects of a large group or of a single calf (many pens have at least one curious calf), the average latency to touch of the first 5 calves was analysed. For the CET_U and the CET_F, the average score per pen was analysed. For the analysis of the UET, the number of calves standing at the start of the test was subtracted from the number of calves standing after one minute. A similar correction was performed for the number of calves standing after 30 min in the RRT. Spearman's rank correlations were also calculated between the different behavioural tests. Furthermore, Spearman's rank correlation of behavioural test data were calculated using residuals of an analysis of variance model with age (at the farm for 13–15 weeks) as a fixed effect. With the exception of lying idle, standing idle and walking, behaviour is expressed without distinguishing between lying and standing. Play behaviour is the sum of running, jumping, mounting, and butting behaviour. Comfort behaviour is the sum of stretching, scratching, nose licking and self-licking behaviour. Farm effects for all variables were analysed with the Kruskal-Wallis test.

Results

At all 23 farms, the UET, NOT, RRT and CET_U were conducted. At one farm, the HAT was not conducted due to practical constraints. Twenty out of 23 farmers were willing to perform the CET_F.

With the exception of the RRT on the second day of observation, a significant farm effect was found for all behavioural tests (Table 2). Although calves seemed to respond differently on the second day, no day effects were found for the behavioural tests. Inter-observer correlations for the behavioural tests were found to be very high and significant (Table 3). Test-retest correlations within pen and within farm were significant for all behavioural tests, except for the RRT (Table 4). Test-retest correlation coefficients were higher at farm level than at pen level, except for the RRT.

Table 2
Mean (± SD) results of behavioural tests and farm effect per behavioural test per observation day.

Test	Variable	Day	Mean (± SD)	df	Farm effect
UET	% standing after 1 min	1	36.2 (± 29.5)	22	P < 0.001
		2	31.6 (± 29.2)	22	P < 0.001
NOT	Latency to touch NO (s)	1	135.0 (± 33.0)	22	P < 0.001
		2	108.5 (± 38.2)	22	P < 0.001
HAT	Latency to touch humans (s)	1	110.2 (± 39.6)	21	P < 0.001
		2	96.1 (± 45.5)	21	P < 0.001
RRT	% standing after 30 min	1	5.9 (± 20.1)	21	P < 0.05
		2	3.4 (± 22.6)	21	ns
CET_U	Average pen score	1	1.7 (± 0.58)	22	P < 0.001
		2	1.8 (± 0.60)	22	P < 0.001
CET_F	Average pen score	1	1.6 (± 0.58)	19	P < 0.001
		2	1.7 (± 0.64)	19	P < 0.001

Table 3
Inter-observer reliability per behavioural test over two observation days.

Test	Variable	Observer			
		1 and 2***	1 and 3***	1 and 4***	Kendall***
UET	% standing after 1 min	0.93 (n = 255)	0.98 (n = 506)	0.96 (n = 266)	0.80
NOT	Latency to touch NO (s)	0.99 (n = 104)	0.99 (n = 128)	0.99 (n = 112)	0.80
HAT	Latency to touch humans (s)	0.99 (n = 96)	0.99 (n = 128)	0.99 (n = 112)	0.81
RRT	% standing after 30 min	0.92 (n = 104)	0.96 (n = 128)	0.96 (n = 102)	0.76
CET_U	Average pen score	0.97 (n = 104)	0.96 (n = 128)	0.97 (n = 112)	0.82
CET_F	Average pen score	0.93 (n = 104)	0.91 (n = 120)	0.89 (n = 68)	0.82

Spearman rank correlation coefficients are given for the pairwise comparison and Kendall's coefficient of concordance for the overall comparison. *** P < 0.001 for all correlations.

Table 4
Reliability between days within pen and within farm per behavioural test.

Test	Variable	Days within pen	Days within farm
UET	% standing after 1 min	0.26*** (n = 516)	0.63*** (n = 23)
NOT	Latency to touch NO (s)	0.70*** (n = 180)	0.88*** (n = 23)
HAT	Latency to touch humans (s)	0.74*** (n = 176)	0.83*** (n = 22)
RRT	% standing after 30 min	0.14 (n = 172)	0.05 (n = 22)
CET_U	Average pen score	0.69*** (n = 180)	0.94*** (n = 23)
CET_F	Average pen score	0.68*** (n = 150)	0.93*** (n = 20)

*** P < 0.001 for all correlations.

On both days, a highly significant positive correlation was found between NOT and HAT, and between CET_U and CET_F (Table 5). A significantly positive moderate correlation was found between the NOT and the HAT and the UET for the first, but not for the second observation day. Both NOT and HAT were negatively correlated with the CET_U and the CET_F on both days. On farms where calves were reluctant to make contact with the human or the novel object (high average contact latencies), these calves were also difficult to approach by a human (low average response scores).

Table 5
Spearman correlation coefficients between behavioural tests at farm level.

Test		NOT	HAT	RRT	CET_U	CET_F
Day 1						
	UET	0.54*	0.52*	0.02	–0.26	–0.07
	NOT		0.92**	–0.03	–0.81**	–0.64**
	HAT			–0.20	–0.74**	–0.54**
	RRT				–0.11	0.09
	CET_U					0.84**
Day 2						
	UET	0.29	0.07	–0.25	–0.12	0.11
	NOT		0.73**	–0.34	–0.68**	–0.50*
	HAT			–0.38	–0.66**	–0.48*
	RRT				0.25	–0.13
	CET_U					0.80**

* P < 0.05 ; ** P < 0.01

Correlations of behavioural test measures were not affected or only marginally so, when residuals after correction for age effects were used (results not shown). This means that test-retest reliabilities obtained in the present study were not as a result of consistent differences between ages.

For several behavioural elements, a significant farm effect was found before and after feeding (Table 6). Significance and strength of test-retest correlations differed per behavioural element and also occasionally before and after feeding (Table 7). Inter-observer correlations were generally high and significant for the different behavioural elements, with the exception of the correlation between observer 1 and 4 for the behaviour, manipulating floor (Table 8).

Table 6
Mean (± SD) percentages of behaviour around feeding and farm effect per behavioural element.

Behaviour	**Day**	**Mean (± SD)**	**Farm effect Before feeding**	**Mean (± SD)**	**Farm effect After feeding**
Lying idle	1	6.2 (± 8.2)	0.001	7.2 (± 12.3)	0.001
	2	7.4 (± 9.9)	0.007	11.4 (± 15.3)	0.001
Standing idle	1	39.3 (± 13.0)	0.001	43.7 (± 21.5)	0.001
	2	29.6(± 12.4)	0.001	39.0 (± 20.4)	0.001
Tongue	1	0.6 (± 1.0)	0.034	0.6 (± 1.2)	0.746
rolling/playing	2	0.9 (± 1.9)	0.001	0.5 (± 0.9)	0.602
Manipulating fence	1	4.9 (± 4.1)	0.002	2.8 (± 3.0)	0.213
	2	6.4 (± 5.5)	0.001	3.6 (± 4.3)	0.027
Manipulating	1	10.1 (± 9.6)	0.001	5.9 (± 7.7)	0.001
feeder	2	12.1 (± 10.0)	0.005	6.6 (± 7.9)	0.002
Manipulating	1	3.6 (± 3.5)	0.001	2.5 (± 2.7)	0.001
pen mate	2	4.3 (± 3.9)	0.001	2.3 (± 2.1)	0.105
Manipulating floor	1	0.6 (± 1.1)	0.069	0.7 (± 1.4)	0.865
	2	1.1 (± 2.0)	0.001	0.7 (± 1.0)	0.243
Play behaviour	1	1.5 (± 2.4)	0.268	0.6 (± 0.9)	0.097
	2	1.8 (± 2.3)	0.138	0.7 (± 1.2)	0.431
(Sham) Ruminating	1	4.3 (± 6.0)	0.001	2.9 (± 3.9)	0.001
	2	6.1 (± 8.6)	0.001	3.0 (± 4.2)	0.002
Confort behaviour	1	8.1(± 2.8)	0.174	5.8 (± 3.2)	0.016
	2	8.2 (± 4.0)	0.004	5.6 (± 3.4)	0.154
Self licking[1]	1	3.0 (± 2.6)	0.153	2.8 (± 2.3)	0.114
	2	3.7 (± 2.7)	0.109	2.7 (± 2.4)	0.009
Walking	1	2.8 (± 2.7)	0.011	2.5 (± 2.2)	0.737
	2	2.6 (± 2.2)	0.090	2.0 (± 1.8)	0.485

[1] Self licking is also included in comfort behaviour.

Table 7
Reliability of behavioural observations around feeding between days at pen level and at farm level.

Behaviour	*Before feeding* Days within pen (n=90)	*Before feeding* Days within farm (n=12)	*After feeding* Days within pen (n=90)	*After feeding* Days within farm (n=12)
Lying idle	0.63***	0.34	0.36**	0.67*
Standing idle	0.37***	0.60*	0.57***	0.76**
Tongue rolling/playing	0.41*	0.73**	0.41	0.36
Manipulating fence	0.56***	0.92***	0.26*	–0.02
Manipulating feeder	0.55***	0.73**	0.66***	0.79**
Manipulating pen mate	0.43***	0.64*	0.37***	0.36
Manipulating floor	0.32	0.63*	0.33	0.36
Play behaviour	0.29*	0.33	0.34	0.22
(Sham) Ruminating	0.33**	0.60*	0.39***	0.75**
Confort behaviour	0.30**	0.50	0.45***	0.82**
Self licking[1]	0.36***	0.67*	0.35**	0.86***
Walking	0.19	0.55	0.26*	0.64*

[1]Self licking is also included in comfort behaviour. * P < 0.05; ** P < 0.01; ***P < 0.001.

Table 8
Inter-observer reliability (observers 1, 2, 3 and 4) for the behaviour around feeding (n = 64).

	Combination of observers							
	Before feeding				*After feeding*			
Behaviour	1 and 2	1 and 3	1 and 4	Kendall	1 and 2	1 and 3	1 and 4	Kendall
Lying idle	0.99*	0.98*	0.96*	0.73*	0.99*	0.94*	0.98*	0.73*
Standing idle	0.89*	0.87*	0.83*	0.75*	0.96*	0.92*	0.92*	0.76*
Tongue rolling/playing	0.75*	0.83*	0.94*	0.75*	0.68*	0.66*	0.89*	0.71*
Manipulating fence	0.64*	0.72*	0.75*	0.73*	0.64*	0.70*	0.74*	0.71*
Manipulating feeder	0.90*	0.89*	0.95*	0.76*	0.88*	0.86*	0.94*	0.74*
Manipulating pen mate	0.75*	0.65*	0.36*	0.71*	0.67*	0.67*	0.57*	0.71*
Manipulating floor	0.68*	0.58*	0.88*	0.63*	0.54*	0.73*	0.13	0.59*
Play behaviour	0.50*	0.76*	0.75*	0.66*	0.53*	0.46*	0.49*	0.56*
(Sham) Ruminating	0.79*	0.53*	0.86*	0.71*	0.54*	0.25*	0.57*	0.66*
Confort behaviour	0.47*	0.63*	0.53*	0.70*	0.75*	0.71*	0.71*	0.73*
Self licking[1]	0.63*	0.69*	0.64*	0.72*	0.73*	0.68*	0.61*	0.72*
Walking	0.40*	0.48*	0.59*	0.68*	0.38*	0.43*	0.65*	0.67*

Spearman rank correlation coefficients are given for the pair-wise comparisons and Kendall's coefficient of concordance for the overall comparison. [1] Self licking is also included in comfort behaviour. * P < 0.05; ** P < 0.01; ***P < 0.001.

Discussion

The results of this study showed that inter-observer reliability was high and significant for all behavioural tests. Thus, the observation methods were clear and feasible, although there is a need for experienced, well-trained individuals to conduct observations. Test-retest reliability was high and significant for all behavioural tests with the exception of the RRT. For the majority of behavioural tests, there were significant farm effects, which indicate that the behavioural observations used could detect differences between farms. This is important when these tests are utilised in order to monitor animal welfare. In this study, however, we did not aim for determining factors causing farm differences for different variables. Test-retest reliability of the behavioural tests was higher at farm level than at pen level which again is relevant for a monitoring system that should assess the level of welfare at farm level. The second visit to a farm, to conduct the retest observations, was within a few days in order to exclude age effects. This increased the risk of habituation to the behavioural tests or calves lowering their threshold to express fear at the second observation day (Forkman et al. 2007). But no such effect was found in our study.

In general, it was feasible to perform all behavioural tests at commercial farms. Nevertheless, some practical constraints need to be discussed.

The UET could be performed several times in barns that were divided into a number of separate units. In some barns, however, units within a barn were not separated by a solid wall. Therefore, calves can easily disturb one another. Furthermore, it was difficult to control whether anyone had been in the barn one hour prior to the test. This may be a reason for the low reliability for the UET between days within pen.

Occasional, minor practical constraints were seen in the NOT. Sometimes it was difficult to move the novel object between pens, e.g. when the ceiling was low, or when milk, water or gas pipes were suspended over the feeding corridor just above head level.

One practical constraint for the HAT was that on occasion the front of a pen differed not only between farms but also within them. It was easy for calves to touch a part of the observer when the front of a pen was made up only of a feeding trough and one bar. Touching the observer became more difficult when the front of a pen was a fence with grating. The effect of this constraint could not be tested in the present study.

The correlations found between NOT and HAT suggest that they evoke similar behavioural responses in calves. It would be attractive therefore to choose one of the tests to include in an animal welfare monitoring system. However, it remains unclear whether the response to a human and to a novel object has the same underlying cause (Andersen et al. 2000; Grignard et al. 2001; van Reenen et al. 2004). For both NOT and HAT we analysed the latency to touch of the first 5 calves to have a variable with a low within-farm bias. This variable, however, is subjected to different dynamics in small and large groups. Assume, for example, 10% of the calves are not fearful of a novel object or of the human. In a pen of ten calves, it may be expected that one calf will touch the novel object or the human, while in a group of 40 calves, four individuals may be expected to touch the novel object or the human. When taking the first 5 calves for analysis, this may affect the average latency. In large groups, however, distance to the novel object or the human may be relatively large due to pen size. Calves may ignore the novel object or the human because they are busy with other activities in the rear of the pen, or they are neither attracted nor fearful of the novel object or the human at such a distance. This would introduce bias when analysing latencies of all calves. Furthermore, in small pens it may be that a fearful calf touches the novel object or the human just because it happens to be standing close to it, which may also affect average latency. As a contrast to the 10% assumption, we can assume that 50% of the calves are not fearful. Here, there is a risk of overestimating fearfulness in a large group. It is highly unlikely that twenty calves will touch the novel object or the human due to limited space around the novel object and in front of the human or as a result of calves simply not being attracted to the novel object or the human. In a group of ten calves, however, it is quite easy for 5 calves to approach the novel object or the human. Thus, although the analysis of latencies of the first 5 calves for the NOT and the HAT has advantages and disadvantages, it is a robust and useful variable to measure reliability of observations.

Both the CET_F and the CET_U had significant interobserver and test-retest reliability which corresponds with the results of Lensink et al. (2003). Although the CET_F had a high reliability, the test had some practical disadvantages, which made this test less feasible. In particular, the farmer had to be persuaded to co-operate. Moreover, although the farmers were provided with instructions beforehand, each performed the test slightly differently. Such variation may attenuate the level of standardisation of a test. Nevertheless, in our study, the scores obtained by the farmer (CET_F) were significantly correlated with those obtained by a trained and experienced observer (CET_U), as found earlier for calves housed individually or in pairs (Lensink et al. 2000; 2001). This indicates that our test was robust. These results also support the idea that veal calves generalised their response across a familiar and an unfamiliar person. This does not necessarily mean that calves are unable to discriminate between people, i.e. under certain conditions, calves were capable of this (Arave et al. 1992; de Passillé et al. 1996). In veal production, a generalised response across different people may be the result of the low intensity of contact between calves and humans under commercial conditions. The farmer is present in the barn twice a day to feed and inspect the calves and there are occasional extra visits to move or to treat calves. Human-animal interactions around feeding may be associated with positive experiences by calves, while other types of interaction may well be associated with negative experiences (e.g. certain veterinary procedures). It remains clear that unravelling discrimination and generalisation is complex (Rushen et al. 1999).

Both the CET_U and the CET_F had practical constraints when calves were two weeks from slaughter. At this stage, the calves were heavy (250–350 kg) and little space remains for moving around in pens. The safety of the individual entering the pen and interacting with calves could not be guaranteed: as such, this test should not be performed with calves at this age and weight.

On the one hand, the negative correlations between the average latencies to contact with the human (HAT) and the novel object (NOT) and, on the other, the average score during the CET_U and CET _F are suggestive that the responsiveness of veal calves to these tests is mediated partly by the same underlying motivational state, possibly fear.

The results of the behavioural observations around feeding are a little more ambiguous. Most inter-observer and test-retest reliabilities were significant and correlation coefficients were higher at farm level than at pen level. Again, this is relevant for the development of an animal welfare monitoring system aimed at estimating animal-based parameters at farm level. Due to the tight time schedule of an observation day, a habituation period of 5 min prior to starting an observation was achievable, maximally. Indeed, calves were not adapted completely to humans standing in front of their pen by then, but all observations were conducted according to the same procedure and therefore assumed to be comparable.

Most inter-observer reliabilities for behaviour around feeding were significant. The procedure for these observations was designed in such a way that two observers could make behavioural recordings without speaking to each other. There was 30 s per pen for recording in small groups and 120 s for large groups. In that time, observers had to record for each individual calf, the behaviour observed. Recordings, therefore, were not conducted exactly simultaneously.

Hence, differences between observers could occur, especially for behavioural elements of short duration. Nevertheless, the procedure for these observations was robust enough to be reliable. The present study was conducted with the aim of examining the reliability of observation methods, and not to identify factors that may affect behaviour. A larger scale study would be needed in order to address this latter aim.

Conclusion and animal welfare implications

Inter-observer reliability was high for behavioural tests and observations around feeding. Test-retest reliability was high for all behavioural tests with the exception of the RRT. The RRT also had a low distinctive power between farms indicating that the value of this measurement is low to detect differences between farms. The RRT is an unreliable test and, therefore, unsuitable as a monitor of animal welfare. For the other behavioural tests, variation between farms was significant. Although inter-observer and test-retest reliability of the CET_F was high, this test was not feasible in practice due to an over-reliance on the farmer. The CET_U was feasible and gave similar results as the CET_F. Therefore, we would advocate against the use of the CET_F in an animal welfare monitoring system. A constraint for the CET_U is that it requires to be performed at an age when interaction with calves in a relatively small enclosure is safe. All the other behavioural tests and the observations around feeding were feasible to perform on-farm, although certain minor practical constraints did exist. It can be concluded that the UET, NOT, HAT, CET_U and the observation method for spontaneous behaviour are all reliable tools to be utilised in an animal welfare monitoring system. Assessing animal welfare with reliable tools is a must as important decisions may ultimately be made on the strength of these; this has implications for both farmers and animals.

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CHAPTER 3

Evaluation of on-farm veal calves' responses to unfamiliar humans and potential influencing factors

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Evaluation of on-farm veal calves' responses to unfamiliar humans and potential influencing factors

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Abstract

The human–animal relationship is an important component of the welfare of farm animals and for this reason animal responsiveness tests to humans are included in on-farm welfare assessment schemes that provide indicators for this. However, apart from the behaviour of stockpersons towards their animals, other factors may also influence animals' reactivity to humans as observed through behavioural tests, which can add a further layer of complexity to the interpretation of test results. Knowledge of these factors may help a better interpretation of differences from one farm to another in the outcome of human–animal relationship tests, and may provide clues for improving the relationship between animals and humans. The main objective of this study was to identify whether management or environmental factors could influence the outcome of human–animal relationship tests in veal calves. Two tests were performed when calves were aged 14.9 ± 1.6 (SD) weeks in 148 veal farms: the voluntary approach of an unfamiliar human standing at the feeding fence and the reaction towards an unfamiliar human who entered the home pen and tried to touch each calf in a standardised way (Calf Escape Test (CET) – score 0 to 4). Questionnaires were filled in and interviews with the stockpersons were performed in order to obtain information on stockpersons, management, animal and building characteristics. The latency to touch an unfamiliar human at the feeding fence was significantly correlated with the CET scores. Total number of calves on the farm, space allowance, breed, environmental enrichment, stockperson's experience and season of observation influenced the percentage of calves that scored 0 in CET (i.e. calves that could not be approached). Type of milk distribution, type of breed and number of calves per stockperson influenced the percentage of calves that scored 4 in CET (i.e. calves could be touched). For both CET0 and CET4, the level of self-reported contacts by the stockperson (analysed only on the French subset of 36 farms) did not influence the results. This paper concludes that according to the tests conducted on veal calves on commercial farms, factors such as milk distribution method, breed of the calves or the level of experience of stockpersons with veal farming can have an impact on the results of tests focusing on human–animal relationships.

INTRODUCTION

The relationship between stockpersons and breeding stock is an important component of the welfare of farm animals (Hemsworth et al. 1993). Several studies have shown that behaviour of the stockperson has an impact on both welfare and productivity of animals (pigs: Hemsworth and Barnett 1991; poultry: Barnett et al. 1994; dairy cattle: Rushen et al. 1999; veal calves: Lensink et al. 2001). Therefore, potential indicators of the relationship between stockpersons and animals are included in on-farm welfare assessment schemes (e.g. Botreau et al. 2007). Observations used for integration in these schemes should be easy and quick to perform, be repeatable, valid and ideally not be influenced by external factors such as the test person or the test location (Martin and Bateson 1993; de Passillé and Rushen 2005).

In on-farm conditions, the human–animal relationship is often assessed through the response of animals to human presence or to physical contact by humans during feeding (e.g. veal calves: Lensink et al. 2000b; dairy cows: Waiblinger et al. 2003), at the feeding place (e.g. veal calves: Bokkers et al. 2009; pigs: Lensink et al. 2009) or in the home pen (e.g. dairy cows: Windschnurer et al. 2008; veal calves: Bokkers et al. 2009). The response of farm animals to the presence of an unfamiliar experimenter who remains stationary has been observed in a special test arena (e.g. dairy cows: Hemsworth et al. 2000; sheep: Tallet et al. 2006). A number of reports support that these farm tests can give an indication of the level of avoidance of the tested animals (for review: Waiblinger et al. 2006). In production systems animals are in contact daily with the stockperson, but also with other people such as veterinarians on a regular basis. Fear of humans might lead to, for instance increased cortisol responses or reduced productivity (for review: Hemsworth and Coleman 2010), which are indicators of low welfare.

For veal calves housed in groups, Bokkers et al. (2009) developed several tests focusing on the human–animal relationship. For two of these tests, the Human Approach Test (HAT) and the Calf Escape Test (CET), the inter-observer and the test–retest reliability were good. Therefore, an on-farm animal welfare assessment scheme for veal calves could consider including these tests. The calves' responses to a familiar or unfamiliar stockperson were highly correlated, suggesting a generalisation of the calves' responses to humans. Responses of veal calves in these types of tests were shown to be reflecting the stockperson's behaviour towards the animals in both experimental and on-farm conditions (Lensink et al. 2000a; 2000b). In addition to the behaviour of the stockperson, other factors may influence responses of farm animals, including veal calves, in tests focusing on the human–animal relationship. Knowledge of these factors may help to explain differences between farms in the outcome of tests focusing on the relationship between animals and stockpersons, and may provide clues for improving farm parameters that directly affect this relationship. Few studies have focused on the human–animal relationship under farm conditions. In dairy cattle, the avoidance distance of cows is correlated with the quality and quantity of daily contacts with the milker and, to a lesser extent, with factors related to animals, management and housing (Waiblinger et al. 2003). In a previous comprehensive study on veal calves housed individually, factors such as attitude towards calves, years of experience with calves and the number of stockpersons working on the farm were linked with calves' responses to humans, next to stockperson's behaviour towards calves (Lensink et al. 2000a). According to current regulations in the European Union, it is compulsory to keep calves in group housing from the age of 8 weeks onwards. Group housing can make handling of calves more difficult, therefore the effect of group size on human-animal interactions must be examined further (Raussi 2003). In studies on (veal and dairy) calves mainly dairy breeds have been studied so far (Lensink et al. 2000a and 2000b; Rousing et al. 2005; Bokkers et al. 2009). In beef cattle, clear breed differences were found in terms of temperament and reactivity to humans (Murphey et al. 1981; Boivin et al. 1992; 1994). Similar differences may therefore also apply between veal calves of dairy and beef breeds.

The objective of the present study was to investigate behavioural responses of veal calves to humans on livestock farms and to determine potential influencing factors other than the stockperson's behaviour. Therefore, at all farms, calves were observed during two behavioural response tests as described by Bokkers et al. (2009), data regarding housing conditions and management of calves were collected and stockpersons were interviewed. An overall analysis of the potential influencing factors for calves' behaviour was performed on the data.

Material and methods

Farm sample

Data were collected between summer 2007 and spring 2009 on 148 veal farms in the Netherlands (n = 88), France (n = 36) and Italy (n = 24). Farms were selected so as to make up a representative cross-section of the veal production in Europe. Calves were housed in small groups of 5 to 15 calves and fed milk replacer and solid feed by a bucket (45) or a trough (103). The sample within each country consisted of farms located in the main regions where veal calves are reared. All farmers took part in this study on a voluntary basis. A single batch of calves was considered for each farm, and the tested batches were distributed across all four seasons. Calves arrived at the farms at the age of ~15 days and were slaughtered at the age of 17 to 33 weeks. Farms showed a variety in type and origin of calves, size of the farm, feeding plan (amount of milk replacer and amount and type of solid feed), climate control, day light intensity and general management.

Behavioural tests

Two behavioural tests were carried out on all farms when the calves were aged 14.9 ± 1.6 (SD) weeks: the HAT and CET previously described by Bokkers et al. (2009).

HAT: the behavioural response of calves to a passive unfamiliar person (experimenter) was measured. An unfamiliar experimenter approached the pen to be tested and stood still in the middle at the front of the pen. The experimenter was leaning against the fence with his/her elbow on top of the fence allowing the calves to voluntarily approach and touch the person. Eye contact was avoided. The test started when the experimenter with a clear but normal voice said a sentence to catch the attention of the calves. All calves could simultaneously approach and touch the experimenter; the number of calves standing was noted and the latency to every first touch was recorded for each individual calf. A maximum time allowance of 3 min was recorded for calves that did not touch the experimenter.

CET: the behavioural response of a calf to an active approach of an unfamiliar person (experimenter) was measured. The experimenter entered the pen and waited for 1 min to let the calves get used to his/her presence. Next, the experimenter standing erect in the pen chose a calf standing with its head oriented towards the experimenter at ~1.5 m distance. The test consisted of four stages: (1) the experimenter can only make eye contact with the calf; (2) the experimenter can make one step towards the calf with one arm stretched forward and stand still with two feet next to each other for 1 s; (3) the experimenter can make a second step and stands still again for 1 s; and (4) the experimenter can touch the calf's muzzle. The test was ended whenever the calf moved one of its forelegs backwards. For each successful stage, one point was given (0- to 4-point scale), with 0 points for calves unable to make eye contact with and with a maximum of three attempts per calf.

In total, 5 experimenters (two men, three women; one experimenter per farm) performed the observations on the farms. They were wearing similar (dark blue coloured) clothing at all farms. Although the experimenters were experienced in behavioural research, they completed a training assessment with videos and photos of calf behaviour and practised together at a farm beforehand. Ten pens per farm were observed for HAT and 20 pens per farm for CET (including the 10 pens observed for HAT). On all farms, the HAT was performed between 10:00h and 12:00h, whereas the CET was performed between 14:00h and 16:00h.

Farm data

At all farms, information on characteristics of the building and equipment was collected by the experimenter using a pre-defined questionnaire with questions concerning, for example, type of milk distribution system within the farm, number of calves, space allowance per calf, prevailing breed of the batch, etc. Stockpersons were asked before the behavioural observations about their management practices (such as number of years of experience with calves, daily time spent in the building, frequency of visits by a technician, etc.). These data were used to build a list of potential risk factors (Table 1).

Stockperson's behaviour

For practical reasons, it was not possible to observe the stockperson's behaviour towards the calves. For the French farms (n = 36) included in this study, an additional questionnaire was filled in by the stockpersons with 6 questions aiming to obtain a description of their interactions with their calves. The questions were asked in the form of 'How often do you x your calves?', where x was either 'touch', 'pet ', 'talk', 'let them suck your fingers', 'slap with your hands' or 'kick'. Answers were given on a 7-point scale with 1 = never, 4 = sometimes and 7 = very often. This data were then grouped into two variables. First, the farmer's answers about the four forms of positive contact ('touch', 'pet', 'talk' and 'let them suck your fingers') were summed up into a score of self-reported level of positive contacts (with possible score ranging from 4 to 28 points). Second, the farmer's answers about the two types of negative contacts ('slap with your hands' and 'kick') were summed up into a score of self- reported level of negative contacts (with possible score ranging from 2 to 14 points). These scores were added to the list of potential risk factors (Table 1).

Table 1
List of the analysed potential factors influencing the human-animal relationship in veal calves

Item	***Average ± SE (range) or proportions***	***Classes and unit***
Milk distribution system	Bucket: 30% Trough: 70%	Bucket feeding or trough feeding
Farm size	800 ± 58 (128 to 5800)	Total number of calves: ≤300, 300<x≤600, 600<x≤1200, >1200
Space allowance	1.8 ± 0.2 m² (1.8 to 2.4)	=1.8 or >1.8 m²/calf
Season of observation	Spring: 20% Summer: 35% Autumn: 30% Winter: 15%	Spring, summer, autumn, winter
Prevalent breed	Holstein or milk type : 70% Dual breed : 15% Crossbred or meat breed: 15%	More than 50% of calves are of: Holstein or milk type, dual purpose breed, crossbred or meat breed type
Frequency of visits by technician	Weekly: 88% Every 2 weeks: 12%	Weekly, every 2 weeks
Frequency of visits by stockperson/day	2.4 ± 0.1 (1 to 3)	Number of time one of the stockpersons visits the calves' building each day: ≤2, >2
Stockpersons' experience	18.6 ± 1.0 year (1 to 45)	≤5, 5<x≤15, 15<x≤25, >25 years
Adoption of the present housing system	7.5 ± 0.4 year (1 to 26)	≤2, 2<x≤10, >10 years
Environmental enrichment	No: 85% /Yes: 15%	Presence of hanging objects in the pens: Yes/no
Number of calves per stockperson	500 ± 26 (32 to 1540)	Number of calves/ number of stockpersons: ≤200, 201<x≤400, 401<x≤600, 601<x≤800, >800 calves per stockperson
Gender of stockperson	Man : 44% Woman : 32% Both: 24%	Man / woman / both a man and a woman working at the farm
Duration visit / calf	1.0 ± 0.1 min (6 to 25)	Average daily time spent in the building by the farmer per calf (daily time spent in the building (min) / number of calves) : <1 min per calf / >1 min per calf
Self-reported positive contacts[1]	17.1 ± 0.8 (6 to 25)	Score: <14 / 14-18 / 18-21 / >21
Self-reported negative contacts[1]	4.0 ± 0.4 (2 to 8)	Score: <2 / 2-4 / 4-6 / >6

[1]Only for French data set (n = 36)

Statistical analysis

Data were analysed by using GenStat software (GenStat Committee 2000), which takes each farm as a statistical unit. Spearman rank correlations were calculated between the behavioural tests. For the CET test, variables used for the analyses were the percentage of calves per farm with a score 0 (CET0) and 4 (CET4) as these variables represent extreme responses of calves to experimenters. For the HAT, mean latency of the first 5 calves (HATlat) was used for analysis. When means are indicated, standard errors are given.

Two series of risk factor analyses were performed with response variables CET0, CET4 and HATlat. The generalised linear model comprised a logit link function and a binomial variance function with an additional multiplicative dispersion parameter. Estimation was by maximum quasi-likelihood. The multiplicative dispersion parameter was estimated from Pearson's X^2 statistics. Significance tests were based on the Wald test. Details may be found in McCullagh and Nelder (1989). For the first series of analyses, explanatory variables were the potential risk factors obtained from the questionnaire (listed in Table 1). For the second series of analyses only performed on the French data, the same explanatory variables were used, together with the farmers' self-reported contacts with the calves. All explanatory variables were expressed in the form of factors. Factor levels were defined such that each level corresponded to a sizeable number of farms in the sample. First, potential risk factors were inspected one at a time. Risk factors with a significance level below 0.10 ($P < 0.10$) were retained for further study. Second, the remaining risk factors were jointly entered into the model. Further analyses comprised variable selections by stepwise backward and forward selection. For all potential risk factors that were selected either by backward or forward selection, best subset selection was performed and significance tests for the selected risk factors were evaluated. Only main effects were considered, avoiding multicollinearity problems. Selection was based on adjusted R2, and final significance ($P < 0.05$) of potential risk factors. For each risk factor that was finally selected, odds ratios and associated 95% confidence intervals were obtained from the final model. Results are presented in such a way that level I is compared with level II. Odds ratios >1 with a t-value level <0.05 indicate a significant risk factor.

Results

General results

For the HAT, the mean latency for touching the experimenter (HATlat) was 113.4 ± 2.0 s (range 52.8 to 172.0 s). For the CET, the average score (CETaverage) was 1.7 ± 0.1 (range 1.0 to 2.8), with average percentages of 5.8% ± 0.7% of calves per farm that could not be approached (CET0; range 0.0% to 38.1%), 54.4% ± 1.4% of calves per farm with score 1 (range 18.4% to 84.5%), 21.5% ± 0.6% of calves per farm with a score 2 (range 3.6% to 41.7%), 4.5% ± 0.4% of calves per farm with score 3 (range 0.0% to 22.4%) and 13.9% ± 0.8% of calves with score 4 (CET4;range 0.9% to 49.3%).

The mean HATlat was positively correlated with CET0 ($n = 148$; $r_s = 0.36$; $P < 0.01$) and negatively correlated with CET4 ($n = 148$; $r_s = 20.38$; $P < 0.01$).

Risk factor analyses

The multiple regression analysis showed that none of the variables studied were significantly linked with the mean latency of the calves to be touched in the HAT.

Total number of calves at the farm, space allowance per calf, environmental enrichment, season of observation, breed and stockperson's experience were found to influence significantly (all $P < 0.05$) the percentage of calves that were scored 0 during CET (Table 2). The variables accounted for 37.89% of the variance. For all the class comparisons (except for farm size <300 calves compared with 300 to 600 calves), a larger farm size was associated with a higher risk of finding a higher proportion of calves with CET0. A space allowance of 1.8 m2 per calf and the absence of environmental enrichment (hanging objects) in the calves' pens were also associated with higher risks of observing a higher percentage of CET0 on the farm. Observations performed during autumn or winter led to a higher risk of observing a higher percentage of CET0 on the farm when compared with summer or spring observations. Herds with mainly milk type calves when compared with dual-breed calves and crossbred calves had higher percentages of CET0. The more experience a farmer had with veal calves, the lower was the risk of finding increased percentages of calves with CET0.

Table 2
Multivariate regression model for the percentage of calves scored 0 (could not be approached; n = 138)

Risk factor[1,2]	**Level of comparison I**	**Level of comparison II**	**OR**	**95% confidence interval**	**t-value of pairwise comparison**
Total number of calves on the farm	< 300	300 to 600	0.95	0.48 to 1.47	0.889
	< 300	600 to 1200	0.59	0.32 to 1.09	0.094
	< 300	> 1200	0.32	0.16 to 0.64	0.002
	300 to 600	600 to 1200	0.62	0.39 to 0.98	0.044
	300 to 600	> 1200	0.34	0.20 to 0.56	0.000
	600 to 1200	> 1200	0.55	0.34 to 0.87	0.011
Space allowance	1.8 m^2/calf	> 1.8 m^2/calf	2.12	1.30 to 3.45	0.003
Enrichment	No	Yes	2.11	1.16 to 3.82	0.015
Season of observation	Summer	Autumn	0.34	0.19 to 0.62	0.000
	Summer	Winter	0.54	0.30 to 0.99	0.048
	Summer	Spring	1.31	0.58 to 2.94	0.519
	Autumn	Winter	1.58	1.04 to 2.39	0.034
	Autumn	Spring	3.80	1.89 to 7.65	0.000
	Winter	Spring	2.41	1.21 to 4.80	0.014
Type of breed	Dairy breed	Dual breed	2.01	1.11 to 3.64	0.023
	Dairy breed	Crossbred	1.65	0.83 to 3.27	0.155
	Dual breed	Crossbred	0.82	0.34 to 1.99	0.662
Stockpersons' experience	< 5 years	5 to 15 years	1.11	0.53 to 2.33	0.777
	< 5 years	15 to 25 years	0.65	0.30 to 1.40	0.271
	< 5 years	> 25 years	0.44	0.21 to 0.92	0.030
	5 to 15 years	15 to 25 years	0.58	0.35 to 0.96	0.036
	5 to 15 years	> 25 years	0.40	0.25 to 0.62	0.000
	15 to 25 years	> 25 years	0.68	0.42 to 1.10	0.119

OR = odds ratio. [1]All risk factors in the multivariate regression model were significant for $P < 0.05$; adjusted R^2 = 37.89. [2]Variable or class is a risk factor when OR > 1 and t-value < 0.05.

When performing statistical analyses on the French subset (n = 36) by including the stockperson's self-reported levels of positive and negative contacts, these factors did not influence the CET0 level when analysed individually and were omitted from further analyses. For the final model for the French subset, the variables space allowance per calf and stockperson's experience influenced significantly ($P < 0.05$) CET0 levels and accounted for 20.71% of the variance observed (Table 3). Space allowance per calf and stock- person's experience gave the same interpretations as for the results presented in Table 2, which means a higher space allowance per calf and increased experience of stockpersons leading to lower CET0 levels.

Table 3
Multivariate regression model for the percentage of calves scored 0 (could not be approached) for the sample with farmer's self-reported contacts (n = 36)

Risk factor[1,2]	**Level of comparison I**	**Level of comparison II**	**OR**	**95% confidence interval**	**t-value of pairwise comparison**
Space allowance	1.8 m^2/calf	> 1.8 m^2/calf	2.37	1.02 to 5.49	0.053
Stockpersons' experience	< 5 years	5 to 15 years	4.90	1.54 to 15.58	0.012
	< 5 years	15 to 25 years	2.54	0.65 to 9.94	0.190
	< 5 years	> 25 years	1.12	0.40 to 3.12	0.830
	5 to 15 years	15 to 25 years	0.52	0.11 to 2.57	0.428
	5 to 15 years	> 25 years	0.23	0.06 to 0.86	0.037
	15 to 25 years	> 25 years	0.44	0.10 to 1.91	0.282

OR = odds ratio. 1All risk factors in the multivariate regression model were significant for P < 0.05; adjusted R2 = 20.71. 2Variable or class is a risk factor when OR > 1 and t-value < 0.05.

With regard to the proportion of calves with CET4, type of milk distribution, breed and number of calves per stock- person explained significantly (all P < 0.05) 16.26% of the variance in the multiple regression model (Table 4). Calves raised on farms with bucket feeding had a higher risk of increased CET4 levels when compared with those with trough feeding. Farms with dual-breed or crossbred calves were more likely to have a higher proportion of calves with CET4 compared with the farms with only dairy breed calves. In general, farms with a lower number of calves per stockperson had a greater chance of higher levels of calves with CET4.

Table 4
Multivariate regression model for the percentage of calves scored 4 (could be touched; n = 147)

Risk factor[1,2]	**Level of comparison I**	**Level of comparison II**	**OR**	**95% confidence interval**	**t-value of pairwise comparison**
Type of milk distribution	Bucket	Trough	1.33	0.97 to 1.83	0.077
Type of breed	Dairy breed	Dual breed	0.70	0.51 to 0.96	0.030
	Dairy breed	Crossbred	0.63	0.46 to 0.87	0.006
	Dual breed	Crossbred	0.90	0.60 to 1.34	0.599
Number of calves/stockperson	< 200	201 to 400	1.03	0.72 to 1.46	0.889
	< 200	401 to 600	1.34	0.90 to 2.00	0.156
	< 200	601 to 800	2.12	1.23 to 3.65	0.007
	< 200	> 800	1.47	0.89 to 2.45	0.138
	201 to 400	401 to 600	1.30	0.94 to 1.82	0.120
	201 to 400	601 to 800	2.07	1.26 to 3.39	0.004
	201 to 400	> 800	1.44	0.93 to 2.23	0.108
	401 to 600	601 to 800	1.59	0.98 to 2.56	0.061
	401 to 600	> 800	1.10	0.72 to 1.68	0.658
	601 to 800	> 800	0.69	0.40 to 1.21	0.197

OR = odds ratio. 1All risk factors in the multivariate regression model were significant for P < 0.05; adjusted R2 = 16.26. 2Variable or class is a risk factor when OR >1 and t-value <0.05.

When performing statistical analyses on the French subset (n = 36) by integrating the stockperson's self-reported levels of positive and negative contacts, these factors did not influence the CET4 level when analysed individually and were omitted from further analyses. For the final model for the French subset, the variables type of breed and frequency of visits by the technician influenced significantly ($P < 0.05$) CET4 levels and accounted for 16.26% of the variance observed (Table 5). Farms with dual-breed or crossbred calves had a higher risk of having a higher proportion of calves with CET4 compared with the farms with dairy breed, and farms with dual-breed calves had a higher risk of having a higher proportion of calves with CET4 compared with farms with crossbreds. Farms that were visited weekly by the technician had a higher risk of increased CET4 levels when compared with those were the technician came every 2 weeks.

Table 5
Multivariate regression model for the percentage of calves scored 4 (could be touched) for the sample with farmer's self-reported contacts (n = 36)

Risk factor[1,2]	Level of comparison I	Level of comparison II	OR	95% confidence interval	t-value of pairwise comparison
Sype of breed	Dairy breed	Dual breed	0.29	0.12 to 0.72	0.012
	Dairy breed	Crossbred	0.80	0.47 to 1.36	0.423
	Dual breed	Crossbred	2.75	1.01 to 7.44	0.056
Frequency of visit by the technician	Weekly	Every 2 weeks	5.10	1.04 to 24.90	0.053

OR = odds ratio. 1All risk factors in the multivariate regression model were significant for P < 0.05; adjusted R2 = 16.26. 2Variable or class is a risk factor when OR > 1 and t-value <0.05.

Discussion

In the present study, two tests were performed on all farms in order to determine the human–animal relationship. The HAT was based on the voluntary approach of calves when the experimenter was standing at the feeding fence, whereas during the CET the experimenter entered a pen and tried to touch calves in a standardised way. A higher latency to touch during HAT was moderately negatively correlated with the CET score 4, demonstrating that calves not approaching an unfamiliar person easily, also avoided this person when he/she approached these calves. At farms with a high HAT latency, a low proportion of calves that could be touched was found. However, stockperson's management and animal characteristics did not influence latency to touch during HAT. It is possible that a voluntary approach of a human during the day is not a challenge for the animals. Animals may be in a situation of conflict between curiosity and fear when an unknown person is present (de Passillé and Rushen 2005). During the CET, a human is clearly provoking a reaction of the animal and it is likely that those that can be touched are not fearful of humans (Boivin et al. 1998). However, animals that cannot be touched may not necessarily be frightened (Waiblinger et al. 2003), and tests like our CET might be a mixture of measuring fear (extreme reactions), disinterest (moderate reactions) and lack of interest (Scott et al. 2009).

Farm animals' reactions to humans are generally an indicator of the quality and quantity of contacts they had with stockpersons (Hemsworth and Coleman 2010). Ideally, the stockperson's behaviour towards the animals should be observed in order to have a more exact evaluation of the human–animal relationship. As for the majority of field studies published, no direct observations of the stockperson's behaviour towards their calves were performed during the present study. For practical reasons it was not possible to perform these observations on such a large scale and within the limited time available for the experimenters. Therefore, for a part of the stockpersons, questions were asked on the quality and frequency of their contacts with calves. In several studies, a clear link was demonstrated between stockperson's attitudes and their behaviour towards animals (Hemsworth et al., 1994 and 2002; Coleman et al., 2000; Waiblinger et al., 2002). In the present study, no relationship was established between the level of contacts with the calves reported by the farmer and the behaviour of calves measured in the CET. Several reasons can be imagined for this apparent lack of relationship. It is possible that the variation in the results from the questionnaire and the number of questions were insufficient to have any effect in the analysis. Furthermore, stock- persons might have moderated their responses to questions concerning negative interactions and might have exaggerated their responses to questions regarding positive interactions (Lensink et al. 2000a). In addition, some aversive contacts such as moving or vaccinating calves and other types of contacts such as visual contact during daily routine procedures were not integrated in the assessment explaining therefore the lack of relationship.

At larger farms, especially those with more than 1200 calves, animals avoided the experimenter more than at smaller farms. Furthermore, on farms with fewer calves per stockperson more calves could be touched during the CET. This supports earlier findings that stockpersons in bigger units have less time to interact individually with animals (English 1991; Lensink et al. 2000a). For this reason, these variables are reflecting a lower level of physical and visual contact per animal, which in turn might influence the animals' reactions to humans (Barnett et al. 1994).

The way calves were fed influenced their response during the CET. Calves fed in a trough were more fearful than those fed in a bucket. It is possible that in the bucket system, calves have more visual and physical contact with the stockperson compared with the trough system. In fact, in the bucket system, for each meal the stockperson stands still in front of each bucket (and therefore each calf) in order to fill the bucket, whereas in the trough system the stockperson stands only directly in front of one calf or troughs are filled automatically without any human presence. This finding seems to support the suggestion that the amount of visual contact with stockpersons during feeding can influence the animal's behaviour towards humans (Jago et al. 1999; Lensink et al. 2000b). Furthermore, higher average space allowance per calf led to a lower proportion of calves with a score 0 (no eye contact) during the CET. Normally, farmers keep their calves at the minimum required space allowance as laid down in the regulations of the European Union (1.8 m^2/calf), but in some farms calves had some more space. Although the test was standardised, these calves might have been able to escape more easily or walk away from the experimenter or were less challenged by the test situation leading to a lower proportion of score 0.

Breed differences were found in the outcome of the CET. Crossbred calves generally tended less to avoid humans compared with dual-purpose breeds and dairy breed calves, while compared with dairy breed calves, dual-purpose breed calves demonstrated also less avoidance of humans. These findings are in accordance with previous studies demonstrating potential differences in reactivity to humans between beef cattle breeds (Murphey et al. 1981; adult cattle: Boivin et al. 1992; calves:

Boivin et al. 1994). However, it is generally believed that beef cattle are more fearful of humans than dairy breeds (Murphey et al. 1981), but this could be partially due to a lower level of contact with humans rather than a genetic effect. In our study, crossbred calves, which were a cross between dairy and beef breeds, and dual-purpose (milk and meat) breed calves were generally less fearful than dairy breed calves. As calves from those different breeds were raised in similar conditions, these effects might be due to genetic differences, but further research is needed to defend this hypothesis.

Seasonal effects were found on the percentage of calves that could not be touched. This result was unexpected as calves are penned and managed in the same conditions throughout the year. A seasonal effect might reflect temperature differences or effects on health. In autumn and winter, calves have a higher risk of lung diseases (Lundborg et al. 2005), which leads to more medical treatments. These treatments can be accompanied with some additional potentially negative handling by the stockperson or the veterinarian explaining potentially the higher number of calves that could not be touched.

In our study, presence of environmental enrichment (hanging objects in the calves' pens) was associated with a lower risk of observing a high percentage of calves avoiding the experimenter. Environmental enrichment was shown to reduce fear of humans in pigs (Pearce et al. 1989) but this impact has not been demonstrated in cattle yet (Raussi 2003). More research is needed to determine the potential links between hanging objects, general fearfulness and reactivity of animals to humans.

Our study indicates that factors other than the stock- person's behaviour towards their animals can affect calves' reaction during a human–animal relationship test performed in on-farm conditions. Stockperson's characteristics and management such as the number of calves they take care of, or years of experience, influenced calves' reactions; in addition, feeding system and breed also affected the outcome of the tests. More detailed studies are needed to clearly identify the importance of different aspects of the stockperson's behaviour because, next to physical contact, factors such as visual contact or being accustomed to human presence seem to have considerable impact on the animals' reactions to humans. However, as our study did not integrate the observation of the stockpersons' behaviour towards their calves, care should be taken with the interpretation of all potential effects as confounding issues might remain. In future, more research is needed integrating all aspects of the human–animal relationship in field studies to clarify and confirm the different results obtained.

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CHAPTER 4

Effects of the observation method (direct versus from video) and of the presence of an observer on behavioural results in veal calves

CHAPTER 4

Effects of the observation method (direct versus from video) and of the presence of an observer on behavioural results in veal calves

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ABSTRACT

This study aimed at assessing the effect of the observation method (direct or from video) and the effect of the presence of an observer on the behavioural results in veal calves kept on a commercial farm. To evaluate the effect of the observation method, twenty pens (4-5 calves per pen) were observed by an observer for 60 min (two observation sessions of 30 min) and video recorded at the same time. To evaluate the effect of the presence of the observer in front of the pen, twenty-four pens were video recorded on four consecutive days and an observer was present in front of each pen for 60 min (two observation sessions of 30 min) on the third day. Behaviour was recorded using instantaneous scan sampling. For the study of the observer's effect the analysis was limited to the posture, abnormal oral behaviour and manipulation of substrates. The two observation methods gave similar results for the time spent standing, but different results for all other behaviours. The presence of an observer did not affect the behaviour of calves at day level, but affected their behaviour when actually present in front of the pens. A higher percentage of calves were standing and were manipulating substrate in presence of the observer, but there was no effect on abnormal oral behaviour. In conclusion, direct observations are a more suitable observation method than observations from video recordings for detailed behaviours in veal calves. The presence of an observer has a short term effect on certain behaviours of calves which will have to be taken into consideration when monitoring these behaviours.

Introduction

Farm animal welfare is a multidimensional concept (Fraser 1995) which includes the "freedom to express normal behaviour by providing sufficient space, proper facilities and company of the animal's own kind" (Farm Animal Welfare Council 1992). Assessing welfare of farm animals therefore requires the evaluation of their behaviour (Botreau et al. 2007).

Behavioural observations can be conducted in different ways, including observations from video recordings and observations directly on the spot (Martin and Bateson 1993). Video recordings have several advantages: they can be stored, permit multiple viewing and can be slowed down to study short or complex behaviours (Martin and Bateson 1993). On the other hand, they request high quality devices to ensure a good quality of image and a broad angle to limit missing data due to animals not visible or behaviours difficult to assess (Tosi et al. 2006). Cameras need to be installed in advance in the animal's environment and the analysis of the recordings might be time consuming. In the context of welfare monitoring on a large number of commercial farms, all information should be collected within a short period of time (often within one or two days). It is unpractical installing cameras for this purpose and therefore direct observations are often preferred. Direct observations have the advantage that they are less sensitive for technical failures.

A major concern related to direct observations, however, is the potential effect of the presence of the observer on the behaviour of the observed animals (Martin and Bateson 1993). This effect has been shown in a number of mammals (Tamarin: Cain 1990; skunks: Lariviere and Messier 1998; non-human primates: Iredale et al. 2010). Humans can elicit three types of responses: attraction, habituation, and avoidance (Whittaker and Knight 1998). Habituation is considered completed when "animals no longer respond to the presence of a human observer" (McDougall 2012). It is often advised (especially for animals which are not habituated to the presence of observers such as wild animals) to spend long periods habituating subjects to the presence of the observer before starting the observation. Due to time constraints, habituation is not possible when behavioural observations are performed as part of welfare monitoring on large numbers of farms. The presence of the observer might influence particularly the expression of subtle behaviours such as play or sexual behaviours (Martin and Bateson 1993) but also abnormal behaviours (Broom 1983). The effect of the presence of humans on the behaviour and expression of abnormal behaviours has been studied in zoo animals (see Fernandez et al., 2009 for review) but has only been little studied in farm animals such as cattle.

Veal calves are raised in an environment providing little stimulation (Le Neindre 1993). A barren environment can induce a high reactivity of calves to novel stimulations (Veissier et al. 1997). In general, veal calves have little physical contact but more frequent visual contact with humans. A potential positive association with humans is related to feed distribution which can induce a high motivation for calves to interact with them (Jago et al. 1999; Lensink et al. 2000). Other contacts veal calves might have with humans are often negatively associated because they are in a fear or pain eliciting context (for example veterinary treatments, moving, transport, blood sampling). These experiences with humans can induce alertness towards humans. Knowing that calves are able to discriminate between people (de Passillé et al. 1996) the presence of an unknown observer in the barn could represent a novel element for the calves. In addition, the observer might be present in the barn at an unusual time (outside feeding hours) and may show unusual behaviour (standing still, observing) compared to the caretaker (moving, providing feed). These aspects again could induce alertness which might be reflected in fear and/or curiosity in calves.

For these reasons, it seems of prime importance to evaluate the effect of the presence of an observer on the observation of the behaviour of veal calves. The aim of the present study was twofold: (i) to compare two methods of on-farm observation of behaviours in calves, direct observation versus observation from video recordings, and (ii) to evaluate the effect of the presence of an observer on veal calves' behaviour.

MATERIAL AND METHODS

Calves and calves' management

Observations were performed at a commercial veal farm in the Netherlands with Holstein-Friesian veal calves, 10 weeks after the arrival of the calves at the farm. Calves arrived at the farm at around 15 days of age and were kept individually in babyboxes until 5 weeks after arrival at the farm. Thereafter, they were group housed in pens of 4 or 5 calves per pen. Calves were raised according to the EU Directive 97/2/EC (EU Council 1997) with 1.8 m^2/calf in an insulated and ventilated building with both natural and artificial lighting. The lights were on between 06:00h and 21:00h. Calves were fed standard commercial milk replacer and solid feed at 09:00h and 18:30h according to a commercial feeding schedule (up to approximately 10 litres of milk replacer and 250gDM of solid feed per calf per day) and provided in a collective trough. They had access to water provided via water nipples. During observation days, the farmer only performed feed distribution activities in the barn (approximately 1 hour in the morning and afternoon).

Pens and video recordings

The barn had 7 units (6 units with ten pens and one unit with 6 pens). Four units were used for this study. Within these units, 24 pens were randomly chosen for video recordings (6 pens per unit). In total 105 calves were observed (9 pens with 5 calves and 15 pens with 4 calves). Eighteen cameras (Type RC516BH, 600 TVL) were installed two days before the start of the study, and were attached to the ceiling of the barn so that they would not be accessible to calves. One camera could film one or two pens and provided good quality black and white digital videos. Pens were video recorded on four consecutive days from 06:00h to 21:00h (15 hours). Data were stored for later observation.

Observations for comparison of direct observation and observation from video recordings.

Direct observations and observations from video recordings were performed by two different observers trained to use the same ethogram (Table 1) and protocol. Both observations (direct and from video) were performed using instantaneous scan sampling (Altmann 1973) with a two minutes interval. For each calf, the posture (standing or lying) and the behaviour was recorded. Direct observations were performed on day 3 of the recording days. Twenty pens out of the twenty four were observed for two times 30 min by an observer (woman, 1.60 m, wearing a dark coloured overall) between the morning (09:30h) and the afternoon (16:00h) feeding (4 hours interval between these two sessions). Three to 5 pens in the same unit were observed simultaneously by the observer standing approximately 1 m from the front of the pens. In total 85 calves were observed (5 pens with 5 calves and 15 pens with 4 calves).

Observation from video for comparison without and with observer.

The video recordings of the four days (3 days without and 1 day with an observer present for two times 30 min) were analysed by a trained observer using the same ethogram (Table 1) and using instantaneous scan sampling (Altmann 1973) for each pen with a 10-minutes interval for the 15 hours of video recordings per day.

Table 1
List of recorded postures and behaviours.

Posture	***Description***
Standing	Calf stands on three or four legs
Lying	Calf lies on the floor either on the sternum or the flank
Behavioural category	***Desciption***
Sleeping (l)	Calf lies with its eyes closed or with the head turned backwards
Idle (s/l)	Calf looks ahead without showing any other activityRunning
Walking (s)	Calf walks (four-beat gait) through the pen
Eating (s)	Calf drinks milk, eats solid feed from a trough, drinks water from the water nipple, or licks a mineral stone
Ruminating (s/l)	Calf makes chewing movements
Oral manipulation of substrates (s/l)	Calf licks, nibbles, suckles or bites an object such as wall, fence, bucket, trough, floor or any other object accessible in the pen excepting feed
Abnormal oral behaviour (s/l)	Calf performs tongue rolling (repeated movement of the tongue inside or outside the mouth) or drinks urine (drinks or licks the urine of a pen mate or his self or sucks at the prepuce of a pen mate), or manipulates a pen mate (takes into its mouth and sucks or bites a part of the body of a pen mate, excluding the prepuce)
Comfort behaviour (s/l)	Calf licks or scratches itself (with leg or against an object) or calf stretches
Play and social play behaviours (s)	Calf gallops, jumps, butts, kicks, shakes its head or calf mounts another calf, performs frontal pushing, or displacement of another calf
Social licking (s/l)	Calf licks, nibbles and sniffs another calf at head, shoulders, flanks, back or tail (excluding legs and under the belly)
Other activity (s/l)	Calf is performing any other activity not described in the previous behavioural descriptions
Not visible (s/l)	Calf is not visible or it is not possible to determine its activity

The ‘l’ and ‘s’ between brackets indicate whether the behaviour can be performed in a standing or lying posture

Statistical analyses

All data were analysed with the SAS® statistical program (SAS® Institute inc. version 9.1). Data were expressed as percentages of calves performing a specific behaviour per scan, or as percentage of time spent on each behaviour by unit of time (either 60 min or 15 hours) with the statistical unit being pen. In addition, the percentage of time calves were in a standing posture by unit of time (either 60 min or 15 hours) was analysed as an estimation of the activity level of calves.

For comparison of the observation method, all behaviours in the ethogram were analysed. The observations from video were conducted exactly at the same period as when the direct observations were conducted for each pen. Therefore, for both methods of observation there were two times 30 min of observation periods. For each pen, data from the two 30 min periods of observation were added up into one period of 60 min of observation. Means were calculated for the two types of observation and compared by a Wilcoxon matched-pairs sign rank test for paired comparisons. Associations between the outcomes of the direct observations and the observation from video were calculated with Spearman rank correlations (rs), the strength of the correlations was described according to Martin and Bateson (1993).

The behaviour of calves was assessed at day level (from 06:00 h to 21:00 h). The percentage of time calves were standing, or spent on performing abnormal oral behaviour, and manipulating substrates were compared between days (without and with the presence of the observer). Means were calculated per day and compared with the Wilcoxon matched-pairs sign rank test for paired comparisons and the Kruskall Wallis test for overall comparison.

The effect of the observer when present in front of the pens was assessed by comparing the percentage of calves standing, performing abnormal oral behaviour, and manipulating substrates (60 min in two observation sessions of 30 min, on day 3) with the exact same moment of the day on the other three days. Means were calculated for the 60 min period and compared with the Wilcoxon matched-pairs sign rank test for pair comparisons and the Kruskall Wallis test for overall comparison.

The duration of the effect of the presence of the observer was evaluated by an analysis of the behaviour of calves around the moment when the observer was present in front of the pen (starting 60 min before the observer arrived and ending 150 min after the observer had left). For each pen, for each scan (4 scans during the observation, 6 scans before the observation and 15 scans after the observations, one scan every 10 min) the number of calves performing one behaviour during the first session was added to the number of calves performing the same behaviour during the second session. Then, for each scan, for the 20 pens, the percentage of calves performing one behaviour on day 3 was compared with the percentage of calves for the average of days 1, 2 and 4 using Wilcoxon matched-pairs sign rank test.

RESULTS

Effect of the observation method (direct versus from video)

There was no difference in the percentage of time calves were standing between the two observation methods (Table 2). The percentage of time calves were not visible was higher for observations from video than for direct observations. The percentage of time spent idle was higher when observed from video than observed directly, although there was a positive correlation between the two measures (Table 2). The observations from video also resulted in a higher percentage of time calves spent manipulating substrates and walking than with the direct observations. In contrast, a higher percentage of time calves spent performing abnormal oral behaviour was obtained through direct observation compared to observation from video. The correlations between the two observation methods were poor but significant for manipulating substrate and walking, and showed a tendency to be correlated for abnormal oral behaviour. No calf was recorded as eating when observed from video while this behaviour represented 9% of the time when directly observed (Table 2). Four behaviours (ruminating, playing, comfort behaviours, and other behaviours) gave different outcomes with low and non-significant correlations between the two observation methods.

Table 2
Percentage of time for the standing posture and the behaviours of calves assessed during 60 min (two observation sessions of 30 min) by two observation methods (direct and from video) (n = 20).

	Direct	**Video**	**Wilcoxon**[1]	**Correlation**[2]	
	Mean + SE	**Mean + SE**	**P**	r_S	**P**
Posture					
Standing (%)	85.0 ± 3.0	84.4 ± 3.1	ns	0.77	<0.01
Behaviour					
Idle (%)	34.7 ± 2.3	43.3 ± 3.0	0.04	0.53	0.01
Manipulating substrates (%)	20.8 ± 1.8	37.9 ± 3.0	<0.01	0.46	0.04
Abnormal oral behaviour (%)	9.0 ± 0.7	3.3 ± 0.5	<0.01	0.40	0.08
Ruminating (%)	6.2 ± 1.3	1.4 ± 0.5	<0.01	0.30	ns
Eating (%)	9.0 ± 1.8	0.0 ±0.0	<0.01	-	-
Playing (%)	1.7 ± 0.3	0.7 ± 0.3	0.02	0.25	ns
Comfort behaviour (%)	9.5 ± 0.6	4.6 ± 0.5	<0.01	–0.13	ns
Walking (%)	2.5 ± 0.4	4.0 ± 0.6	0.04	0.58	<0.01
Other (%)	6.3 ± 0.7	1.2 ± 0.3	<0.01	–0.02	ns
Not visible (%)	0.4 ± 0.1	3.7 ± 0.8	<0.01	0.24	ns

[1]Wilcoxon matched-pairs sign rank test. 2Spearman rank correlation

Effect of the presence of the observer on the behaviour of calves

When analysing the percentage of time calves spent standing, performing abnormal oral behaviour, and manipulating substrates between days, we found that on average, calves spent 33.0 ± 0.5% on standing, 3.5 ± 0.1% on abnormal oral behaviour, and 12.6 ± 0.2% on manipulating substrates during the four days. A day effect was found for these three behaviours (Kruskall Wallis test, P < 0.01). The percentage of time calves spent standing was higher at day 1 than all other days, and day 2 and 3 were higher than day 4 (Table 3). Calves performed more abnormal oral behaviour on days 1 and 2 than on days 3 and 4, whereas manipulating substrates was performed more on day 1 than on the other days.

Table 3
Mean (±SE) percentage of time spent standing, performing abnormal oral behaviour and manipulating substrates on day 1, 2, 3 and 4 (n = 24).

	Day 1	Day 2	Day 3	Day 4	P
Posture					
Standing (%)	36.40 ± 1.2[a]	33.1 ± 0.7[b]	32.1 ± 0.8[b]	30.0 ± 0.6[c]	< 0.01
Behaviour					
Abnormal oral behaviour (%)	4.4 ± 0.4[a]	3.7 ± 0.3[a]	2.8 ± 0.3	2.9 ± 0.2[b]	< 0.01
Manipulating substrates (%)	14.1 ± 0.6[a]	12.2 ± 0.4[b]	12.2 ± 0.5[b]	11.6 ± 0.4[b]	< 0.01

[a, b, c]: values within the same row with different superscripts differ significantly ($P < 0.05$, Wilcoxon matched-pairs sign rank test). [1]: Kruskall Wallis test.

Calves were standing more often and spent more time on manipulating substrates when the observer was present than at the same moment on the other three days when the observer was not present (Table 4). They also spent a higher percentage of time standing (81.0% ± 2.7) during the presence of the observer than on average during the 15 hours of observation on that day (32.1% ± 0.8, Table 3). No day effect was found for the percentage of time calves spent performing abnormal oral behaviour.

Table 4
Mean (±SE) percentage of time spent standing, performing abnormal oral behaviour and manipulating substrates during the 60 min of presence of the observer in front of the pen on day 3 and at the same time on days 1, 2 and 4.

	Day 1	Day 2	Day 3	Day 4	P[1]
	n=19	n=20	n=20	n=19	
Posture					
Standing (%)	27.4 ± 4.6[a]	37.5 ± 5.7[a]	81.0 ± 2.7[b]	31.3 ± 4.2[a]	< 0.01
Behaviour					
Abnormal oral behaviour (%)	4.1 ± 1.0	3.6 ± 0.7	3.7 ± 0.8	4.1 ± 1.1	ns
Manipulating substrates (%)	10.3 ± 2.0[a]	12.4 ± 1.9[a]	30.7 ± 2.8[b]	13.7 ± 2.4a[a]	< 0.01

[a, b]: values within the same row with different superscripts differ significantly ($P < 0.05$, Wilcoxon matched-pairs sign rank test). [1]: Kruskall Wallis test.

Figure 1a shows that more calves were standing on day 3 during the presence of the observer and during the 20 min after the observer had left compared to the average at the same moment for the other three days. A lower percentage of calves were standing between 40 and 100 min after the observer had left compared to the same moment on the other days. Figure 1b shows that less calves performed abnormal oral behaviour on day 3 during the first ten minutes of the presence of the observer but not during the rest of the time the observer was present compared to the other days. Differences in the percentage of calves performing abnormal oral behaviour between day 3 and the other days can also be seen at the same time points before and after the presence of the observer. Figure 1c shows that manipulating substrates followed nearly the same pattern as standing behaviour with a higher percentage of calves manipulating substrates during the presence of the observer and during the following 10 min compared to the average at the same moment for the three other days. Then they performed less manipulating substrates between 50 and 110 min after the observer had left compared to the same moment on the other days.

Discussion

The objectives of the study were to assess the effect of the observation method (direct or from video recordings) on the evaluation of the behaviour of calves and the effect of the presence of an observer during direct observations on behaviour in calves.

The direct observations and the observations from video gave comparable results for the percentage of time spent standing. Standing is an obvious posture which can be easily distinguished from lying. The two methods of observing gave different outcomes for all other behaviours analysed. More manipulation of substrates and less abnormal oral behaviour were observed with observations from video observation than with direct observation. These results were similar to those found by Tosi et al. (2006). Except for idle and walking, all other behaviours were observed in lower percentages with the observations from video recordings. With respect to the behaviour eating, it was not possible to observe from the video recordings if there was feed in the trough. Therefore, the observer might have recorded eating behaviour as manipulation of substrate (trough). A behaviour like tongue rolling also might be difficult to observe from video recordings because it can only be clearly observed when calves face the camera, whereas with direct observations, the observer can move and detect tongue rolling easier. The observations from video recordings also resulted more often in calves being not visible. This can be due to the presence of blind spots on the video recordings, calves hiding behind other calves, or the light being of a too low intensity. Given these results, we could assume that observation from video recordings were less precise for a certain number of behaviours and therefore not suitable for precise observations for abnormal oral behaviour in calves. This is in accordance with the conclusions of Tosi et al. (2006) that video recordings would badly replace direct observations and only should be used to assess inactivity, lying bouts or lying postures. The choice of using direct observations for the assessment of veal calf welfare focusing on abnormal oral behaviour seems to be suitable with the objectives of that type of observation.

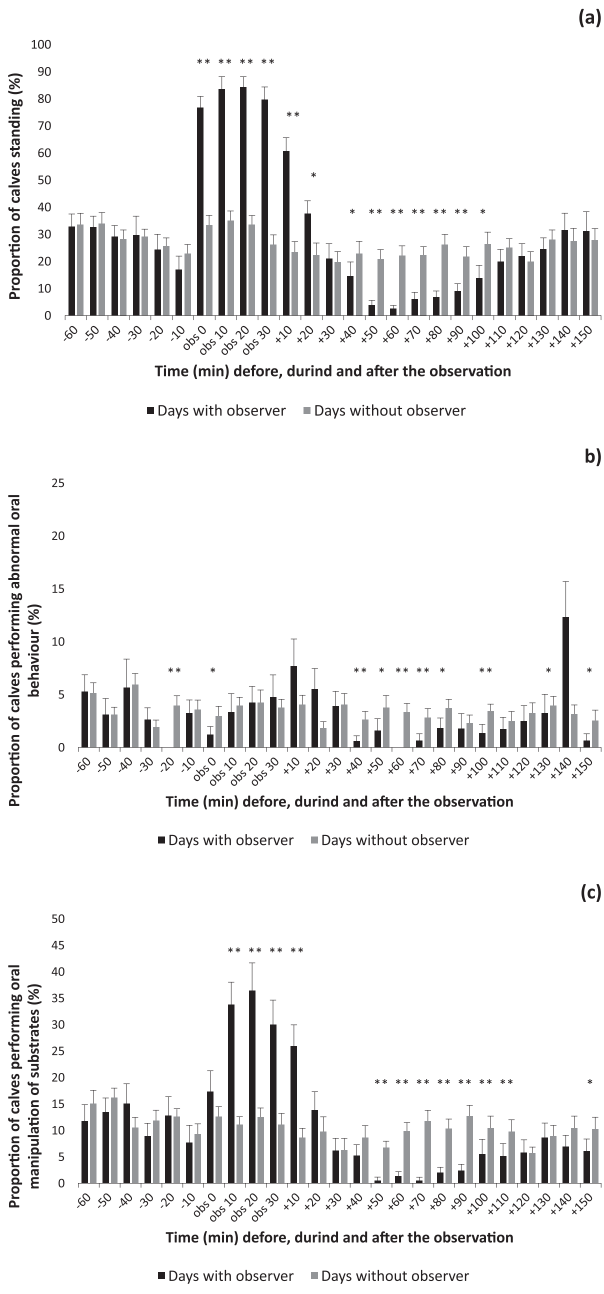

Figure 1
Percentage of calves (+SE) standing (a), performing abnormal oral behaviour (b) or oral manipulation of substrates (c) 60 min before, during, and 150 min after the presence of the observer in front of the pen on day 3 (black bar) and at the same time of the day on day 1, 2 and 4 (grey bar) (n = 20). On the x-axis, obs = the observer is present in front of the pen on day 3 at t=0 (obs0), t=10 min (obs10), t=20 min (obs20) and t=30 min (obs30). Wilcoxon matched-pairs sign rank test *P < 0.05; ** P < 0.01.

Variation in the behaviour of calves was found between the four days for the three observed behaviours (standing, abnormal oral behaviour and manipulating substrates). However, average levels of these behaviours on day 3 – when the observer was present during 60 min of direct observations – were not systematically different from those obtained on the other three days (1, 2 and 4). Rather, they seemed to be well within the normal day-to-day variation. This suggests that the effect of the presence of the observer was short-lived, without affecting the daily average levels of behaviours.

There was an effect of the presence of the observer for the 60 min of observation. Calves stood more when the observer was present than at the same moment on days without an observer. Calves also stood much more than what was recorded on a 15-hours basis on the same day. No effect of the presence of the observer was found on abnormal oral behaviour. At some observation points the percentage of calves performing abnormal oral behaviour was different when the observer was present compared to the same moment on the other days. Such differences, however, were also found at other observation points when the observer was not present. This suggests that abnormal oral behaviour was consistent within days but variable between days. In this study, abnormal behaviours consisted of two behaviours: tongue rolling and manipulation of penmates (including urine drinking). Webb et al. (2012) found that the expression of tongue rolling was relatively constant throughout a day. In contrast, sucking and manipulation of pen mates seem more related to specific events during the day as it has been found to be mostly observed after meals (Veissier et al. 1998). More substrate manipulations were observed in the presence of an observer. This result could be expected for two reasons. First, this behaviour is performed generally while standing and most of calves stood when the observer was present. Secondly, calves might associate humans with feed (Jago et al. 1999), and manipulating substrates such as nibbling objects is mostly observed just before meals (de Passillé et al. 1992; Veissier et al. 1998; Webb et al. 2012). The behaviour manipulation of penmates (included in abnormal oral behaviours) could have been affected by the presence of the observer just like the behaviour manipulating substrates was. Thirty minutes after the observer had left, calves showed an increased level of lying compared to the days when the observer was not present, which might be compensating behaviour for the increased activity level when the observer was present. The monitoring of calves' behaviour for welfare purposes using direct observations, therefore, might induce an overestimation of the behaviours standing and manipulating substrates. When, however, the aim of monitoring is to benchmark farms relative to other farms or to see whether farms comply with certain welfare certification standard, it is of less importance. Relative values instead of absolute values therefore do not necessarily impair the welfare assessment of farms.

Conclusion

In this study, observations from video were not accurate for subtle behaviours such as abnormal oral behaviour. Some behaviours can be easily confused with other behaviours when observed from video. There was an effect of the presence of an observer on the behaviour of calves as it elicited more standing and manipulating substrates. Direct observations for welfare monitoring purposes can be a suitable method with regard to the effect of the observer's presence and the quality of observation. Nevertheless, when the exact level of certain behaviours needs to be evaluated, one should first habituate calves to the presence of the observer before starting the observations.

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CHAPTER 5

Prevalence and potential influencing factors of abnormal oral behaviours of veal calves on commercial farms

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Prevalence and potential influencing factors of abnormal oral behaviours of veal calves on commercial farms

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Abstract

Veal calves raised under intensive conditions may express abnormal oral behaviours as signs of mental suffering and reduced welfare due to a mismatch between environment and management features, and the animals' needs. The aims of this study were to estimate the prevalence of abnormal oral behaviours in a large sample of veal farms in Europe and to determine the potential influencing factors present at farm level. Data were collected on 157 commercial veal farms in the 3 main producing countries in Europe (The Netherlands, France and Italy). Observations of 3 abnormal oral behaviours (manipulating substrates, tongue playing and manipulating a penmate) were performed when calves were aged 14 weeks and the prevalence of abnormal oral behaviours were calculated. Information on management practices, characteristics of the building and equipment were collected on all farms to assess the potential influencing factors for each of the 3 behaviours. Odds Ratios and 95% confidence interval were calculated to evaluate the effect of each individual factor within a generalized linear model. Results showed that calves spent on average 11.0 ± 0.46% of the observation time on manipulating substrates, 2.8 ± 0.18% on performing tongue playing and 2.7 ± 0.09% on manipulating a penmate, with a high variation between farms. Allowing more space to calves than the legal requirement of 1.8 m² and housing them in groups of >10 calves/pen reduced manipulating substrates and tongue playing. Occurrence of manipulating substrates was lower for calves fed maize silage compared to calves fed cereal grain, pellets or muesli. A higher risk of tongue playing was found when babyboxes were not used. Risk of calves manipulating a penmate was higher for calves of milk or meat type breeds compared to dual purpose breeds and for calves fed from 280 to 308 kg compared to those fed over 380 kg of milk powder in total for the fattening period. The study allowed assessing multiple factors across farms that showed a variety in terms of conditions and level of abnormal behaviours. The identification of the factors influencing each abnormal oral behaviour is helpful to define potential actions that could be taken on farms to improve the welfare of calves and to reduce the prevalence of these unwanted behaviours.

Introduction

Abnormal behaviours differ from the norm because they are directed towards inappropriate objects, differ from the animal's specific range of behaviour in its nature or frequency, have no function or are harmful to the individual (Mason 1991; Garner 2005). They are mostly performed by animals living in inappropriate captive environments (Mason 1991) and are increased by social deprivation (Veissier et al. 1998). Abnormal behaviours are a serious sign of mental suffering and reduced welfare (Broom and Fraser 2007).

Veal calves raised in intensive husbandry systems might experience environmental and management conditions that do not entirely fulfill their behavioural and physiological needs. Veal calves, for example, are separated from their dams at an early age and, therefore cannot drink mother milk in a natural way. Instead same-aged calves of different farms are brought together in fattening units where they are commonly fed twice-a-day with milk-replacer from a bucket, a trough or an Automatic Milk Delivery device (AMD) and a limited amount of solid feed. This condition of unnatural feeding of calves (no dam, imposed time) may result in more abnormal oral behaviours than calves with free access to suckle their dam (Fröberg and Lidfors 2009).

Veal calves express different types of abnormal behaviours amongst which the most frequent are abnormal oral behaviours such as manipulating (licking, nibbling or biting) substrates of their homepen (Le Neindre 1993; Veissier et al. 1998), cross sucking (Jensen 2003), and tongue playing (Le Neindre 1993). Abnormal oral behaviours were first studied in individual housing where calves, for example, spent 10-20% of the observed time on manipulating substrates of their homepen (Le Neindre 1993; Veissier et al. 1998). In group housing, abnormal oral behaviour represented between 15 and 35% of the activity of calves, with 15% to 20% of the observed time spent on manipulating substrates (Bokkers and Koene 2001; Webb et al. 2012). These behaviours are mostly performed around feeding (Veissier et al. 1998) and are suggested to be stimulated by milk drinking itself (de Passillé et al. 1992). Nibbling and biting of substrates probably derive from the normal ontogeny of grazing in (pre-)ruminants (Veissier et al. 1998) and from an intrinsic need for exploring (Sato and Wood-Gush 1988). These behaviours, therefore, indicate that without the possibility to graze, the absence of an appropriate amount of roughage, or a poor stimulating living environment, calves redirect their grazing, ruminating and exploring behaviours towards inappropriate objects. Licking and sucking of objects might be more related to suckling behaviour (de Passillé et al. 1992). Calves have a strong motivation for suckling. In absence of their dam or a teat they might redirect this behaviour towards elements in their environment. In group housing, suckling also can be directed towards conspecifics resulting in calves performing cross-sucking or urine drinking (Jensen 2003). Cross-sucking can lead to hair loss and inflammation of the sucked part of the body of the calf being suckled (Jensen 2003).

A third category of abnormal oral behaviour observed in calves is tongue playing. Tongue playing is described as a repeated movement of the tongue which can be performed inside and outside the mouth. Tongue playing represented 1.5 to 5% of the activity of calves in individual (Le Neindre 1993; Veissier et al. 1998) or group housing (Veissier et al. 1998; Bokkers and Koene 2001; Webb et al. 2012, 2013). Tongue playing (and to a lesser extent biting of objects) is often expressed in a stereotypical way. Stereotypies were defined by Mason (1991) as a repetitive, invariant behaviour without obvious goal or function. Stereotypical tongue playing can indicate frustration or lack of stimulation experienced by the animal (Mason 1991).

In the European Union, all calves are housed in groups and are provided daily at least with a minimum amount of fibrous feed of 50 - 250 g for calves aged 8 to 20 weeks (Council Directives 97/2/EC and 2008/119/EC). Studies carried out on a small number of farms and under experimental conditions, however, showed that even group-housed veal calves provided with some solid feed perform abnormal oral behaviours (Bokkers and Koene 2001; Webb et al. 2012, 2013). Next to inappropriate feed, environment and lack of stimuli, the prevalence of abnormal oral behaviours does increase with age (de Passillé et al. 1992; Bokkers and Koene 2001; Webb et al. 2012), probably because the motivation of a ruminant to ingest solid feed and to ruminate increases with age. Although the prevalence of abnormal oral behaviours in veal calves is documented rather extensively, no studies have been conducted to analyse potential influencing factors on commercial farms.

The objectives of this study therefore were to estimate the prevalence of abnormal oral behaviours in veal calves housed in groups on a large number of commercial farms and to determine the potential influencing factors present at farm level. The results may facilitate the implementation of remedial measures on housing conditions and management of veal calves to reduce abnormal oral behaviours on commercial veal farms.

Material and methods

Farm Sample

Data were collected between Summer 2007 and Spring 2009 on 157 veal farms in the Netherlands (98 farms), France (45 farms) and Italy (14 farms). Farms were selected in a way that they were assumed to represent a cross-section of farms producing veal in Europe. Calves were either housed in small groups of 4 to 6 calves per pen or intermediate groups of 7 to 15 calves per pen and milk-fed by a bucket (39 farms) or a trough (98 farms), or in groups of 25 to 80 calves per pen and milk-fed with an AMD (20 farms). All farms complied with the EU Council Directives 97/2/EC and 2008/119/EC. The sample within each country consisted of farms located in the main regions where veal calves are raised and it was selected among farms belonging to integrators / owners willing to participate in the study. A single batch of calves (= group of same aged calves) was selected for each farm, and the tested batches were evenly distributed across all four seasons. Calves arrived at the fattening units at the age of approximately 15 days and were slaughtered at the age of 17 to 30 weeks. Farms showed variation in type and origin of calves, size of the farm, diet (amount and composition of milk replacer and amount and type of solid feed), climate control, day light intensity, and general management.

Behavioural Observations

Behavioural observations were performed at all farms when the calves were aged 14.9 ± 1.6 (SD) weeks by one observer per farm (ten observers in total). Observers (men and women) wore similar dark-coloured clothing at all farms. Although the observers were experienced in behavioural research, they completed a training with videos and photos of calf behaviour and practiced together at a farm beforehand (for methodology, see Bokkers et al. 2009). Observers were considered as sufficiently trained when they reached a 80% agreement with the gold standard.

At all farms, observations were performed in three sessions corresponding to the same moments of the day in each farm. The morning session took place approximately one hour after the start of the morning meal. The noon session took place around 12 pm. The afternoon session took place at least one hour before the start of the afternoon meal. During the day of observation lights were on in the building(s). In each session (morning, noon, afternoon) 3 observation bouts of 10 minutes were performed on different pens. Observation pens and the order of observation were randomly selected beforehand. In farms with groups of less than 15 calves per pen, 4 pens were observed simultaneously for each observation bout and 36 pens in total (3 sessions x 3 observation bouts x 4 pens). In farms with groups of more than 15 calves per pen, one pen was observed for each observation bout and 9 pens in total (3 sessions x 3 bouts x 1 pen). All pens were observed in case there were less than 9 pens.

For each observation bout, the observer waited 5 minutes before starting the observation, so that calves could get accustomed to the observer's presence. Thereafter, the observer recorded every 2 minutes the posture (lying / standing) and the behaviour of all calves in each pen using instantaneous scan sampling (Altman 1973). A complete ethogram was used during observation (see Leruste et al. 2013). For this study, only abnormal oral behaviours were analysed. The following behaviours were recorded: oral manipulating substrates (calf licks, nibbles, suckles or bites an object such as wall, fence, bucket, trough, floor or any other object accessible in the pen, except for feed), tongue playing (calf performs a repeated movement of the tongue inside or outside the mouth), and manipulating a penmate (calf takes into its mouth and sucks or bites a part of the body of a penmate, under which the prepuce that in case of manipulation might result in urine drinking).

Farm Data

At all farms, information was collected by means of a questionnaire on the characteristics of the building and equipment such as the type of milk distribution system, number of calves, space allowance, prevalent breed etc. (Table 1). The stockpersons were interviewed on another day but before the behavioural observations about their management practices such as number of years of experience with calves, daily time spent in the building, frequency of the visit by the technical adviser etc. These data were used to determine a list of potential influencing factors for the prevalence of abnormal oral behaviour.

Table 1
Parameters recorded on veal farms through the questionnaire.

Item	Levels
Parameters related to production and housing system	
Farm size (total number of calves)	≤ 300 | 300 < x ≤ 600 | 600 < x ≤ 1,200 | > 1,200
Space allowance	= 1.8 | >1.8 m^2 / calf
Type of floor	Slatted wooden floor | Slatted concrete floor | Slatted rubber floor or straw
Estimated luminosity of the barn	Light | Half-light | Dark
Environmental enrichment	No | Yes
Renovation of the barn	≤ 4 | 4 < x ≤ 8 | > 8 years
Parameters related to batch characteristics	
Quality of the batch at arrival[1]	Good | Average | Bad
Season at arrival at the farm	Spring | Summer | Autumn | Winter
Calves origin	National | One foreign country | Several countries
Prevalent breed	Holstein or other milk breed | Dual purpose breed | Crossbred or meat breed
Percentage of females	0 | 0 < x ≤ 5 | > 5%
Average hemoglobin level at moment of observation	≤ 5.7 | 5.7 < x ≤ 6.2 | > 6.2 mmol/L
Average number of calves/pen	≤ 6 | 7 ≤ x ≤ 9 | ≥ 10
Age of calves at observation	Less | More than 15 weeks
Duration of fattening cycle	< 24 | 24 ≤ x ≤ 30 | > 30 weeks
Parameters related to management and farmer experience	
Prophylaxis treatment	No | Yes
Use of individual baby-boxes	No | Yes
Duration of baby-boxes use	0 | 0 < x ≤ 4 | 4 < x ≤ 6 | > 6 weeks
Sorting/regrouping practice	No | Yes
Frequency of visits by technician	Weekly | Every 2 weeks | More than 2 weeks between visits
Frequency of visits by veterinarian/ fattening cycle	< 3 | ≥ 3
Frequency of visits by farmer/day	≤ 2 | >2
Farmers' experience	≤ 5 | 5 < x ≤ 15 | 15 < x ≤ 25 | > 25 years
Years of adoption of the existing rearing system	≤ 2 | 2 < x ≤ 10 | > 10 years
Number of stockpeople	1 | 2 | 3 or more
Urine drinkers are separated	No | Yes
Parameters related to feeding system	
Type of milk delivery system	Bucket | Trough | AMD
Total amount of milk-replacer powder	≤ 280 | 280 < x ≤ 330 | 330 < x ≤ 380 | > 380 kg / calf / fattening cycle
Calves always receive ≥14 liquid meals/week	No | Yes
Prevalent type of solid feed	Maize silage | Pellets or muesli | Cereal grain2 | Treated maize3
Total amount of solid feed fattening cycle	≤ 50 | 50 < x ≤ 100 | 100 < x ≤ 150 | 150 < x ≤ 3 00 | > 300 kg DM / calf /
Type of roughage distribution	On floor | trough or bucket | separated trough or bucket | automatic distribution | start period in trough, fattening period on floor | trough and separated trough | trough and automatic distribution
Water provision	Ad libitum | Limited | No water
Water origin	Tap | Well
Drinker type	Bucket | Trough | Nipple | Bowl | Other

[1] Estimation by the farmer 2Barley or maize. 3Rolled and/or flaked maize.

Statistical Analysis

Data were analysed by using GenStat software (GenStat Committee 2000) with farm as statistical unit. For each farm, data from the three observation sessions (morning, noon, afternoon) were summed up. Data were expressed as average percentages with standard error (SE) of calves performing a specific behaviour by unit of time. Risk factor analyses were performed for each of the following response variables: percentage of calves manipulating substrates, percentage of calves performing tongue playing and percentage of calves manipulating a penmate.

The explanatory variables used for the construction of the models were obtained from the questionnaire and are listed in Table 1. Levels of factors were defined according to the frequency of farms per level and covariates were transformed into factors. Potential influencing factors were at first inspected individually by Generalized Linear Model (GLM) univariate analyses, and those factors significantly associated with the dependent response variable ($P < 0.10$) were further included in the multivariate analysis. Further final multivariate models were built using both stepwise backward and forward selection. On the union of the final models of both selection procedures, best subset selection was performed and significance tests for the effects of the selected influencing factors were evaluated. Main effects only were considered precluding interactions and avoiding multicollinearity problems. The model selection procedure was based on an increase in R^2 and retaining factors that were significant ($P < 0.05$), where R^2 is the square of the multiple regression coefficient and represents the explained portion of the variance in the dependent variable. The final model was fitted to the data, and for each risk factor retained in the final model, an odds ratio (OR) and a corresponding 95% confidence interval (CI) was obtained. All GLMs were logistic regression models, comprising a multiplicative overdispersion factor with respect to the binomial variance function. Occasionally some farms are missing in the OR analyses ($n < 157$) because information on particular parameters was missing of those farms.

RESULTS

General Results

The mean percentage of calves per farm performing manipulating substrates was 11.0 ± 0.46% (Figure 1), performing tongue playing 2.8 ± 0.18% and manipulating a penmate 2.7 ± 0.09%.

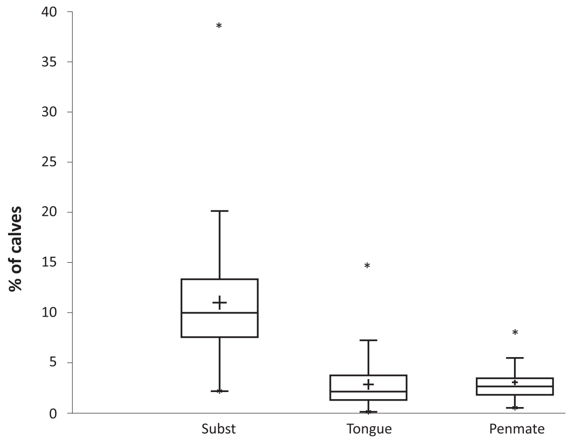

Figure 1

Prevalence of calves manipulating substrates (SUBST), tongue playing (TONGUE) and manipulating a penmate (PENMATE) amongst the 157 farms. *Dots represent minimum-maximum values of the sample, line and cross in the box represent respectively median and average values of the variable.*

Risk Factor Analyses

Manipulating Substrates: Prevalent breed of calves, farmer's experience, prevalent type of solid feed, space allowance per calf, average number of calves per pen and season when the batch entered the farm were found to influence ($P < 0.05$) the percentage of calves manipulating substrates (Table 2). The variables accounted for 41.6% of the variance. Crossbred or meat breed calves were associated with a higher risk of manipulating substrates in comparison with dual purpose breeds. In comparison to a farmer's experience of 5 to 15 years, the other experience classes showed an increased risk for the development of manipulating substrates in calves. Calves fed maize silage showed a lower level of manipulating substrates compared to calves fed pellets or muesli, or cereal grain. A space allowance above the legal requirement (1.8 m^2) was associated with a lower risk of calves manipulating substrates. Farms with calves housed in pens of less than 10 calves were associated with a higher risk of calves manipulating substrates compared to farms with calves housed in pens of more than 10 calves. Calves exhibited more manipulating substrates when the batch arrived at the farm during summer (i.e. behavioural observations performed during autumn) compared to winter.

Table 2
Multivariate regression model for the percentage of calves performing manipulating substrates (n = 153).

Factor	**Level of**	**Pred. prevalence**	**OR**	**95% confidence interval**	**t**	**P**
Breed	Holstein or milk breed	10.42^{ab}	1.24	0.99 – 1.55	0.066	0.024
	Dual purpose breed	8.62^{b}	-	-	-	
	Crossbred or Meat breed	11.80^{a}	1.43	1.10 – 1.84	0.007	
Farmer experience	≤ 5 years	11.32^{a}	1.43	1.15 – 1.80	0.002	< 0.001
	5 < x ≤ 15 years	8.21^{b}	-	-	-	
	15 < x ≤ 25 years	10.20^{a}	1.27	1.04 – 1.56	0.021	
	> 25 years	11.40^{a}	1.45	1.21 – 1.72	< 0.001	
Prevalent type of solid feed	Maize silage	8.24^{b}	-	-	-	0.001
	Pellets or muesli	10.79^{a}	1.35	1.14 – 1.61	0.001	
	Cereal grain	12.15^{a}	1.55	1.26 – 1.91	< 0.001	
	Treated maize	9.93^{ab}	1.23	0.89 – 1.70	0.207	
Space allowance	= 1.8	11.78^{a}	1.39	1.17 – 1.66	< 0.001	< 0.001
	> 1.8 m^2 / calf	8.58^{b}	-	-	-	
Nb calves /pen	≤ 6	11.13^{a}	1.60	1.25 – 2.05	< 0.001	0.001
	7 -9	12.42^{a}	1.82	1.34 – 2.46	< 0.001	
	≥ 10	7.29^{b}	-	-	-	
Season at arrival at the farm	Spring	9.49^{b}	1.05	0.81 – 1.35	0.735	< 0.001
	Summer	12.90^{a}	1.48	1.20 – 1.83	< 0.001	
	Autumn	9.62^{b}	1.06	0.85 – 1.32	0.598	
	Winter	9.12^{b}	-	-	-	

Adjusted R^2 = 41.6 ^{a,b} Values within the same column with different superscripts per level differ significantly ($P < 0.05$)

Tongue Playing: Number of calves per pen, space allowance per calf, and use of babyboxes were found to influence (P < 0.05) the percentage of calves performing tongue playing (Table 3). The variables accounted for 20.9% of the variance. A higher risk of calves performing tongue playing was found in calves housed in pens of less than 10 calves compared to calves housed in pens of more than 10 calves. Allowing more space to calves than the legal requirement (1.8 m²) reduced the risk of calves performing tongue playing. When no babyboxes were used, calves showed an increased level of tongue playing compared to farms were babyboxes were used.

Table 3
Multivariate regression model for the percentage of calves performing tongue playing (n = 143).

Factor	Level of	Pred. preval	OR	95% CI	t	P
Nb calves/pen	≤ 6	3.24^{b}	1.85	1.06 – 3.23	0.032	0.002
	7 -9	4.40^{a}	2.56	1.46 – 4.46	0.001	
	≥ 10	1.78^{c}	-	-	-	
Space allowance	= 1.8	3.79^{a}	1.55	1.16 – 2.07	0.003	0.002
(m²/calf)	> 1.8	2.49^{b}	-	-	-	
Duration of	No babybox	5.76^{a}	2.89	1.83 – 4.54	< 0.001	< 0.001
babybox use	0 – 4	2.09^{b}	-	-	-	
(weeks)	4 – 6	2.31^{b}	1.11	0.82 – 1.49	0.498	
	> 6	2.41^{b}	1.16	0.78 – 1.72	0.460	

Adjusted $R^2 = 20.9$ ^{a,b,c} Values within the same column with different superscripts per level differ significantly (P < 0.05)

Manipulating a Penmate: Breed of calves and amount of milk powder were found to influence (P < 0.05) the percentage of calves manipulating a penmate (Table 4). The variables accounted for 12.2% of the variance. Compared to dual purpose breed, Holstein or milk breed and crossbred or meat breed showed a higher risk of calves manipulating a penmate. Compared to calves fed more than 380 kg of milk powder in total for the fattening period, calves fed between 280 and 380 kg of milk powder were associated with an increased risk of calves manipulating a penmate.

Table 4
Multivariate regression model for the percentage of calves performing manipulating a penmate (n = 111).

Factor	Level of	Pred. p.	OR	95% CI	t	P
Breed	Holstein or milk breed	2.90^{a}	1.53	1.19 – 1.97	0.001	0.004
	Dual purpose breed	1.91^{b}	-	-	-	
	Crossbred or meat breed	2.57^{a}	1.35	1.01 – 1.82	0.047	
Amount of milk	280 – 330	2.73^{a}	1.36	1.07 -1.73	0.014	0.010
(kg / calf per	331 – 380	2.64^{a}	1.32	1.09 – 1.60	0.006	
fattening cycle)	> 380	2.02^{b}	-	-	-	

Adjusted $R^2 = 12.2$ ^{a,b} Values within the same column with different superscripts per level differ significantly (P < 0.05)

DISCUSSION

The objectives of this study were to estimate the prevalence of abnormal oral behaviour of group-housed veal calves in a large sample of commercial farms and to identify potential influencing factors at farm level. The variability between farms was high, especially for manipulating substrates, justifying the importance of a risk factors analysis. In the present study, the average prevalence of the observed behaviours were comparable to values found in previous studies in group-housed calves observed around 3 - 4 month of age (Bokkers and Koene 2001; Webb et al. 2012, 2013) with tongue playing and manipulating a penmate representing around 3% of the time-budget of calves and manipulating substrates reaching about 10%.

The final risk factor model for manipulating substrates explained more than 40% of the variance with factors related to calves, farmer, feed, housing and season. The final risk factor model for tongue playing explained 20% of the variability with risk factors only related to housing (group size, space allowance and use of babyboxes). These results suggest that housing can be an important factor for the development of tongue playing as previously suggested in individually-housed calves by Le Neindre et al. (1993) and in group-housed calves by Bokkers and Koene (2001). The final risk factor model for manipulating a penmate explained 12% of the variability with factors related to both calves and feed.

Differences between calves of different types of breed were found. Crossbred/meat breed calves showed a higher risk of manipulating substrates compared to dual purpose breed calves and both Holstein/milk breed calves and crossbred/meat breed calves showed a higher risk of manipulating a penmate than dual purpose breed calves. Genetic differences in the expression of some abnormal oral behaviours were reported between strains of cattle of the same breed (Fuerst-Waltl et al. 2010). Although genetic factors are involved in the expression of these behaviours, breed differences in the expression of abnormal oral behaviours have, to our knowledge, never been reported in cattle. Further research is needed to support the hypothesis that breed might affect the expression of abnormal oral behaviour.

Some characteristics of feeds provided on commercial farms had an impact on abnormal oral behaviours of calves. There were less risks of manipulating substrates when the solid feed was based on maize silage compared to cereal grain, pellets or muesli. Maize silage and treated maize contain more fibres (50 to 60% of raw cellulose) than cereal grain, pellets or muesli (INRA 2007). Cereal grain, and pellets or muesli can be considered as concentrated feed which are ingested and digested relatively quickly (Morisse et al. 2000) and will induce little rumination (Morisse et al. 1999). Therefore, this finding agrees with experimental data in veal calves showing that, in comparison with an all-milk diet, provision of solid feeds with high fibre content (such as hay or straw) next to milk replacer reduces abnormal oral behaviours such as manipulating the trough (Webb et al. 2013) whereas more concentrated feeds (such as beet pulps) have a smaller effect on calves' behaviour (Mattielo et al. 2002). The overall effect of solid feed on calves' oral behaviour depends on a combination of type of feed, feed quality and quantity of feed (Webb et al. 2013). More information is needed about the impact of the type of solid feed on characteristics of feed ingestion (e.g. duration of ingestion, duration of rumination, nitrogen release, saliva production) to explain the effects of solid feed provision on the expression of manipulating substrates in calves.

It seems surprising that the quantity of solid feed was not a risk factor in the present study neither for manipulating substrates, nor for tongue playing. One explanation could be that a significant impact of the quantity of solid feed is only seen when there is a substantial difference between low and high levels of solid feed provision. In recent experimental studies of Webb and co-workers, for example, differences in the amount of solid feed given to calves next to milk replacer ranged from 0 to 1.2 kgDM/calf per day (Webb et al. 2012). On commercial farms involved in the present study, calves received on average 0.534 ± 0.018 kgDM/calf per day of solid feed during the total fattening period with the average daily intake ranging from 0.040 to 1.360 kgDM/calf per day. On half of the farms participating in the current study, the average daily intake of solid feed provided to calves next to milk replacer ranged between 0.39 and 0.66 kgDM/calf per day. It is possible that differences between the majority of farms in the present study were too low to result in differences in the behaviour of the calves. This suggestion seems to be supported by the recent observation that the voluntary intake of solid feed by calves around 14 weeks of age was approximately 2 kgDM/calf per day (Webb et al. unpublished data) which is about 4 times the amount of what they received on farms involved in this study. It is also possible that calves received equivalent amounts of solid feed at the moment of observations (around 14 weeks) even if the total amount of solid feed distributed during the entire fattening period was different. Finally, calves were observed at a relatively young age (14 weeks of fattening). We could expect a higher level of abnormal oral behaviours, especially tongue playing, in older calves (Mason 1991; Bokkers and Koene 2001; Webb et al. 2012, 2013).

Calves with the highest quantity of milk powder consumed during the fattening period showed less manipulating a penmate. These results are in accordance with the literature. Cross sucking is a behaviour that is elicited by the ingestion of milk (Jensen 2003) and especially by the motivation to suckle after a meal. Hunger and restricted milk feeding can increase this phenomenon (Rushen and de Passillé 1995). In addition to the quantity of milk powder consumed, we could have expected the type of milk delivery system (bucket, though or AMD) to have an impact (Jensen 2003) on manipulating a penmate. It could have been interesting to record also more information about the milk meal such as milk program, milk flow rate, use of a separation gate in AMD systems or use of teats in bucket systems, as those factors may have an impact on the level of cross sucking, as suggested by Jensen (2003).

Characteristics of the housing of calves had an effect on tongue playing behaviours. Calves showed more tongue playing behaviour when housed in groups of relatively small size (less than 10 calves per pen) than in larger groups regardless of the type of milk distribution system. A space allowance above the legal requirement (more than 1.8 m² / calf) was associated with a reduced risk of tongue playing. The positive effect of a higher space allowance on oral behaviours has been shown before (Schlichting et al. 1990 in Jensen et al. 2003). In the current study, calves had a higher risk of performing tongue playing when no babyboxes were used. Here we might have expected an effect of the use of babyboxes on the behaviours manipulating a penmate rather than on tongue playing behaviours. Early group housing allows calves to develop cross sucking behaviours at a very young age (Jensen 2003). Housing calves in babyboxes at the beginning of the fattening period has been shown to reduce cross sucking in veal calves (de Wilt 1985) which is one of the reasons why babyboxes are used in commercial practice. As far as we know there is no evidence in the literature suggesting that the use of babyboxes would also interfere with the later development of tongue playing.

The experience of the farmer - in number of years - had an impact on the percentage of calves manipulating substrates. Farmers with less than 5 years of experience and more surprisingly farmers with more than 15 years of experience were found to increase the risk for calves to perform oral manipulating substrates in comparison with farmers with 5 to 15 years of experience. These results seem to support the idea that stockpersons influence the behaviour and welfare of calves (Hemsworth and Coleman 1998; Lensink et al. 2000a). However, the present findings do not provide a clear explanation as to which components of the experience of a farmer would explain a higher risk of manipulating substrates. Although experience was not confounded with other factors analysed, there could be other underlying causes than experience itself. Experienced farmers might, for instance, have an experience in raising calves partly based on a former housing and feeding system (individual crates and no solid feed) that was predominant before 2004. They could therefore have had a different way to adapt to the present system, for instance, in terms of feed management and distribution. The differences in number of years of experience could also relate to personal differences between farmers (age, attitudes towards calves, behaviour) or to differences in job related factors such as, for example, knowledge and education (Lensink et al. 2000b). Lensink et al. (2001) showed that the attitude and behaviour of the farmer has an impact on the behaviour of calves (reactivity and ease of handling). In our study, the attitudes of the farmers were not analysed. More information is therefore needed on the farmer's experience, practices, attitude and behaviour to explain their impact on the behaviour of calves.

Unexpectedly, calves exhibited more manipulating substrates when behavioural observations were performed during autumn (calves arrived at the farm in summer). There were no indications that housing conditions or management of calves were different throughout the year. Therefore, we speculate that there are other underlying factors such as luminosity, humidity, temperature, or duration of the day at the start of the batch or at the time of observation that influenced the calves' behaviour.

Conclusions

This study allowed the quantitative examination of association between prevalence of abnormal oral behaviours in veal calves and multiple husbandry factors as observed on commercial veal farms in practice. The recorded prevalence of abnormal oral behaviours – even if comparable with data from the literature - varied between farms and was associated with factors related not only to housing and feeding but also to the farmer. These results partly confirm and partly complement those from experimental studies. Additional studies are necessary to (further) elucidate underlying causal mechanisms. Collectively, these findings will help the definition of concrete remedial actions that could be taken on commercial farms to improve the welfare of veal calves.

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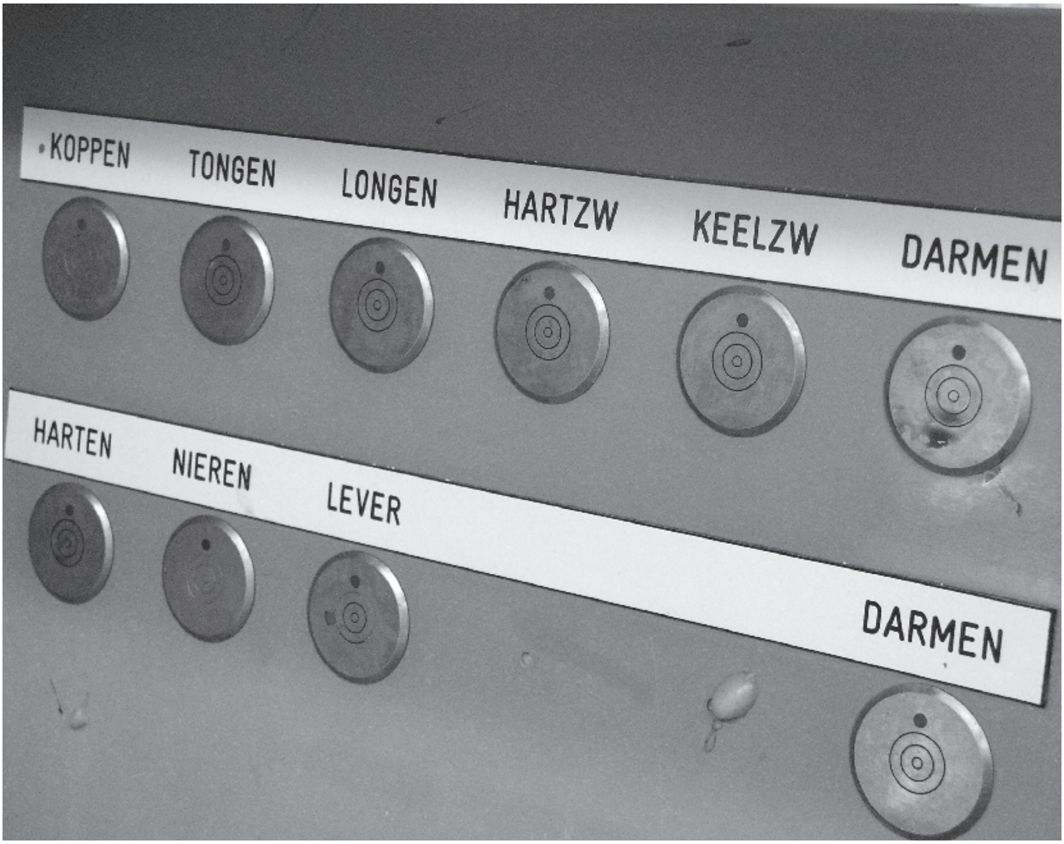
CHAPTER 6

The relationship between clinical signs of respiratory system disorders and lung lesions at slaughter in veal calves



CHAPTER 6

The relationship between clinical signs of respiratory system disorders and lung lesions at slaughter in veal calves

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ABSTRACT

The presence and severity of lung lesions recorded post-mortem is commonly used as an indicator to assess the prevalence of respiratory problems in batches of bovines. In the context of a welfare monitoring based on on-farm measures, the recording of clinical signs on calves at the farm would be more convenient than the recording of lung lesions at slaughter. The aim of the present study was to investigate the relationship between clinical respiratory signs at farm and post-mortem analyses of lung lesions observed at slaughter in veal calves. If clinical signs were a good predictor of lung lesions it could be possible to integrate only those measures in a welfare monitoring system. One-hundred-and-seventy- four batches of calves were observed 3 times: at 3 and 13 weeks after arrival of the calves at the unit and at 2 weeks before slaughter. For each batch a maximum of 300 calves was observed and the proportions of calves showing abnormal breathing, nasal discharge and coughing were recorded. Post-mortem inspection was carried out on a sample of lungs belonging to calves from the observed batches. Each examined lung was classified according to a 4-point scale for pneumonia from healthy lung (score 0) to severe lesions (score 3). The clinical signs recorded *infra vitam* were significantly correlated with moderate and severe lung lesions for observations at 13 weeks and 2 weeks before slaughter and the level of the correlation was highly variable (r_{sp} from 0.16 to 0.40). Receiver operating characteristic (ROC) curves were created and the area under the curves showed that batches with a high proportion of lungs with moderate or severe lesions could not be accurately detected by the three clinical signs of respiratory disorders. These results suggest that both clinical signs and post-mortem inspection of lung lesions must be included in a welfare monitoring schemes for veal calves.

Introduction

In Europe, approximately 5.6 million dairy and cross- bred calves are kept for veal production every year (Institut de l'Elevage 2010). Calves generally arrive at specialized fattening units at the age of approximately 15 days, where they are kept until 4–6 months of age before being slaughtered. During the fattening period, calves are mainly fed milk replacer with some solid feed supplementation. The predominant housing system in Europe is the following: calves are kept individually for the first 6–8 weeks; thereafter they are housed in group pens of 5–15 calves with 1.8m2 space allowance per animal. These pens have wooden slatted floors (or less frequently concrete slatted floors) and a bucket or a trough feeding system. In some farms calves are kept in large groups of 15–80 animals per pen. These calves are fed through automatic milk delivery devices (AMD) and kept mainly on wooden slatted floors (or less frequently on straw) with a space allowance of 1.8 m2 per animal. These systems of intensive rearing of veal calves have been long time criticized for their potential detrimental effects on the welfare of calves (EFSA 2006).

On-farm animal welfare monitoring schemes have been developed for different farm animal species including calves kept for veal production (Botreau et al. 2007a; Bokkers et al. 2009; Welfare Quality® 2009). These schemes are mainly based on animal based measures including health and behavioural measures such as lameness or abnormal oral behaviours. Ideally only one visit per production cycle (e.g. lactation or fattening period) should be performed after which the results will be used to define an action plan for welfare improvements on farms where the situation does not fulfill the minimal requirements (Welfare Quality® 2009).

Respiratory disorders are one of the major contributors to reduce welfare in veal production (EFSA 2006). Around 25% of veal calves are treated for respiratory disorders (Martineau et al. 2007) and 17% of the calves show extensive lung lesions post-mortem (Van der Mei and Van den Ingh 1987). The most frequent symptoms observed in veal calves treated for respiratory disorders are fever, abnormal breathing and coughing (Martineau et al. 2007). The inclusion of animal-based respiratory disorders in a health protocol as part of an on-farm welfare monitoring scheme is only possible if the measures are non-invasive, valid, reliable and easy to be recorded in practice. This condition excludes, for example, individual body temperature measurements (fever), because they are time-consuming and require animal handling. In contrast, clinical symptoms of respiratory disorders such as abnormal breathing, nasal discharge and coughing can be easily recorded on farm on a large number of animals without a need for handling. The inspection of lungs at the slaughterhouse represents also a feasible retrospective tool for collecting information on respiratory disorders in veal calves. However, a farm visit is needed in any case because of all other measures needed to monitor welfare. When additionally to that visit, a visit to the slaughter house is needed to collect post-mortem data, that will result in extra time and costs. Furthermore, it is not clear if for example lung lesions give information on respiratory disorders that occurred at a certain moment in time or over the complete fattening period.

The objective of the present study was to determine which respiratory clinical sign would be the best estimate for lung lesions. Therefore, the relationship was studied between three respiratory disorders (abnormal breathing, coughing and nasal discharge) recorded on farm at three moments (early, mid, late stage) during the fattening period and pathological findings on lungs obtained for the same batches of calves at slaughter. The hypothesis was that the level of respiratory clinical signs recorded in a given batch of calves would be a good measure to detect batches with high levels of lung lesions.

MATERIALS AND METHODS

Farm sample

Data were collected between the summer of 2007 and spring of 2009 on 174 veal farms located in the Netherlands (n = 100), France (n = 50) and Italy (n = 24). The sample within each country consisted of farms located in the main regions where veal calves are reared and it was selected among farmers willing to participate in the study. The sample is considered as a judgment sample as described by Dohoo et al. (2003). A single batch of calves (group of calves that arrived at the farm at the same time to be fattened and slaughtered together) was considered for each farm, and the selected batches were distributed across all four sea- sons. One hundred and forty nine batches were housed for the whole fattening period in groups of 5–15 calves (small groups) and fed milk replacer with a collective trough or an individual bucket. Twenty-five batches were housed in groups of 25–80 calves (large groups) and fed milk replacer by AMD. Calves (mostly males of Holstein breed) arrived at the fattening units around 15 days of age. All batches received one collective prophylactic treatment for respiratory disorders at the start of the fattening. The calves were slaughtered after a fattening period of on average 26 weeks (range 15–31 weeks). The study farms showed a large variability in size (number of calves), feeding plan (amount of milk replacer and amount and type of solid feed), microclimate control and management.

Clinical observations

On all farms, the selected batch was observed 3 times during the fattening period for clinical inspection: the first observation was performed at about 3 weeks (range 2–6 weeks) after arrival of the calves at the fattening unit, the second at 13 weeks (range 11–17 weeks) after arrival and the third at the final stage of fattening period which was about 2 weeks (range 1–4 weeks) before slaughter. At the onset of fattening period, calves that were later on housed in small groups were individually housed by temporary separators placed inside the group-pen. These separators were removed after a maximum of 6 weeks and thereafter animals were group housed until the end of fattening period. Consequently, calves were individually housed at the time of the first visit, while they were group housed at the second and the third visit. The protocol for detecting respiratory disorders (including conditions of the observation and a description of the symptoms) was developed in collaboration with field veterinarians specialized in veal production. Three symptoms were taken into account to evaluate the prevalence of respiratory disorders: abnormal breathing, nasal discharge and coughing. A calf exhibited abnormal breathing when at least one of the following signs was observed: tachypnea (breathing frequency higher than 40 breaths/min), enforced abdominal breathing, breathing in a pumping way and excessive nostril movements. Nasal discharge was defined as obvious visible drops from the nostrils that could be transparent to yellow/green. Coughing was recorded when calves dis- played an audible expulsion of air through the mouth.

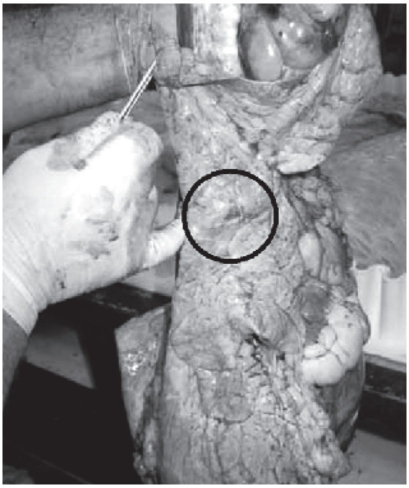
Clinical observations were performed after the morning feeding in small group systems or after the morning visit of the farmer in large groups systems. Visual observation of individual calves was performed from the corridor for calves housed in small groups and from inside the pen for calves housed in large groups. When the size of the batch was ≤200 calves, all calves were observed. When the size of the batch was >200 calves a maximum of 300 calves were observed, which was considered the maximum number of calves that could be assessed for the visit. When calves were housed in small groups, pens were randomly chosen before the observation using pen numbers and all calves in these pens were observed. In large groups, calves were randomly chosen before the observation using their ear-tag numbers from a list. As the farmer could move some calves from one pen to another between farm visits, observations for the 3 visits were performed on the same pens but not necessarily on the same calves.

Observations were performed by experienced field veterinarians who were trained prior to the start of the study to use the protocol mentioned above. Observers were considered sufficiently trained when reaching a sensitivity of at least 80% after judging 240 veal calves from video and 60 calves in live observations on farm.

Post-mortem observations

Post-mortem observations were performed at the abattoir. Since some batches were slaughtered over several days and in several slaughterhouses, for practical reasons, one slaughter day was chosen per farm with the aim to examine lungs of at least 100 calves per batch. Calves that were inspected in the slaughterhouse were not necessarily the same calves that were observed at the farm but in all cases they belonged to the same batch. The assessment was performed directly at the slaughter line before the routine veterinary inspection, in order to include lungs that would have been discarded during inspection. Observations were performed by experienced veterinary pathology technicians or veterinary students who were trained to the procedure prior to the onset of the study. Only assessors who reached a minimum agreement of 0.80 with the gold standard during training were selected for the inspections. The observer visually examined each lung (cranial and ventral lobes) evaluating signs of pneumonia. A pneumonia score was given to each lung using a 4-point scale with score 0 for healthy lungs (pale orange colour with no sign of pneumonia), score 1 for minimal lesions (one spot of grey- red discoloration), score 2 for mild or moderate lesions (one larger or several small spots of grey-red discoloration with a total surface of less than 1 lobe), and score 3 for severe lesions (grey-red discoloration area of at least one full lobe and/or presence of abscesses) (Figure 1).

Score 0



Score 1

Score 2

Score 3

Figure 1
Description of the scores used for the evaluation of lung lesions at post-mortem inspection.
Score 0: no pneumonia (healthy lung with a normal pale orange colour); score 1: minimal pneumonia (one spot of grey-red discoloration); score 2: mild/moderate pneumonia (one larger or several small spots of grey-red discoloration with a total surface of less than 1 lobe); score 3: severe pneumonia (grey-red discoloration area of at least one full lobe and/or presence of abscesses). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

Statistical analyses

Data recorded at the three clinical observations on farm were expressed as percentages of observed calves that showed abnormal breathing, nasal discharge and coughing per batch. Data obtained during post-mortem inspections were expressed as percentages of lungs for each lesion score (scores 0, 1, 2 and 3) per batch. Batch was used as statistical unit and statistical analyses were performed using Genstat software (GenStat Committee 2000).

Comparison of proportions of calves with clinical signs of respiratory disorders at different time points was analysed by estimating the differences between two time points using Wilcoxon matched-pairs sign rank test. Associations between clinical signs of respiratory disorders observed on farm at different time points and lung lesions recorded at slaughter were assessed by calculating correlation coefficients between variables using Spearman rank correlations (rsp). Both analyses considered $p < 0.05$ as minimum threshold of statistical significance.

A further set of analysis compared the prevalence of moderate plus severe (score 2 plus 3) lung lesions observed for the 174 batches with a benchmark value above which the health situation of the batch should be considered seriously compromised (Botreau et al., 2007b). Four veterinarians specialized in veal calves were asked independently to define this threshold that was set as average of their opinion (being 21% of lungs showing moderate or severe lesions). Those batches with a prevalence of (moderate plus severe) lung lesions that exceeded the threshold (21%) were referred to as 'positive' batches. The evaluation of the possibility to detect positive batches by use of clinical signs recorded on farm was focused on clinical data measured 2 weeks before slaughter, since at this time clinical data had the highest correlation with lung lesions recorded post-mortem (see Section 3). A receiver operating characteristic (ROC) curve was constructed for each clinical sign, and the area under the ROC curve (AUC) was calculated to acquire the accuracy of the prediction. The optimal cut-off points on the ROC curves (percentage of calves expressing the symptom) were determined by selecting the optimal sensitivity (SE) and specificity (SP) using the following formula: minimal value of $[(1 - SE)2 + (1 - SP)2]$ (Akobeng, 2007a). Sensitivity, specificity and positive predictive values were calculated for the selected cut-off point for each clinical sign. In order to examine whether the detection of batches of calves with a high prevalence of lung lesions based on clinical examinations would improve if multiple clinical signs were considered, a logistical regression analysis was carried out with lung lesion scores as a binary response variable (1 for a prevalence above 21% and 0 otherwise). The 9 proportions in the three classes for clinical signs at the three time points (i.e., abnormal breathing, nasal discharge and coughing at 3 weeks, 13 weeks, and 2 weeks before slaughter) were introduced as so many explanatory variables on the logit scale. The optimal linear combination of the explanatory variables was selected, using maximum adjusted R2 as a criterion. From a range of boundary values for this linear combination, and several others, sensitivities and specificities were evaluated to construct an ROC curve. This is equivalent to the use of a range of boundary values running from 0 to 1 for the fitted values on the original probability scale of the logistic regression.

Results

Prevalence and evolution during fattening of clinical signs of respiratory disorders

The size of the observed batches ranged from 75 to 1985 calves. On average, 60% of the calves of a batch were observed during the farm visits (range 15–100%).

Descriptive statistics for the 3 clinical signs of respiratory disorders recorded on farm during fattening are reported in Table 1. The proportion of calves showing abnormal breathing was 3.7 ± 0.4% (SE) at 3 weeks of fattening and showed a significant decrease with the progress of the fattening period. A relatively high prevalence of this respiratory symptom was found in some batches, with a maximum of 28% of calves, at 3 weeks of fattening. Nasal discharge was the most prevalent respiratory disorder observed, with higher prevalence at 3 weeks of fattening and at two weeks before slaughter (6.3 ± 0.5% and 6.8 ± 0.6% of calves) as compared to 13 weeks of fattening (5.0 ± 4.5%). High values of nasal discharge (over 30% of calves) were detected in some batches at any stage of the fattening period. The proportion of calves with coughing was comparable between 3 weeks and 13 weeks of fattening (4.7 ± 0.3% and 4.2 ± 0.3% of calves) while it was lower 2 weeks before slaughter (3.2 ± 0.3%). The maximum prevalence for coughing was 39% in one batch of calves and it was recorded at the end of fattening.

Table 1
Respiratory disorder prevalence (% of calves) in 174 batches of veal calves at three stages of the fattening period.

Item	3 weeks of fattening		13 weeks of fattening		2 weeks before slaughter	
	Mean ± SE	**95% range**	**Mean ± SE**	**95% range**	**Mean ± SE**	**95% range**
Number of calves observed per batch	252 ± 5.8	75 - 323	253 ± 5.7	99 - 328	248 ± 5.7	95 - 314
Abnormal breathing (%)	3.7 ± 0.4^{a}	0 - 18.3	1.2 ± 0.1^{b}	0 - 5.0	0.7 ± 0.1^{c}	0 - 3.6
Nasal discharge (%)	6.3 ± 0.5^{a}	0 - 24.8	5.0 ± 0.5^{b}	0 - 22.6	6.8 ± 0.6^{a}	0 - 28.5
Coughing (%)	4.7 ± 0.3^{a}	0 - 15.0	4.2 ± 0.3^{a}	0 - 15.9	3.2 ± 0.3^{b}	0 - 12.1

$^{a, b, c}$: values within the same row with different superscripts differ significantly ($p < 0.05$, Wilcoxon matched-pairs sign rank test).

Prevalence of lung lesions at post-mortem inspection

The lungs of 32% (range 7–99%) of the calves were inspected post-mortem at the slaughterhouse on 170 batches. More than half of the inspected lungs showed one or more signs of lesions (Table 2); 29.1% had minimal lesions (score 1), 13.9% had mild or moderate lesions (score 2) and 7.7% had severe lesions (score 3).

Table 2
Lung lesion prevalence in 170 batches of veal calves.

		Mean ± SE	95% range
Number of lungs observed		137.9 ± 4.5	30 - 200
Lungs with no lesion,%	Score 0	49.3 ± 1.3	12.9 - 79.5
Lungs with lesions,%	Score 1	29.1 ± 0.8	11.5 - 51.0
	Score 2	13.9 ± 0.6	2.0 - 32.9
	Score 3	7.7 ± 0.7	0.0 - 36.5

Correlations between clinical signs of respiratory disorders and lung lesions

Abnormal breathing at 3 weeks of fattening was significantly positively correlated with the proportion of lungs without lesions and negatively correlated with minimal lesions (score 1; Table 3). Nasal discharge at 3 weeks was negatively correlated with minimal lesions, while nasal discharge and coughing were both positively correlated with moderate lesions (score 2). At 13 weeks of fattening, abnormal breathing was not correlated with any of the lung lesion scores whereas nasal discharge and coughing were positively correlated with moderate and severe lesions (scores 2 and 3) and negatively correlated with no or minimal lesions (scores 0 and 1). Two weeks before slaughter, abnormal breathing, nasal discharge and coughing were positively correlated with the percentages of lungs showing moderate (score 2) and severe (score 3) lesions with correlations between 0.18 and 0.40. The three respiratory disorders were all negatively correlated with no lung lesions (score 0; Table 3).

Table 3
Spearman rank correlations between respiratory disorders observed on farms and lung lesions at slaughter in 170 batches of veal calves.

	3 weeks of fattening			13 weeks of fattening			2 weeks before slaughter		
	Ab. breath[a]	Nasal dis[b]	Cough[c]	Ab. breath[a]	Nasal dis[b]	Cough[c]	Ab. breath[a]	Nasal dis[b]	Cough[c]
Lungs with no lesions (Score 0)[d]	0.16*	0.02	0.00	0.14	-0.18*	-0.11	-0.30**	-0.16*	-0.22**
Lungs with lesions[d]									
Score 1	-0.17*	-0.29**	-0.13	-0.15	-0.19*	-0.16*	-0.02	-0.10	-0.10
Score 2	0.04	0.22**	0.21*	-0.06	0.32**	0.19*	0.23**	0.18*	0.27**

[a] % of calves with abnormal breathing. [b] % of calves with nasal discharge. [c] % of calves coughing. [d] % of calves. * p < 0.05 ; ** p < 0.01.

Receiver operating characteristic (ROC) curve analysis for clinical signs to detect batches with high levels of lung lesions

When considering the threshold of 21% of lungs with a moderate or a severe lesion, the prevalence of 'positive batches for lung lesions' was 37.5%. The ROC curves for the three clinical respiratory signs at 2 weeks before slaughter are shown in Figure 2. The areas under the curves (AUC) were 0.72 for coughing, 0.64 for nasal discharge and 0.69 for abnormal breathing. For abnormal breathing the optimal cut-off value was 0.4% with a sensitivity of 60.0% (95% CI of 47.1–72.0), a specificity of 72.2% (95% CI of 62.8–80.4), and a positive predictive value of 56.5% (95% CI of 44.0–68.4). For nasal discharge the optimal cut-off value was 5.0% with a sensitivity of 55.4% (95% CI of 42.5–67.8), a specificity of 70.4% (95% CI of 59.8–78.0), and a positive predictive value of 52.2% (95% CI of 39.8–64.4). For coughing the optimal cut-off value was 2.5% with a sensitivity of 61.5% (95% CI of 48.6–73.4), a specificity of 75.0% (95% CI of 65.8–82.8), and a positive predictive value of 59.7% (95% CI of 47.0–71.5). The logistic regression model that predicted high prevalence of lung lesions best, comprised the following four clinical measures: abnormal breathing at 3 weeks, coughing at 3 weeks, nasal discharge at 13 weeks, and abnormal breathing 2 weeks before slaughter. Using this multivariable model for the detection of batches with high prevalence of lung lesions did not significantly improve the quality of the ROC curve (Figure 2). The AUC using a multivariable approach was only slightly higher than the AUC for ROC curves for single clinical signs: 0.77.

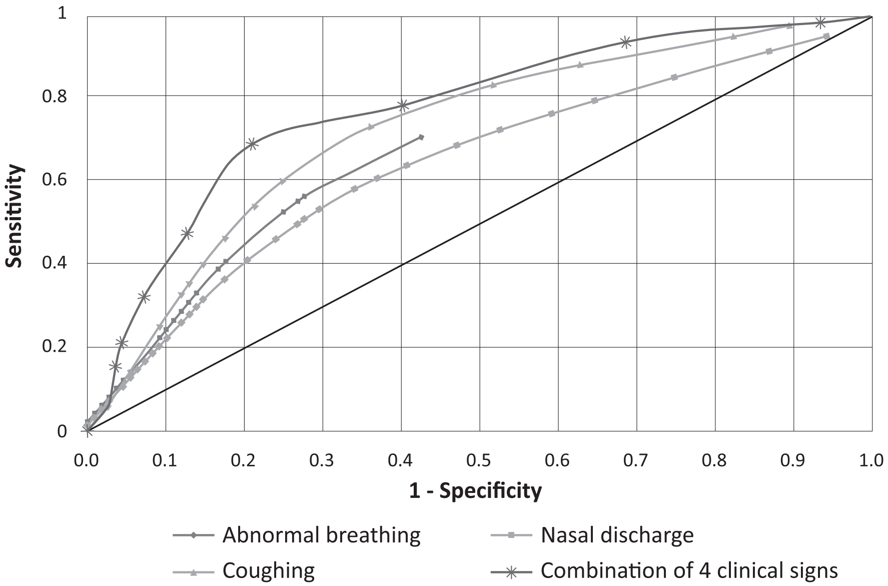


Figure 2
Receiver operating characteristic (ROC) curves for the detection of severe lung lesions using the 3 clinical respiratory signs 2 weeks before slaughter and a combination of four clinical signs of respiratory disease recorded during the fattening cycle at 3 weeks, 13 weeks, and 2 weeks prior to slaughter.

Discussion

The current study aimed at investigating the relationship between respiratory disorders in batches of veal calves observed at three stages of the fattening period and lung lesions recorded at the abattoir.

The proportion of veal calves in a batch expressing one of the clinical respiratory disorders in vivo did not exceed 6.8% on average at either stage of the fattening period. The highest level of abnormal breathing was observed at the early stage of the fattening period. This is in accordance with other studies that concluded that respiratory disorders are most often encountered in the early stage of fattening in veal calves (Miller et al. 1980; Wilson et al. 2000; Timmerman et al. 2005). Wilson et al. (2000) reported a prevalence of audible breathing and/or coughing in veal calves of 12.7% at day zero and 13.4% 3 weeks after arrival at the fattening unit, whereas the same measure had the lowest prevalence at the end of the fattening period (1.7%). Predisposing factors for these respiratory disorders include the low energy status of calves at arrival in the unit, the decrease in time of maternal immunity that impair the coping with the receiving environment made of new housing facilities, pen-mates, stockmen, and management (Miller et al. 1980; Autio et al. 2007). This status may be aggravated by other risk factors such as the conditions on the farm of birth, conditions during transport, and the exposure to pathogens at the veal unit. In the present study, the prevalence of abnormal breathing and coughing showed a decrease over time during the fattening period. The decrease in observed proportion of calves with abnormal breathing towards the end of fattening may be due to medical treatments, recovery of sick calves or mortality of calves. Nasal discharge did not show a change in time except for a slight decrease at 13 weeks of fattening. This result suggests that nasal discharge in calves may not be only due to pathogens. For instance dust particles can stimulate the production of mucus to clean the respiratory tract (Curtis 1972); this production of mucus can result in nasal discharge as well.

The prevalence of lung lesions was much higher than the prevalence of clinical signs. On average, about half of the calves showed lung lesions at slaughter. In 1987, Van der Mei and Van den Ingh recorded 17% of veal calves with extensive lung lesions but no more recent field data are available for veal calves. Lung lesions are frequently found in 10–15 month-old feedlot steers as a prevalence between 33% and 72% of lungs lesions were found by Gardner et al. (1999), Thompson et al. (2006), Wittum et al. (1996) and Schneider et al. (2009).

Although most correlations between clinical signs and lung lesions were significant, their range was very wide (from 0.16 to 0.40). The strongest positive correlations were found between all clinical respiratory disorders observed 2 weeks before slaughter and moderate and severe lung lesions at slaughter. In the same way, the strongest negative correlations were found between clinical respiratory signs and no or minimal lesions, mostly 2 weeks before slaughter. Post-mortem recording of lung lesions at the abattoir, however, also gave an indication of respiratory disorders encountered earlier in the fattening of calves since nasal discharge and coughing recorded in the middle of the fattening period (13 weeks) were positively correlated with prevalence of moderate and severe lung lesions. There is a lack of literature on the biological relevance of the level of correlations (Thompson et al. 2006; White and Renter 2009). On the one hand, post-mortem lesions such as chronic inflammation, parenchymal fibrosis, non functional or flat tissues (atelectasis) can result from chronic respiratory disorders (Le Frapper et al. 2003) and probably indicate respiratory disorders encountered by a calf for the past weeks or even the full length of the fattening. On the other hand, early respiratory disorders will not necessarily result in lung lesions as these might heal or become minimal by the time of slaughter, as suggested by Thompson et al. (2006). This is consistent with the current findings that correlations between clinical signs and lung lesions increased with increasing age at which the clinical inspection was performed. The dataset of our study did not include the medical treatments received by the calves during fattening. The quality of these treatments, however, could be a predominant factor in the recovery of calves and, correspondingly, on the level of healing of the lungs at the time of slaughter. This would mean that, in order to produce an accurate assessment of the respiratory status of a given batch of veal calves, data obtained at farm level and at the slaughterhouse should be integrated with farm records of medical treatments. Furthermore, in the present study calves were not identified individually, therefore, results obtained on-farm and post-mortem were likely based on a different sample of calves within batch, which may have weakened the correlations. Therefore, to identify the best set of animal-based measures reflecting respiratory disorders in veal calves, additional research is required such as a longitudinal follow up of the same animals.

The possibility to detect batches with an abnormal level of lung lesions by recording the prevalence of clinical signs on farm two weeks before slaughter was assessed by ROC curve analyses. The three clinical signs recorded 2 weeks before as well as a multivariable approach gave equivalent results. The positive predictive values found can be considered as low given the fact that only 50–60% of the batches considered as positive according to one of the clinical signs were above the positive threshold for lung lesions. In the sample, the prevalence of 'positive batches for lung lesions' was relatively high and we could expect, therefore, high positive predictive values (Akobeng 2007b). Furthermore, the ROC area under curve barely reached the values of 0.70 for individual clinical signs and 0.77 for a multivariable model, indicating moderate accuracy (Swets 1988). Therefore, we can consider that clinical signs recorded at 3 separate time-points during the fattening period did not allow an acceptable detection of 'positive batches' for lung lesions. Several factors could explain these results. As indicated earlier, data obtained on-farm and post-mortem could be based on a slightly different sample of calves in the batches, which may explain the moderate predictive values found. In addition, the correlations between clinical signs and lung lesions remained relatively moderate and the biological link between clinical signs and pathological findings on lungs at slaughter still has to be confirmed. At last, the threshold set for the evaluation of 'positive batches for lung lesions', even if reflecting a consensus between experienced veterinarians, is only partly objective. Further studies are required to evaluate if the predictive values of clinical signs could be improved using different thresholds. As found earlier by White and Renter (2009), our results preclude the exclusive use of clinical signs and would argue in favour of including post-mortem observations in a monitoring system for veal calf welfare.

At individual calf level, a respiratory disorder results in impaired welfare. At batch level, one of the objectives in terms of welfare is to minimize the prevalence of respiratory disorders in order to avoid a deterioration of the level of welfare. In relation to the development of an on-farm animal welfare monitoring scheme a scientifically sound selection of reliable measures for the assessment of the occurrence of respiratory problems is required (Botreau et al. 2007a). The present findings show that reducing the number of measures for the assessment of respiratory dis- orders might result in a less precise evaluation of welfare as the measures are not strongly correlated.

Conclusions

The prevalence of clinical signs remained low at whatever stage of the fattening period. Nevertheless half of the calves showed lung lesions post-mortem, indicating that respiratory disorders are a relevant matter in terms of welfare for veal calves. Lung lesions at slaughter were correlated with clinical signs two weeks before slaughter and, to a lower extent, in the middle of the fattening period. However, clinical signs observed two weeks before slaughter do not seem to be an accurate measure to detect batches with a high proportion of calves with moderate/severe post-mortem lung lesions. Therefore, we suggest that clinical observation cannot be used as the sole welfare indicator for respiratory health in a reliable monitoring scheme of veal calves' welfare.

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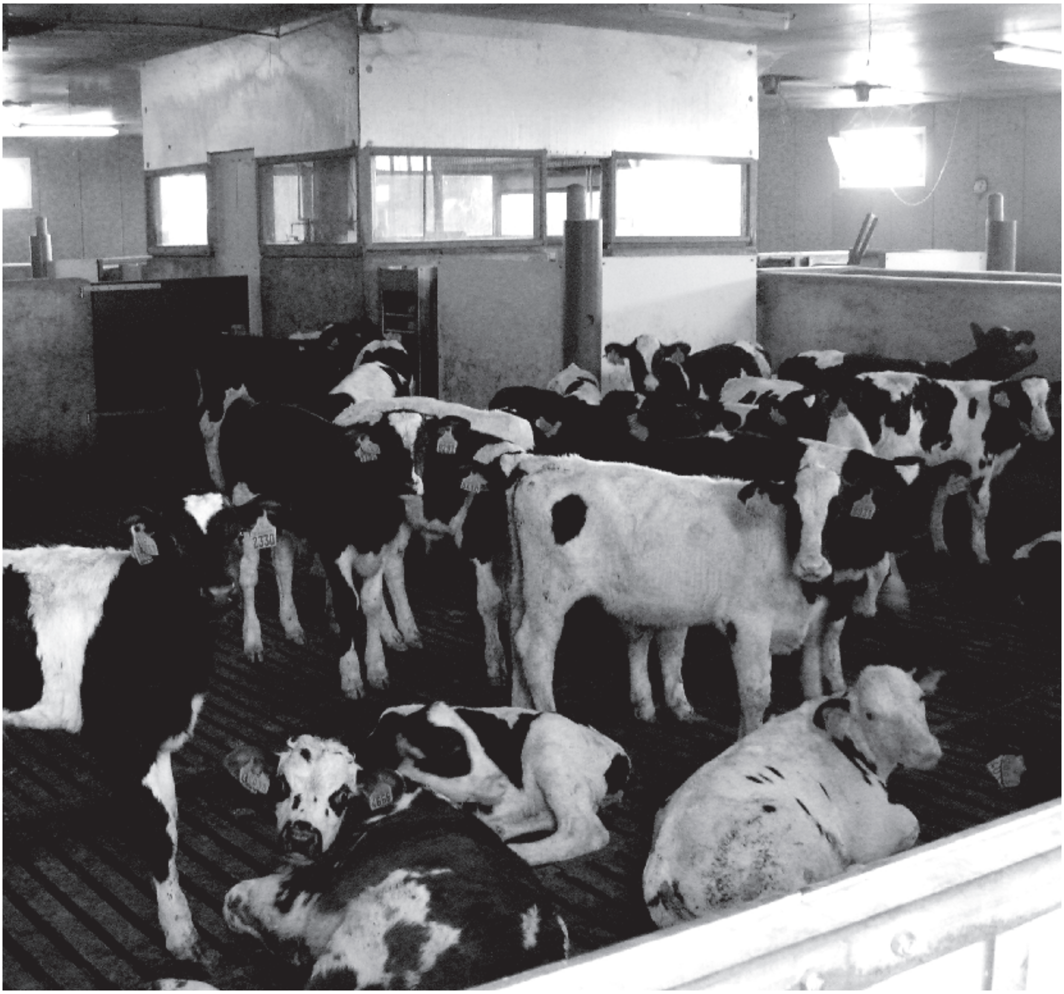
CHAPTER 7

Prevalence of respiratory disorders in veal calves and potential risk factors



CHAPTER 7

Prevalence of respiratory disorders in veal calves and potential risk factors

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Abstract

The study aimed to assess the in vivo and postmortem prevalence of respiratory disorders in veal calves and investigate risk factors associated with them. A cross-sectional study was carried out in 174 farms in the 3 major veal meat–producing countries in Europe (50 in France, 100 in the Netherlands, and 24 in Italy). Trained veterinarians visually evaluated individual calves of 1 batch per farm at 3 and 13 weeks after arrival and at 2 weeks before slaughter to assess the prevalence of hampered respiration, nasal discharge, and coughing. A random sample of lungs belonging to calves of the same batch was monitored at the slaughterhouse for mild to moderate or severe signs of pneumonia, and presence of pleuritis. Data regarding veal calf housing, feeding, and management and specific characteristics of the batch were collected through an interview with the stockperson, and the potential of these as respiratory disease risk factors was assessed. Regardless of the stage of fattening, the prevalence of in vivo signs of respiratory disorders in calves was always <7%. This low prevalence was likely the outcome of the general implementation by veal producers of standardized practices such as prophylaxis, all-in/all-out, and individual daily checks of the calves, which are recognized tools for effective disease prevention and management. However, at postmortem inspection, 13.9% and 7.7% of lungs showed mild to moderate and severe signs of pneumonia, respectively, and 21.4% of the inspected lungs had pleuritis. Thus, even mild clinical signs of respiratory disorder in calves at specific time points during the fattening period may be associated with high prevalence of lungs with lesions at slaughter. Alternatively, clinical symptoms recorded during routine visual inspections of veal calves on-farm may be poor predictors of the true prevalence of respiratory disease in calves. Among all potential risk factors considered, those concerning the characteristics of the batch were predominant but factors related to housing, management and feeding equipment were also relevant. Different risk factors were involved at different stages of the fattening period. Therefore, to overcome respiratory disorders in veal calves, different solutions may apply to different stages of the fattening period.

Introduction

Respiratory disorders are a relevant health problem in young cattle, especially those kept indoors (Virtala et al. 1999; Nikunen et al. 2007), and they affect both the health and welfare status of the animal per se and the economic results of the producers. Bovine respiratory disease is complex, characterized by many types of infection, each of them showing peculiar clinical signs and having different economic implications (Snowder et al. 2006). Main clinical signs include nasal discharge, coughing, fever, hampered respiration, inappetence, and depression. Signs may vary from subclinical to severe, which may lead to calf death. In addition to calf mortality and medical treatment costs, respiratory disorders imply economic losses due to slower growth of calves compared with healthy animals (Snowder et al. 2006). A compensatory gain over the entire 7-months fattening period reduces the growth difference between healthy and infected calves in feedlots if the latter are effectively treated (Bateman et al. 1990). Moreover, costs are reported to be significantly greater when labour, calf isolation, increased time on feed, prophylaxis, and metaphylaxis treatments are considered (Snowder et al. 2006).

The etiology of respiratory disorders in young cattle is multifactorial, with different microbiological agents (bacteria, virus, mycoplasma) acting as initiating pathogens or as exacerbating and complicating factors in synergy with other factors mainly related to the rearing environment (Virtala et al. 1999; Arcangioli et al. 2008; Radaelli et al. 2008). Predisposing and causative factors are equally important because inhalation makes the respiratory system constantly exposed to the farm micro-climatic conditions. Most of the extrinsic risk factors impairing respiratory health are related to in- door housing: insufficient space allowance, overcrowded pens, unsuitable air temperature, quick temperature changes, high humidity, air dust, and high ammonia concentration (Lundborg et al., 2005). Therefore, farm micro-climate and environment should be appropriate to give calves a suitable aerial and thermal comfort (Cozzi et al., 2009).

Intrinsic predisposing factors are linked to the animal's innate and adaptive immunity. Veal calves are separated early from their dams, gathered from different farms, and transported to specialized fattening units when they are about 10 to 20 d old. Mixing calves from different farms during collection, transportation, and housing in a new environment exposes them to a heavy infection load including microorganisms against which they may not have colostral antibodies (Autio et al. 2007). Traditionally, veal calves were housed in individual crates for their entire life as a measure to minimize calf-to-calf contact for disease prevention. Today, the mandatory use of group housing imposed by the European regulation (European Council Directives 91/629/EC and 97/2 EC; European Council 1991; 1997) has increased the risk of pathogen dissemination among calves. Veal farmers adopt several strategies to manage this problem. The all-in/all-out system is a biosecurity tool for the control of infectious diseases that implies the cleaning and disinfection of the empty barn before restocking. As an additional preventive measure, several veal units use individual separators (baby-box) during the first 6 to 8 weeks of fattening (European Council Directives 91/629/EC and 97/2 EC; European Council 1991; 1997) to prevent quick spreading of pathogens and cross-sucking (Virtala et al. 1999).

Several studies have investigated the prevalence of respiratory disease in preweaned calves focusing mainly on dairy calves (Sivula et al. 1996; Virtala et al. 1996; 1999) and young beef cattle (Thomas et al. 2002; Hägglund et al. 2007). The main goal of these studies was to identify the pathogens through bacteriological and serological surveys. Incidence and etiology of respiratory diseases were also monitored in nonweaned calves by several authors (Assié et al. 2004; Autio et al. 2007; Arcangioli et al. 2008). No studies are available on the in vivo and postmortem prevalence of respiratory disorders in veal calves and the associated risk factors. The current study aimed to investigate the risk factors for the occurrence of problems at the respiratory system in veal calves, through a cross-sectional study carried out in a large sample of farms in the 3 major veal-producing countries in Europe.

MATERIALS AND METHODS

Farm Sample

To reflect a cross-section of European veal production, the prevalence of respiratory disorders in veal calves was assessed on 174 farms located in the 3 main veal-producing countries in Europe. One-hundred veal farms were monitored in the Netherlands, 50 in France, and 24 in Italy; the sample of farms was chosen to reflect the proportions of the prevailing rearing systems operating in Europe in terms of type of housing (small or large groups) and milk replacer delivery systems (bucket, trough, or automatic feeding device for individual teat feeding). Further details of the sample of farms and husbandry are described in Brscic et al. (2011).

On-Farm Clinical Examination of the Calves

The assessment protocol for examination of calf health status consisted of 3 visits carried out by trained veterinarians on 1 batch per farm. A batch was considered as a group of calves that arrived at the farm at the same time to be fattened and slaughtered together. Arrivals of the tested batches at the fattening units were distributed across all 4 seasons. Three visits were planned at different stages of the fattening period: the first at about 3 weeks after arrival (early stage), the second at almost 13 weeks of the fattening period (middle stage), and the third at about 2 weeks before slaughter (final stage). During each visit, the veterinarian visually assessed the respiratory status of 200 to 300 calves/ farm, avoiding any kind of animal manipulation. The veterinarian recorded the number of animals with evidence (presence/absence as binary measure) of respiratory disorders, including (1) hampered respiration (difficult or laboured breathing, calf performing belly movements, increased respiratory frequency) indicative of acute lower airway disorders; (2) coughing (visible, sudden, and noisy expulsion of air) indicative of acute or chronic lower and/or upper airway disorders; and (3) nasal discharge (clearly visible flow/discharge from the nostrils, transparent to yellow/green and often of thick consistency) indicative of upper airway disorders.

Farm Environment and Management Data

Specific information regarding veal calf housing, feeding plan, and management, along with some distinctive characteristics of the tested batch, were collected through an interview with the stockperson (Table 1). The interviewer used a specific questionnaire developed to obtain this information in a short time avoiding their direct collection by the veterinarian. These data were considered variables potentially associated with an increased hazard of disease in the risk factor analysis (Table 1).

Table 1
Potential risk factors recorded and tested for their association with respiratory disorders in veal calves

Item	*Levels*			
Related to the rearing facilities				
Housing system, calves/pen	Small group (≤ 15)	Large group (> 15)		
Farm size, total number of calves	≤ 300	301-600	601-1200	>1200
Space allowance, m²/calf	≤ 1.8	>1.8		
Type of floor	Slatted wooden	Concrete (slatted or full)	Rubber on top of slats or straw on full floor	
Presence of a specific sickbay	No	Yes		
Environmental enrichment	No	Yes		
Separated lying area	No	Yes	Partly (during a period or for some calves)	
Renovation of the barn, yr	≤ 4	5-8	> 8	
Floor age, yr	≤ 4	5-8	> 8	
Ventilation	Natural	Mechanical	Both	
Presence of a ridge (opening on the roof that may be opened or closed)	No	Yes	Both (present in part of the barn)	
Manure	Under the calves	Scraped outside		
Related to the rearing facilities				
Quality of the batch at arrival	Good	Medium	Poor	
Season at arrival	Spring	Summer	Autumn	Winter
Calves origin	National	Foreign	More Countries (national and foreign)	
Prevalent breed	Dairy type	Dual purpose	Meat x dairy cross	
Percentage of females	0	1-5	> 5	
Estimated calf BW at arrival, kg, average of the batch	≤ 43	44-47	48-51	> 51
Hemoglobin level at 3 and 13 weeks of fattening, mmol/L, average	≤ 5.7	5.8-6.2	> 6.2	
Average number of calves/pen at each visit	≤ 6	7-9	10-15	> 15
Duration of fattening period, wk	< 24	≥ 24		
Related to management and farmer experience				
Prophylaxis treatment of the batch (vaccination and/or antibiotics and/or antiparasites)	No	Yes		
Use of individual baby-boxes	No	Yes		
Duration of baby-boxes use, wk	0	1-4	5-6	> 6
Use of heating	No	Yes		
Sorting/regrouping practice	No	Yes		
Cleaning for all-in/all-out	Everything	Partial	Brush only	No cleaning
Frequency of visits by technician (veal calf feeding expert)	Weekly	Every 2 weeks		
Frequency of visits by veterinarian/ fattening cycle	< 3	≥ 3		
Frequency of visits by farmer/day	≤ 2	> 2		
Farmers' experience, yr	≤ 5	6-15	16-25	> 25
Use of the operating rearing system, yr	≤ 2	3-10	> 10	

Table 1 (Continued)

Item	Levels			
Related to feeding system				
Type of milk delivery system	Bucket	Trough	Automatic milk delivery device	
Total amount of milk-replacer powder, kg/calf per fattening period	280-330	331-380	> 380	
Calves always received ≥14 liquid meals/wk	No	Yes		
Prevalent type of solid feed	Corn silage	Pellets or mixture	Cereal grain[1]	Treated corn[2]
Total amount of solid feed, kg of DM/calf per fattening period	≤ 50	51-100	101-150	151-300
Water provision	Ad libitum	Limited	No water	
Type of drinker	Bucket	Trough	Pipe	Bowl

[1] Barley or corn. [2] Rolled or flaked corn, or both.

Postmortem Inspection

At the slaughterhouse, a random sample of about 100 pairs of lungs belonging to 100 calves of each batch observed during the fattening period were inspected to assess signs of lung disorders. Prior to the regular veterinary inspection, a trained examiner visually and manually examined both lungs and recorded those showing mild or moderate signs (presence of one large or several small spots of grey-red discoloration involving less than 1 lobe) or severe signs of pneumonia (presence of a grey-red discoloration area involving at least 1 full lobe or presence of abscesses, or both) on at least one side of the lungs. Involvement of the pleura by signs of pleuritis (presence of fibrinous attachment) was recorded as binary measure after assessing the whole lungs.

Training

To ensure that accurate and comparable observations were obtained, all examiners involved in clinical or pathological examinations in the 3 countries were subjected to a rigorous joint training prior to the start of the actual assessments on-farm or at the slaughter- house. The training comprised theoretical and practical parts. During the theoretical part, trainees assessed sets of photos and videos with a known score (gold standard). Each trainee had to pass a final exam and reach at least 80% agreement with the gold standard (binomial test, $P < 0.001$). The practical part considered the clinical assessment of live animals on-farm, and the in-line assessment of lungs at the slaughterhouse. Trainees teamed up with an experienced examiner, and a trainee was assumed to have successfully passed the practical test when the percentage of agreement with the experienced examiner was at least 80% out of 40 successfully assessed calves or lungs.

Data Processing and Risk Factor Analysis

Raw data from clinical examination of calves and postmortem inspection of lungs were expressed as percentages of observations and analysed using GenStat (GenStat Committee 2000), with farm as experimental unit. Prevalences of respiratory disorders were considered as response variables (Y), and the explanatory variables (X) were the potential risk factors obtained from the questionnaire as listed in Table 1. The distribution of farms for each continuous risk factor (e.g., covariable, such as farm size or total amount of solid feed) included in the dataset was displayed with the use of histograms. Subsequently, all covariables were transformed into class variables (Table 1). In case of normally distributed covariables, thresholds for classes were chosen mainly according to location parameters such as lower, median, and upper quartile. For non- normally distributed covariables, thresholds were arbitrarily chosen in such a way that sufficient numbers of farms were present in each class. In the case of the space allowance covariable, a cut-off point equal to the minimum legal requirement of 1.8 m2/calf for calves with a BW >220 kg (European Council Directive 97/2 EC; European Council 1997) could be conveniently applied. Risk factor analyses were carried out separately for each respiratory disorder recorded in vivo at each of the 3 stages of fattening and for all postmortem measurements (% lungs with mild/moderate signs, % lungs with severe signs, and % lungs with pleuritis), regardless of the possible correlations that could be found between them. Correlations between in vivo and postmortem signs were statistically significant but moderate to low (H. Leruste, unpublished data).

Potential risk factors were at first inspected individually adopting generalized linear model univariate analyses, and those significantly associated with the dependent animal measurement for $P < 0.10$ were further included in the multivariate analysis. A two-way ANOVA was then carried out to test, for each pair of risk factors, the significance of main effects and their interaction and to evaluate potential multicollinearity between risk factors through the inspection of the distribution of farms across combinations of risk factors and calculation of the variance inflation factor. High variance inflation factors for the dummy variables for discrete risk factors may point to multicollinearity, and partial confounding between risk factors was automatically signaled. Selection of risk factors was performed both by stepwise forward and stepwise backward selection. The criterion in backward and forward selection was the adjusted R2. On the union of final models of both selection procedures, best subset selection was performed and significance tests for the effects of the selected risk factors were evaluated. Only risk factors that added significance ($P < 0.05$) to the model were retained. Consistent with the methodological approach used by Somers et al. (2005) and Cramer et al. (2009) in previous risk analysis on dairy cattle, risk factors that were (partially) confounded were not simultaneously maintained in the same model and only main effects were considered without interactions.

Because the response variables were always prevalences; that is, percentages per farm (batch), logistic regression models were used, specifying a binomial variance function with a multiplicative dispersion parameter. Estimation was by quasi likelihood. The dispersion parameter in the variance was estimated from the data (McCullagh and Nelder 1984). Soundness of the model was assessed according to the normal distribution of the residuals. Predicted prevalence, odds ratio, and the 95% CI were obtained, and the lowest predicted prevalence of a given problem was used as the reference value for the pairwise comparison between levels.

RESULTS AND DISCUSSION

Observed Batch Size and Farm Characteristics

The average size of the evaluated batch was 252 ± 77 (SD) calves, and about 32% of calves were followed at the slaughterhouse, with an average of 138 ± 59 (SD) pairs of lungs inspected per batch. The majority of farms included in the sample (149/174, 86%) had a group size of fewer than 16 calves/pen, most (73%) had fewer than 7 calves/pen, whereas the remaining farms had a group size of more than 15 calves/pen. The study was dominated by male dairy-bred calves, reared throughout the year: 70% of batches had less than 5% heifers, and farms raising dual- purpose and crossbred calves comprised 16.5% and 17% of the total, respectively. Farm size varied across the sample and 4 categories were identified: 22% of farms reared ≤300 calves, 29% reared 301 to 600 calves, 35% reared 601 to 1,200 calves, and 13% of farms reared >1 200 calves per year. The fattening period lasted an average of 25.8 ± 3.7 (SD) weeks.

Seventy-nine percent of farms complied with the legal requirement for space allowance of 1.8 m2/ calf for a calf weighing >220 kg, and 21% of farms exceeded the space allowance for the entire fattening period. Regarding the type of pen floor, 79% of the farms had fully slatted wooden floors, 15% had concrete floors (slatted or solid), and only 6% of the farms used rubber-covered slatted or straw-bedded floors. None of the farms offered calves access to an outdoor area. Individual baby-boxes to separate pen-mates in the early stage of fattening were used on 83% of the farms. Calves were regrouped during fattening on 94% of farms to harmonize growth within pen.

Prevalence of Respiratory Disorders Recorded In Vivo and at Postmortem Inspection

Prevalence of respiratory disorders recorded during the 3 clinical visits are shown in Figure 1; among the 3 criteria considered, nasal discharge always showed an incidence of 5% or higher. Hampered respiration and coughing were less frequently observed and their prevalence tended to decrease as calves aged. The prevalence of these 2 disorders at 3 weeks of fattening could arise from the common practice of commingling calves originating from different dairy herds in a single batch. These very young animals may not have antibodies to counteract the spread of diseases. The prevalence of hampered respiration showed a large reduction in the later stages of the fattening, likely because of the metaphylaxis provided by the farmers. Farmers consider this clinical sign to be related to a severe respiratory disorder and are likely to promptly treat calves exhibiting this sign (Callan and Garry, 2002; Assié et al., 2004). Recorded prevalences of hampered respiration at 13 weeks of fattening and at 2 weeks before slaughter were too low to allow us to conduct a risk factor analysis. On the contrary, nasal discharge and coughing are not considered signs of severe disease; therefore, farmers likely do not seek specific pharmacological treatments until a large part of the batch is involved or when single calves show signs of severely impaired health. Respiratory disorders are reported within the main causes of mortality in dairy calves (Virtala et al. 1996; Svensson et al. 2006), and the progressive decline of hampered respiration and coughing could be related to calf mortality. The cumulative average values for mortality recorded in this study were 1.0, 2.1, and 3.1% of calves by week 3 and 13 of fattening and 2 weeks before slaughter, respectively.

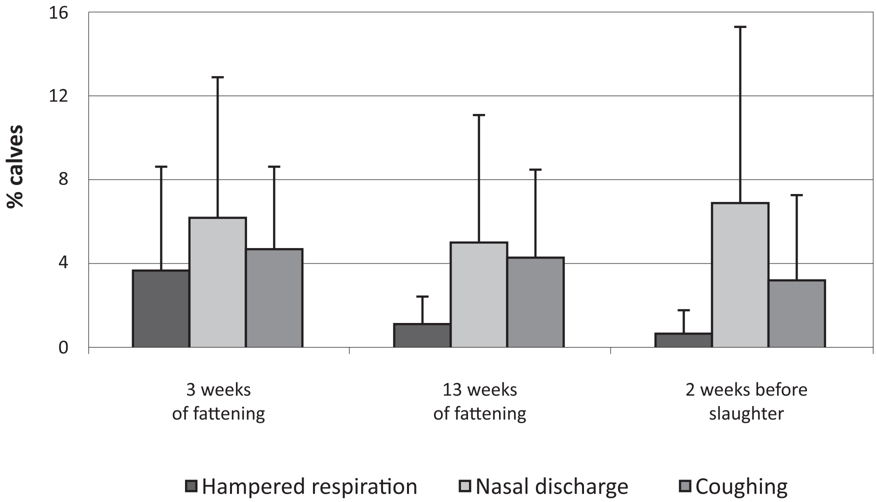

Figure 1
Prevalence of respiratory disorders as a percentage of observed calves (mean ± SD) in 174 veal calf farms in 3 stages of the fattening period.

In this study, the signs of respiratory disorders recorded in vivo showed a generally low incidence compared with the outcomes of studies carried out on replacement dairy calves (Virtala et al. 1996; Nikunen et al., 2007). This might be due to a combination of 2 factors. The first is the use of pharmaceutical treatments at the group level implemented in veal production to control disease outbreak that could cause apparent recovery with decline of symptoms. The second is the different assessment methodology used for group-housed veal calves compared with that of replacement dairy calves, whose housing conditions differ (Virtala et al. 1996; Lago et al. 2006; Nikunen et al. 2007).

Prevalence of signs of respiratory diseases recorded postmortem was moderately high: on average 13.9 and 7.7% of lungs had mild to moderate and severe signs of pneumonia, respectively, and 21.4% of lungs exhibited signs of pleuritis. The apparent difference be- tween prevalences obtained at the in vivo examination and the postmortem inspection in the present study suggests that even mild clinical signs of respiratory disorders in veal calves at specific time points during the fattening period may be associated with a high prevalence of lungs with lesions at slaughter, perhaps because silent pathogens persisted in clinically healthy animals, as observed by Jaeger et al. (2007). Therefore, clinical symptoms scored during routine visual inspections of veal calves on-farm may be poor predictors of the true prevalence of respiratory disease in calves. In our study, on-farm clinical examinations took place only at specific time points; therefore, we were unable to determine the actual severity of episodes of respiratory disease (e.g., for how many days animals exhibited clinical signs). Moreover, episodes of respiratory disease outside the specific time points when clinical examinations took place were missed entirely. In contrast, lung lesions likely reflected the cumulative effect of all respiratory diseases experienced across the entire fattening period. It seems unlikely that clinical symptoms revert by time of slaughter or during transportation to the slaughterhouse because detected signs on lungs were chronic and transport of finished veal calves was shown not to compromise their health status (Grigor et al. 2004).

Risk Factors for Respiratory Disorders

Considering all potential risk factors for the occurrence of respiratory disorders in veal calves, the avail- able literature is mainly addressed toward poor indoor housing conditions (Lundborg et al. 2005; EFSA 2006; Cozzi et al. 2009). Some of the risk factors identified in the present study were linked to characteristics of the rearing facilities and to the equipment necessary to ensure a good farm environment but they were not predominant. Respiratory disorders were mainly associated with specific traits of the batch but also to farm management, the farmer's experience, and the feeding system. Practices that could be related to the control of respiratory disease outbreaks such as prophylaxis, cleaning for all-in/all-out, presence of a specific sick- bay, and frequency of calf health checks did not show significant relevance. This might be due to the lack of variability between farms because these practices are commonly used in the majority of the veal-producing units and therefore they could have already efficiently reduced the prevalence of respiratory disorders, justifying their general low prevalence observed in vivo.

Specific batch characteristics, particularly the estimated BW of the calves at arrival at the fattening unit, were risk factors for hampered respiration at the early stage of fattening (Table 2). Calves arriving at the farm with lower BW showed a higher risk of hampered respiration compared with heavier calves at the 3-weeks assessment. This was likely due to a poorer energy or immunological status impairing their ability to cope with the receiving environment of new housing facilities, pen-mates, stockmen, and management (Miller et al. 1980; Autio et al. 2007). However, a lower BW was not a significant risk factor for hampered respiration at subsequent inspections. It is possible that farmers promptly treated the lightest and weakest calves, determining their quick recovery and this would justify the reduction of hampered respiration prevalence at the middle and end of the fattening period.

Batches judged by the farmer to be good quality at arrival showed an increased risk of nasal discharge at the end of the fattening (Table 3). A skilled stockperson should guarantee constant attention toward all animals throughout the fattening period, regardless of their general appearance and BW at arrival. Gulliksen et al. (2009) reported that risk of respiratory disorders might increase with reduced individual inspection of calves. In our study, a higher prevalence of hampered respiration in young calves was observed when the number of calves/pen increased (Table 2), likely due to less accurate control of individual calves' health. The quick detection of sick calves by the stockperson during milk feeding is probably delayed in larger groups, allowing the spread of pathogen among pen-mates (Lundborg et al. 2005; Svensson and Liberg 2006).

Table 2
Multivariate regression model for occurrence of hampered respiration in veal calves at 3 weeks of fattening (adjusted R^2 = 25%)

Risk factor and levels	Predicted prevalence (%)	Odds ratio	95% CI	*t*-value of pairwise comparison	P-value
Estimated calf body weight at arrival, kg					
≤ 43	7.6^a	3.13	1.63-5.99	0.001	0.004
44-47	6.1^a	2.43	1.31-4.51	0.006	
48-51	6.6^a	2.68	1.44-5.00	0.002	
> 51	2.6^b	-	-	-	
Average number of calves/pen					
≤ 6	2.8^b	-	-	-	0.018
7-9	$3.8a^b$	1.36	0.75-2.47	0.317	
10-15	$6.8a^b$	2.58	0.92-7.28	0.075	
> 15	9.5^a	3.75	1.48-9.52	0.006	
Type of floor					
Slatted wooden	$6.8a^b$	2.20	0.83-5.85	0.115	0.058
Concrete	3.3^b	-	-	-	
Rubber or straw	7.1^a	2.31	1.10-4.87	0.029	
Season at arrival					
Spring	5.0^b	1.34	0.71-2.51	0.364	0.010
Summer	5.5^b	1.50	0.86-2.61	0.158	
Autumn	8.6^a	2.46	1.44-4.23	0.001	
Winter	3.8^b	-	-	-	

[a,b] Values in columns with different superscript letters indicate difference between predicted prevalence of the levels of a factor for P < 0.05.

In dairy cattle, increased risk for respiratory disorders is reported for larger compared with smaller dairy herds (Norström et al. 2000). In the current study, in the early stage of fattening, the risk of nasal discharge increased as farm size decreased. On the contrary, at the end of the fattening period, the risk was lowest in small- to mid-size farms (301 to 600 calves), and this farm size seemed a better option for controlling the prevalence of respiratory disorders (Table 3). However, according to results reported in the literature, factors such as prophylaxis, disease management, and particularly the animal/stockperson ratio, rather than farm size, play a relevant role in the prevalence of respiratory disorders in calves (Waltner-Toews et al. 1986; Assié et al. 2004; Lundborg et al. 2005). In this study, the frequency of calf health checks carried out by the farmer was not relevant in the control of the disease, but frequency of veterinarian visits did influence the prevalence of respiratory disorders. Fewer visits by the veterinarian throughout the fattening period increased the risk of coughing at the end of the period (Table 4). The farmer's skills were shown to be important, because calves reared by farmers who had been using the housing and feeding system for a shorter period had a higher risk of occurrence of nasal discharge at 13 weeks (Table 3) and of mild pneumonia postmortem (Table 5) compared with those reared by farmers with more than 10 years of experience. Timely supervision by the farmer and the veterinarian is likely to allow quicker detection of respiratory problems and earlier treatment of sick calves.

Gathering information from farm health records could have allowed us to validate this hypothesis, but consultation of farm records is not always easy and this was the reason not to implement it in the current study.

The use of individual baby-boxes and the duration of their use (legal up to 8 weeks of age) have shown to be relevant management factors for the prevention of calf respiratory disorders. As expected, the use of individual baby-boxes in the early stage of the fattening period reduced the prevalence of nasal discharge (Table 3) and coughing (Table 4), likely by reducing contact among neighbouring calves. This is in agreement with results reported for dairy calves within 90 d of age by Virtala et al. (1999) and Svensson et al. (2003). It is interesting to notice that prolonged use of these individual separators over 4 weeks was a risk for nasal discharge at 13 weeks (Table 3) and their use for 5 to 6 weeks was a risk factor for coughing at the end of the fattening period (Table 4). Perhaps prolonged forced isolation from pen-mates acted as a chronic stressor due to the lack of opportunity to perform social behaviours, resulting in immunosuppression (Glaser and Kiecolt-Glaser 2005). However, this hypothesis needs to be confirmed in other studies.

Table 3
Multivariate regression model for occurrence of nasal discharge in veal calves at 3 weeks of fattening (adjusted R2 = 31%), 13 weeks of fattening (adjusted R2 = 39%), and 2 weeks before slaughter (adjusted R2 = 46%)

Observation	**Risk factor and levels**	**Predicted prevalence (%)**	**Odds ratio**	**95% CI**	***t*-value pairwise comparison**	**P-value**
3 weeks of	Use of individual baby-boxes					
fattening	No	10.3	1.94	1.43-2.64	< 0.001	< 0.001
	Yes	5.6	-	-	-	
	Farm size, total number of calves					
	≤ 300	10.9[a]	2.28	1.30-4.02	0.005	0.017
	301-600	8.7a[b]	1.76	1.01-3.08	0.047	
	601-1200	7.2[b]	1.43	0.82-2.49	0.209	
	> 1200	5.2[b]	-	-	-	
	Space allowance, m^2/calf					
	≤ 1.8	5.8	-	-	-	< 0.001
	> 1.8	10.2	1.87	1.36-2.56	< 0.001	

Table 3 (Continued)

Observation	**Risk factor and levels**	**Predicted prevalence (%)**	**Odds ratio**	**95% CI**	***t*-value pairwise comparison**	**P-value**
13 weeks of fattening	Duration of baby-boxes use, wk					
	0	1.8[b]	-	-	-	0.003
	1-4	4.2[b]	2.39	0.83-6.89	0.110	
	5-6	6.8[a]	4.00	1.50-10.68	0.006	
	> 6	7.7[a]	4.62	1.75-12.23	0.002	
	Prevalent type of solid feed					
	Corn silage	4.8[ab]	1.46	0.85-2.51	0.174	0.007
	Pellets or mixture	3.4[b]	-	-	-	
	Cereal grain[1]	6.7[a]	2.12	1.28-3.50	0.004	
	Treated corn[2]	5.7[a]	1.78	1.13-2.79	0.013	
	Years of use of the operating rearing system					
	≤ 2	7.7[a]	3.18	1.65-6.16	0.001	0.003
	3-10	5.0[b]	1.98	1.15-3.40	0.014	
	> 10	2.7[c]	-	-	-	
	Type of milk delivery system					
	Bucket	6.6[a]	2.33	1.31-4.16	0.005	0.022
	Trough	3.0[b]	-	-	-	
	AMD[3]	5.9[ab]	2.06	0.55-7.70	0.286	
	Season at arrival					
	Spring	3.2[b]	-	-	-	< 0.001
	Summer	6.2[ab]	2.04	1.29-3.23	0.003	
	Autumn	7.6[a]	2.53	1.54-4.17	< 0.001	
	Winter	3.6[b]	1.11	0.55-2.25	0.765	
2 weeks before slaughter	Quality of the batch at arrival					
	Good	7.7[a]	1.73	1.16-2.57	0.008	0.022
	Medium	4.8[b]	-	-	-	
	Poor	8.2[a]	1.88	0.90-3.90	0.094	
	Farm size, total number of calves					
	≤ 300	7.4[a]	1.97	1.18-3.27	0.010	0.007
	301-600	4.1[b]	-	-	-	
	601-1200	7.4[a]	1.97	1.21-3.20	0.007	
	> 1200	8.6[a]	2.36	1.29-4.32	0.006	
	Duration of the fattening period, wk					
	< 24	14.5	4.17	2.61-6.66	< 0.001	< 0.001
	≥ 24	4.1	-	-	-	
	Ventilation					
	Natural	3.1[b]	-	-	-	< 0.001
	Mechanical	10.1[a]	3.85	1.98-7.49	< 0.001	
	Both	7.5[a]	2.68	1.22-5.89	0.015	
	Ridge					
	No	4.5b	-	-	-	0.014
	Yes	8.5a	2.10	1.30-3.40	0.003	
	Both	7.7ab	1.85	0.88-3.87	0.106	

[a-c] Values in columns with different superscript letters indicate difference between predicted prevalence of the levels of a factor for P < 0.05. 1 Barley or corn. 2 Rolled or flaked corn or both. 3 Automatic milk delivery device.

Table 4
Multivariate regression model for occurrence of coughing in veal calves at 3 weeks of fattening (adjusted R2 = 16%), 13 weeks of fattening (adjusted R2 = 36%), and 2 weeks before slaughter (adjusted R2 = 43%)

Observation	**Risk factor and levels**	**Predicted prevalence (%)**	**Odds ratio**	**95% CI**	***t*-value pairwise comparison**	**P-value**
3 weeks of fattening	Type of milk delivery system					
	Bucket	3.1^{b}	-	-	-	0.019
	Trough	9.1^{a}	3.21	1.09-9.49	0.037	
	AMD[1]	4.8^{ab}	1.58	0.82-3.05	0.173	
	Use of individual baby-boxes					
	No	8.9	3.95	1.18-13.20	0.028	0.015
	Yes	2.5	-	-	-	
13 weeks of fattening	Type of milk delivery system					
	Bucket	5.0^{a}	1.67	1.13-2.46	0.011	0.003
	Trough	3.1^{b}	-	-	-	
	AMD[1]	5.6^{a}	1.88	1.30-2.72	0.001	
	Prevalent breed					
	Dairy-type	5.0^{a}	1.66	1.08-2.55	0.023	0.025
	Dual purpose	3.1^{b}	-	-	-	
	Crossbred	5.6^{a}	1.86	1.16-2.98	0.011	
	Percentage of females, %					
	0	5.1^{a}	1.45	1.02-2.05	0.038	0.036
	1-5	3.6^{b}	-	-	-	
	> 5	5.1^{a}	1.46	1.06-2.02	0.024	
2 weeks before slaughter	Season at arrival					
	Spring	3.6^{ab}	1.54	0.96-2.47	0.076	0.021
	Summer	4.2^{a}	1.82	1.16-2.86	0.010	
	Autumn	2.7^{b}	1.15	0.72-1.84	0.553	
	Winter	2.4^{b}	-	-	-	
	Duration of baby-boxes use, wk					
	0	5.1^{a}	2.63	1.53-4.51	0.001	0.001
	1-4	2.0^{c}	-	-	-	
	5-6	3.3^{b}	1.67	1.08-2.60	0.024	
	> 6	2.4^{bc}	1.21	0.70-2.06	0.497	
	Frequency of visits by veterinarian/ fattening period					
	< 3	3.9	1.60	1.14-2.23	0.007	0.008
	≥ 3	2.5	-	-	-	
	Type of drinker					
	Bucket	4.6^{a}	2.75	1.64-4.60	0.001	0.001
	Trough	1.8^{b}	-	-	-	
	Pipe	3.4^{a}	1.96	1.33-2.89	0.001	
	Bowl	3.9^{a}	2.31	1.32-4.05	0.004	

$^{a-c}$ Values in columns with different superscript letters indicate difference between predicted prevalence of the levels of a factor for P < 0.05. 1 AMD = Automatic milk delivery device.

Table 5
Multivariate regression model for occurrence of mild or moderate signs of pneumonia (adjusted R2 = 18%), severe signs of pneumonia (adjusted R2 = 18%), and pleuritis in veal calves (adjusted R2 = 42%)

Item	Risk factor and levels	Predicted prevalence (%)	Odds ratio	95% CI	*t*-value pairwise comparison	P-value
Mild pneumonia	Years of use of the operating rearing system					
	≤ 2	13.2[ab]	1.35	0.93-1.92	0.118	0.040
	3-10	13.2[a]	1.35	1.07-1.71	0.014	
	> 10	10.1[b]	-	-	-	
	Floor age, yr					
	≤ 4	14.5[a]	1.43	1.16-1.76	0.001	0.003
	5-8	10.6[b]	-	-	-	
	> 8	11.3[b]	1.07	0.85-1.35	0.557	
	Ventilation					
	Natural	9.7[b]	-	-	-	0.001
	Mechanical	11.5[b]	1.21	0.96-1.53	0.112	
	Both	15.2[a]	1.68	1.24-2.20	< 0.001	
Severe pneumonia	Season at arrival					
	Spring	7.7[ab]	2.31	1.20-4.44	0.013	
	Summer	10.5[a]	3.27	1.80-5.95	< 0.001	
	Autumn	6.9[b]	2.05	1.09-3.87	0.027	
	Winter	3.5[c]	-	-	-	
	Water provision					
	Ad libitum	9.7[a]	1.76	1.24-2.49	0.002	
	Limited	5.8[b]	-	-	-	
	No water	6.0[b]	1.04	0.67-1.61	0.853	
Pleuritis	Duration of the fattening, wk					
	< 24	9.3	-	-	-	< 0.001
	≥ 24	27.6	3.75	2.70-5.21	< 0.001	
	Water provision					
	Ad libitum	24.7[a]	1.75	1.22-2.52	0.003	< 0.001
	Limited	16.4[b]	1.03	0.73-1.45	0.880	
	No water	16.1[b]	-	-	-	

[a-c] Values in columns with different superscript letters indicate difference between predicted prevalence of the levels of a factor for P < 0.05.

The season of arrival at the fattening units influenced most respiratory disorders (Tables 2 to 5), confirming that the outcome of these diseases is closely related to the farm micro-climatic conditions (Virtala et al. 1999; Arcangioli et al. 2008; Radaelli et al. 2008). A higher risk of hampered respiration was observed for calves that arrived at the fattening unit during autumn compared with those housed in winter (Table 2), despite a greater expectation of respiratory disorders in the latter season (Diesel et al. 1991; Lundborg et al. 2005; Lago et al. 2006). Calves housed in autumn could have received weakened passive immunity from their dams due to the dams' exposure to summer heat stress during late pregnancy (Callan and Garry 2002). Although it was clear that the season at arrival affected nasal discharge at 13 weeks of fattening (Table 3), coughing 2 weeks before slaughter (Table 4), and the prevalence of severe pneumonia recorded postmortem (Table 5), the time between the outset of the fattening and the observation session should be considered. Indeed, calves housed in spring, spending the warm season in the barn, had lower risk of developing respiratory problems compared with calves housed later in the year. We observed a clear association of an increased risk of nasal discharge and postmortem signs of pneumonia with cold season arrival, which confirms the results obtained by Lago et al. (2006) and underlines that environmental temperature is highly relevant when discussing respiratory problems prevalence, even in standardized indoor housing systems such as those used for veal calves. Although the duration of the fattening cycle was also standardized, averaging about 25.8 ± 3.7 (SD) weeks, contradicting results were observed, with the risk of nasal discharge increasing for calves finished in a shorter period (Table 3), whereas signs of pleuritis were associated with an extended finishing period (Table 5). The latter effect could be associated with slower growth in calves suffering from respiratory disease but no evident conclusions in this regard may be drawn. No firm conclusions can be made for the effects of breed and percentage of female calves, which were significant risks for the occurrence of coughing at 13 weeks of fattening (Table 4). Snowder et al. (2006) reported that relatively small differences in resistance to bovine respiratory disease exist between breed types and that no strong evidence exists that composite breeds are less susceptible than purebreds to disease.

In the literature, factors related to the characteristics of the rearing facilities have been associated with respiratory disorders, indicating that the farm environment should be appropriate to provide calves a suitable aerial and thermal comfort (Cozzi et al. 2009). However, in the present study, some strategies usually applied to increase calf comfort did not yield the expected results, particularly in the early stage of fattening. A higher risk of hampered respiration was found in calves housed on rubber or straw compared with those housed on concrete floors (Table 2), and housing animals with a space allowance >1.8 m2/calf increased the risk of occurrence of nasal discharge compared with a lower space allowance (Table 3). The increased prevalence of respiratory signs when calves were housed on straw-bedded floors was likely due to dust inhalation (Takai et al. 1998). However, straw and rubber flooring solutions are not frequently applied in veal fattening units even though they offer better thermal comfort and avoid potential health risks for calves exposed to a cold environment (Hänninen et al. 2003). A lesser space allowance at the onset of the fattening acting as a preventive measure suggests that calves of very young age cope well in a constrained environment. A limited space allowance may reduce animal activity, thus decreasing exposure to dust and airborne pathogens that have adverse effects on both mucociliary clearance and respiratory defense mechanisms (Callan and Garry 2002). We speculate that a higher concentration of noxious chemical constituents in new wooden floors treated with protective urethane or varnish might explain newer floors (less than 4 years) being risk factors for mild pneumonia signs (Table5). However, other factors may have been confounded with age of the floor that caused this effect.

Prevalence of respiratory disorders was decreased for calves housed in barns with natural compared with mechanical ventilation or a combination of the two, making the presence of mechanical ventilation a risk factor for nasal discharge at the end of the fattening (Table 3) and mild pneumonia signs (Table 5). The presence of a ridge on the roof of the barn to allow air circulation was a risk factor for nasal discharge in the late stage of the fattening (Table 3). This underlines that ventilation is very important for the prevention of respiratory disease in cattle, but it also suggests that calves are very susceptible to drafts. Lundborg et al. (2005) reported an increased risk for respiratory sounds at lung auscultation in calves exposed to draft, whereas they were less likely on farms where ventilation was natural and where the lack of the ridge did not allow air exit from the roof.

Factors related to the feeding system were significantly associated with occurrence of respiratory disorders. In the early stage of fattening, a common trough increased the risk of coughing (Table 4), whereas at the later assessments, it reduced the prevalence of both nasal discharge and coughing (Tables 3 and 4). Perhaps, at an early stage of fattening, when calves are not fully adapted to the new rearing environment, the higher drinking speed allowed by trough feeding along with the absence of teats might increase the risk of accidental inhalation of milk-replacer into the airways, stimulating coughing. In the later stage of fattening, the positive effect of trough feeding on reducing the prevalence of respiratory disorders signs could be related to improved hygienic conditions. The proper cleaning of individual buckets is more time- and labour-consuming than that of either troughs or automatic milk delivery devices. A similar hypothesis might be drawn also for drinking water delivery systems. Compared to the trough, buckets, pipes, and bowls acted as risk factors for coughing, likely because of their lower level of cleanliness and hygiene (Table 4).

Unexpectedly, ad libitum availability of drinking water significantly increased severe signs of pneumonia and pleuritis (Table 5). Drinking water is an important issue for calf welfare because, rather than covering a shortage in calf water requirement, it prevents the arousal of nonnutritive oral behaviours (Gottardo et al. 2002). In our study, this risk factor might be related to drinking behaviour because it is likely that, when water is constantly available, calves play with the drinker, wasting the water and wetting the pen floor, and penmates lay underneath the drinkers. Calves exposed to a moist and cold environment are more sensitive to respiratory problems (Diesel et al. 1991).

The amounts of milk replacer powder and solid feed provided throughout the fattening period, which were highly relevant risk factors for gastrointestinal problems (Brscic et al. 2011), did not affect respiratory disorders. Type of solid feed, however, was associated with nasal discharge; in particular, calves fed cereal grain or treated corn showed a higher prevalence compared with those receiving pellets or mixtures. This might be due to the provision of high-starch solid feeds negatively interfering with calf health. Donovan et al. (2003) showed that the immune response of calves fed an acidogenic diet is weakened, increasing their susceptibility to other pathogens.

Conclusions

The present paper investigated the prevalence of respiratory disorders and associated risk factors in veal calves in a large sample of farms in the main veal-producing countries of Europe. The prevalences detected in vivo were low but the results from postmortem lung inspection suggested that even mild clinical signs of respiratory disorders during fattening should not be neglected. Different risk factors related to batch characteristics, housing, management, and feeding were relevant at different observation time points and, in some cases, controversial findings were observed. This excludes the possibility of carrying out a single visit to a farm or slaughterhouse to identify both problems and risks. Moreover, it is unrealistic to suggest a unique solution to overcome all respiratory disorders. Continuous monitoring of calf health by an experienced farmer, along with repeated herd screenings at strategic time-points by a veterinarian, seems to be the best strategy to plan time-effective interventions to manage disease outbreaks.

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CHAPTER 8

General discussion

CHAPTER 8

General discussion

The aim of this thesis was to contribute to the development of a reliable tool for on-farm assessment of the welfare of veal calves with special emphasis on human-animal relationship, abnormal oral behaviours and respiratory disorders, and to define potential remedial actions that could be taken on farms to address these welfare issues. In this chapter, I will discuss the specific challenges related to the development and the on-farm application of animal-based measures for human-animal relationship tests, abnormal oral behaviours and respiratory problems. Before they can be used on farms, animal-based measures for the assessment of welfare must be validated and fulfill a number of conditions such as feasibility and reliability. The first part of the discussion is related to the development of measures and will address this validation of measures that was examined in chapters 2, 4 and 6. The results of the on-farm assessments, using the animal-based measures that were developed, were associated with various housing and management characteristics of the veal fattening units as demonstrated in chapters 3, 5 and 7. In the second part of the discussion, the risk factors will be discussed to determine how the analysis of influencing factors can contribute to promoting housing and management conditions on farms that could contribute to welfare improvement. The last part of the discussion will address some practical issues related to the on-farm use and the refinement of the welfare assessment tool for veal calves.

Feasibility and reliability of animal-based measures for the assessment of welfare

Welfare is a multidimensional concept (Fraser 1995). Its assessment is a search to collect pieces of information that can be very different in nature. This information should preferably be animal based (e.g. behaviour, health, and post mortem data), but when this is not feasible environmental based information (e.g. water availability, housing conditions) can also be used (Veissier et al. 2011). In order to be included in a welfare assessment tool, measures must comply with minimal standards in terms of validity and reliability (Engel et al. 2003; Knierim and Winckler 2009). Measures should measure what they are intended to measure (validity), should show a high inter-observer and test-retest repeatability (reliability), and should be easy to collect without being too laborious (feasibility). In this thesis, validity, reliability and feasibility were assessed on measures related to human-animal relationship tests, abnormal oral behaviours and respiratory problems (Table 1).

Table 1
List of the three welfare criteria and seventeen measures studied in the thesis (for detailed description see chapters 2, 4 and 6)

Welfare criteria	***Welfare measure***
Good human-animal relationship	% of calves that could be touched (calf escape test score =4)
	% of calves that could not be approached (calf escape test score = 0)
Expression of (abnormal oral) behaviours	% of calves manipulating substrates
	% of calves manipulating substrates
	% of calves tongue playing
	% of calves manipulating penmates
Absence of (respiratory) disease	% of calves coughing (at 3 weeks / at 13 weeks / 2 weeks before slaughter)
	% of calves with nasal discharge (at 3 weeks / at 13 weeks / 2 weeks before slaughter)
	% of calves with hampered respiration (at 3 weeks / at 13 weeks / 2 weeks before slaughter)
	% of calves with mild lung lesions at slaughter
	% of calves with severe lung lesions at slaughter
	% of calves with pleuritis at slaughter

Several behavioural tests were developed measuring the response of calves to humans and to an unexpected stimulus. Out of these tests, the voluntary approach of an unfamiliar human (human approach test, HAT), the reaction of calves to an unfamiliar human approaching (calf escape test, CET) and the voluntary approach of a novel object (novel object test, NOT) were suitable for the evaluation of these aspects. The response of animals to this kind of tests is an indicator of a mixture of emotions such as fear and curiosity they have towards humans and novel objects (Forkman et al. 2007). The measures in these three tests were reliable, farms showed variation for the measures and the measures were correlated to each other (Chapter 2). The CET was the most promising test to include into the welfare assessment protocol for the following reasons. First it showed validity as the reaction of calves to an experimenter was predictive for the reaction of calves to their usual caretaker, i.e. the veal farmer (Chapter 2). Second, the CET was feasible on all farms in both small and large groups of calves; this in contradiction to the other two tests that were influenced by pen size and pen design. In addition, the test made sense to the farmers as they themselves evaluate the 'calmness' of their animals to their reaction when they are entering the barn/pen for routine procedures (farmers' personal communications). The response in the CET is assumed to be a reflection of the type and frequency of contacts calves encounter during their daily interactions with their caretaker, but also visitors such as veterinarians might affect the response (Lensink et al. 2000). A potential source of bias for this type of test is that the responses of calves could be unstable and depend more on the testing conditions such as home environment or behaviour of the experimenter than on the actual fear or curiosity towards humans of the tested calves (de Passillé and Rushen 2005). The good repeatability of the test strongly suggests that this particular bias can be excluded. For all these reasons the CET was selected for the welfare assessment tool.

The second type of measures developed were observations to detect and record abnormal oral behaviours. The most challenging aspect was to find a good compromise between the sampling strategy (number of calves observed and duration of observation) and feasibility of the observations on the farm. The protocol to collect data should permit to detect behaviours that differ from the norm. The observations had to include behaviours that are considered as abnormal in themselves (for instance stereotypic behaviours) but also behaviours that are considered as abnormal when performed at an unusual rate (such as repetitive mounting or excessive licking) (Mason 1991).

The gold standard for a valid measure is considered to be continuous 24h observations, but that is not very feasible. Welfare assessments on farms are generally restricted to a certain maximum duration (e.g. 4 hours, one day maximum), otherwise costs will be too high and farmers will be less willing to cooperate. Moreover, 24h measurement of behaviour usually requires video observation. Direct observations were found to be a more suitable observation method than observations from video recordings because direct observations were more precise for behaviours that are difficult to determine through the observation from video (e.g. tongue rolling) (Chapter 4). Furthermore, direct observations are more flexible and better applicable to perform on many farms as installing cameras at different farms is time consuming and technically difficult. Future technical developments like automated behaviour recording may help to assess behaviours over 24 h periods. This type of assessment, of course, should be of such quality that it is allows accurate recording of the specific behaviours of interest.

Results of the observations of spontaneous behaviours can be affected by the presence of the observer and the moment of the day. The presence of the observer had a short term effect (i.e. disturbance during the observation) but no long term effect (i.e. at day level) on the expression of abnormal oral behaviours in calves. Recorded levels of these behaviours were in general higher than when an observer was absent (Chapter 4). These observed level of (e.g. abnormal oral) behaviours corresponded to the expression of these behaviours in the presence of a human stimulus. The extent of this disturbance should be checked on a larger sample of farms (with both direct observations and observations from video recordings) to verify that the extent of this disturbance is consistent amongst farms. The sampling strategy and sampling moment has to be similar amongst farms as well. As calves, for instance, tend to express more abnormal oral behaviours around feeding (Veissier et al. 1998), the start of observation should be at a fixed moment before or after feeding at all farms (for instance at least more than one hour before or after a meal). Some behaviours - such as abnormal oral behaviours or stereotypies - are known to evolve with the age of animals (Mason 1991; Bokkers and Koene 2001; Webb et al. 2012), so age of the animals also should be considered in on-farm assessment and when farms are compared.

The challenge with the measurement of respiratory disorders was to develop observations that were representative as much as possible for the level of respiratory disorders in a batch of calves throughout the fattening period. Given that some respiratory diseases (such as pneumonia) encountered by calves during their fattening can result in lung lesions that are still visible at slaughter (Wittum et al. 1996), the correlations between clinical signs of respiratory disorders and lung lesions were analysed (Chapter 6). Clinical signs of respiratory disorders recorded during the fattening of calves at specific time points (early fattening, middle of fattening, end of fattening) were not sufficiently predictive for lung lesions recorded post-mortem (Chapter 6) especially when recorded at an early stage of the fattening of calves. The clinical measures nasal discharge, coughing and abnormal breathing give indications of the health status of different parts of the respiratory tract (i.e. lower and upper parts) but their prevalence within a batch of calves can change rapidly over a short period of time. For these reasons, I suggest that clinical signs should not be used as a sole indicator for respiratory disorders in veal calves. The evaluation of lung lesions at slaughter is a promising measure which even might be automated in the future, e.g., through the use of cameras and image analysis.

CAN RISK FACTOR ANALYSIS CONTRIBUTE TO WELFARE IMPROVEMENT?

The welfare assessment tool can be used to identify practical solutions on farm to improve welfare (Veissier et al. 2005). The welfare improvement process requires identifying risk factors for welfare on farms (see Figure 1, introduction section). A risk factor analysis was, therefore, conducted for each of the seventeen welfare measures of human-animal relationship, abnormal oral behaviours and respiratory disorders studied in this thesis (Table 1). In the following section, I will first discuss the outcome of these analyses (proportion of the variability of the measures explained by the models, type of factors influencing welfare measures), then I will discuss the potential use of these results for improvement of welfare of calves by housing and management modifications.

For each welfare measure, a risk factor analysis was conducted. A generalized linear model analysis permitted to select the best set of factors explaining each measure and then multivariate models were selected with the highest adjusted R2 and all factors significantly associated with the welfare measure. Amongst all welfare measures studied in the present thesis, between 12% (for the measure manipulation of penmates) and 46% (for the measure nasal discharge 2 weeks before slaughter) of the variability was explained by the multivariate risk factor models (Table 2). This indicates that although part of the variability remains unexplained, a significant part of the variation could be explained with the selected factors.

Table 2
A summary of the proportion of the variability explained by the risk factor analysis for the seventeen measures of welfare presented in this thesis (chapters 3, 5 and 7).

Welfare measure	***R^2***
% of calves that can be touched (CET =4)	16%
% of calves that cannot be approached (CET = 0)	38%
% of calves manipulating substrates	42%
% of calves tongue playing	21%
% of calves manipulating penmates	12%
% of calves coughing	
- at 3 weeks	16%
- at 13 weeks	36%
- 2 weeks before slaughter	43%
% of calves with nasal discharge	
- at 3 weeks	31%
- at 13 weeks	39%
- 2 weeks before slaughter	46%
% of calves with hampered respiration	
- at 3 weeks [1]	25%
% of calves with mild lung lesions at slaughter	18%
% of calves with severe lung lesions at slaughter	18%
% of calves with pleuritis at slaughter	42%

[1] The prevalence of hampered respiration was too low at 13 weeks and at 2 weeks before slaughter to conduct a risk factor analysis

Most of the welfare measures analysed were explained by factors which are different in nature and related to characteristics of production and housing system, batch of calves, management and farmer experience, characteristics of the feed and feed distribution system. For instance, manipulation of substrates was influenced by factors related to production and housing system (space allowance) as well as characteristics of the calves and the batch (breed, number of calves/pen, season at housing), characteristics of the management and farmer experience (stockperson' experience) and characteristics of the feed and feed distribution system (type of solid feed) (Figure 1).

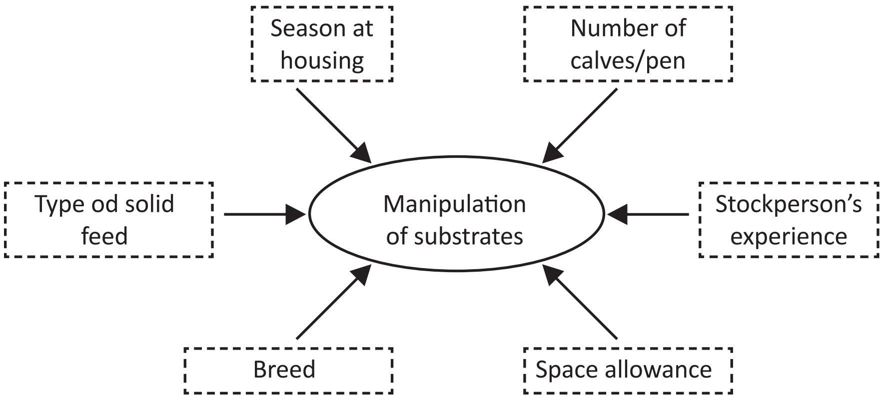

Figure 1
Example of a welfare measure influenced by several factors of different nature. Circle represents the welfare measure, squares represent influencing factors

For on-farm use of the results of risk factor analyses and to provide advice on remedial actions, it is important to know if a factor is influencing only one or several welfare measures (Figure 2). For instance, space allowance above the legal requirement of 1.8 m²/calf would reduce the proportion of calves that cannot be approached (CET) and would also reduce the manipulation of substrate and tongue playing behaviours but would increase nasal discharge at three weeks (Figure 2).

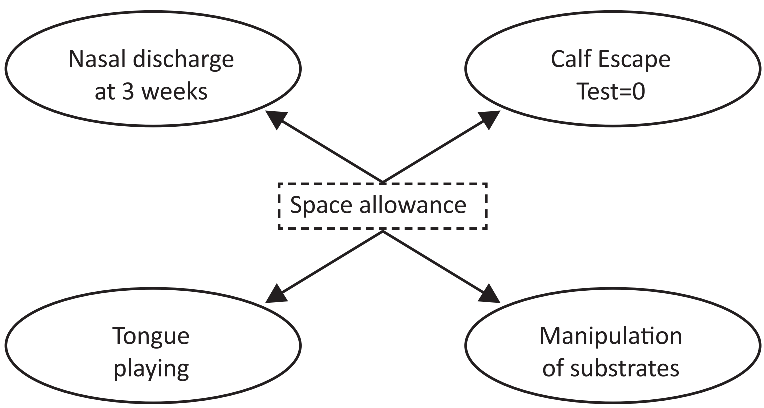

Figure 2
Example of multiple effects of one risk factor on several welfare measures. Circles represent welfare measures, square represents the influencing factor

Within a potential risk factor, the different modalities (or levels) can have a positive impact (protective factor) or a negative impact (risk factor) on the welfare measure. The odds ratios from the risk factor analysis provided information on whether a modality of a factor has a positive or a negative impact on a welfare measure compared to the other modalities of a factor, but also the strength of its influence on the given measure (e.g. high odds ratios mean high risks). For instance, different types of solid feed provided to calves will have different effects on their welfare. The type of solid feed influenced the manipulation of substrates with providing pellets or muesli and cereal grain to calves being a risk factor for an increased expression of manipulating substrates compared to feeding calves with treated maize or maize silage (Chapter 5). In contrast, pellets or muesli would reduce the occurrence of nasal discharge at the middle of the fattening period compared to when cereal grains or treated corn is provided (Chapter 7). Considering all risk factors together, the lowest risk of manipulation of substrates would be achieved by a farmer with 5 to 15 years of experience, raising dual purpose breed calves housed in winter, feeding maize silage in addition to the milk replacer in group pens of more than 10 calves per pen with a space allowance above the legal requirement of 1.8 m^2/calf. It is of course not possible to reproduce ideal conditions for all welfare measures but the risk factor analysis provides information on how various welfare measures are influenced by one factor and which modality within factors has a positive (or negative) impact on each welfare measure.

Each factor does not have the same impact on the overall welfare of calves. It is easier to work with factors that influence only one measure because it avoids possible interactions with other welfare measures. For instance, enrichment is a factor with only two modalities (whether enrichment is provided in the pens or not). It influenced only the results of the CET with providing enrichments in the pens reducing the proportion of calves that could not be approached. This factor will potentially have no impact on the other welfare measures. Therefore, it is easy to advise farmers to equip pens with enrichments. Some factors, however, influence several welfare measures; this introduces the risk that one modality would improve one aspect of welfare but would be detrimental to another one. For instance, the duration of fattening influenced the occurrence of nasal discharge 2 weeks before slaughter and pleuritis at slaughter with opposite effects and odds ratios above 4 in both cases. When the duration of fattening was below 24 weeks, three times less calves showed nasal discharge and three times more calves showed pleuritis at slaughter. Thus, providing advice for welfare improvement is complex when considering these measures.

The next step for the improvement of veal calf welfare is to go from a list of risk factors and their impact on welfare measures to advise farmers (see introduction, Figure 1). For that, not only biological relevance is important, but also causality. Existing scientific papers can provide evidence for causality (and an underlying biological mechanism) of the effect of a risk factor on a welfare measure. The risk factor analyses conducted in the present thesis permitted to detect potential influencing factors of measures of veal calf welfare. However, relationships between welfare criteria and influencing factors should be further analysed for the following reasons:

- By definition, associations between measures and potential risk factors following an epidemiological approach do not necessarily refer to causal relationships. Such associations could be based on chance, or the causal factor(s) behind them could in fact be other factors confounded with the factors that were actually recorded. For some associations found in the present thesis, causality may already have been established in studies published in the scientific literature. Otherwise, causality should be examined in carefully controlled conditions, involving an experimental setup with predefined levels of certain factors and other factors remaining constant. In accordance with the risk factors that were found in this thesis, it has been demonstrated, for example, that type of solid feed affects manipulation of substrates (Webb et al. 2012, 2013). The effect of season on human animal relationships, however, remains unexplained so far.

- Behaviour of calves can be influenced by other factors that were not included in the analysis such as characteristics of the milk distribution programs (Jensen and Holm 2003), more detailed characteristics of solid feed such as particle size or feed composition (Webb et al. 2013) and the behaviour of farmers (Lensink et al. 2001). The prevalence of respiratory problems could also have been affected by the use of preventive or curative health treatments which could be assessed through inventories of number and type of individual and collective treatments or a survey on the health prevention and treatment strategies of the farmer.

- The risk factor analyses for behavioural observations and behavioural tests were based on data collected at a given time point ('cross-sectional data'). It could be interesting to perform such analyses on data of calves of different ages. For instance, older calves are expected to show more abnormal oral behaviours and the influencing factors might differ from those in younger calves (Webb et al. 2013).

When no scientific evidence is available, additional controlled experiments could be needed to confirm or support causal relationships that are hypothesized. The process would be to go from epidemiology to controlled experiments, and back to epidemiology to examine whether a causal relationship also exists under practical conditions. For instance, the relationship between the modalities of a factor and the outcome of the welfare measures should be further analysed to be able to quantify the range of improvement than can be expected in regard to the costs of the changes made.
Risk factors can be classified in four groups according to two criteria: (i) whether these factors can be controlled (i.e. it is possible to advise modifications on farms on this factor given the constraints of the production system), and (ii) whether their impact on the welfare of calves can be explained by biologically relevant causes (i.e. by scientific evidence on causal relationships in veal calves or possibly other species) (Table 3).

Table 3
Classification of influencing factors in four groups according to the existence of a causal relationship with welfare and the possibility to control them in of veal units

		Can we use the factor for changes in management/ farm advice/ can it be controlled on farm (by the farmer or the integrator)? [1]	
		YES	**NO**
Is there support from experimental data for a causal relationship with welfare? [2]	**YES**	**Group 1** Farm size (HA, RD)[3] Number of calves/pen (RD) Number of calves/stockperson (HA) Amount of milk powder (AB) Babybox use (RD) Ventilation/ridges (RD) Enrichments (HA) Type of solid feed (AB, RD) Space allowance (AB, RD) Space allowance (HA)* Type of milk delivery system (HA) Type of milk delivery system (RD)* Quality of the batch (RD)* Frequency of veterinarian visits (RD)* Duration of fattening (RD)* Type of water drinker (RD)* Water provision (RD)* Type of floor/ Floor age (RD)*	**Group 2** Season (RD) Body weight (RD) Breed (HA, AB) Breed (RD)* Stockperson's experience (in years) (RD)*
	NO	**Group 3** Babybox use (AB) Number of calves/pen (AB)	**Group 4** Season (HA,AB) Experience with the use of the system (HA,AB) % females (RD)

[1] is it possible to advise modifications on farms on this factor given the constraints of the production system?
[2] Are there scientific publications on a causal relationship in veal calves or possibly other species?
[3] (HA) (AB) (RD) = indicates that the factor was classified in that group when influencing measures of (HA) human-animal relationship, (AB) Abnormal oral behaviours or (RD) respiratory disease.
* for these factors a causal relationship with the measure was never published yet, but a relationship is assumed and described in the discussion of chapter 3, 5 or 7.

Some potential risk factors for veal calf welfare problems that were identified in the present thesis also represented factors that can be controlled in veal production systems in practice and were related to feed and feed distribution, housing, equipment and management features. For those factors, when a modality is expected to improve the welfare of calves, advice could be given to farmers who want to improve the welfare of their calves. For factors in group 1, the effect on the welfare of calves is known and therefore by adapting the management changes can be made at farm level or at another level of the veal production chain. This group contains most of the factors analysed in the present thesis. These promising results support the idea that a lot can be done in practice to improve the welfare of veal calves. For instance, the effect of quantity and type of solid feed on abnormal oral behaviours is well known (Webb et al. 2013). The best balance between type, quantity and modalities of distribution of solid feed could be achieved by farmers in collaboration with their feed supplier/integrators based on the recent findings on that topic. For factors in group 3, there is a need for further investigation before practical advice could be proposed. For instance housing calves in groups of more than 10 calves/pen seems to reduce the expression of abnormal oral behaviours but as the underlying biological mechanism is not yet completely understood more research is needed to provide a solid scientific basis.

For factors in **group 2**, the causal relation with the welfare criteria is known but we either cannot or can only to a limited extent influence the factor. This is the case for example for the impact of the season on respiratory disorders. The type of breed of calves had impacts on the three aspects of welfare studied, with each of the breed types representing either a risk or a protective factor according to the welfare criteria. For instance, crossbred calves and dual purpose breed calves showed less avoidance of humans than calves from dairy breeds (Chapter 3). Also when observed at the middle of fattening, dual purpose breed showed less coughing, less manipulation of penmates than the other breeds and less manipulation of substrates than crossbreds (Chapter 5 and 7). Therefore we cannot say that one type of breed of calves should be preferred and in practice it is not possible to select specific breeds for the production of veal that would perform best on all welfare criteria studied in the present thesis. For factors in **group 4**, so far there is no scientific background to explain the impact of these factors on the welfare measures and it is not possible to work on these factors on farms as these factors are related to time, season or availability of calves. For instance, season had an effect on several measures. Batches that entered the fattening unit in summer had more calves that could not be approached by humans than calves that entered the fattening unit in autumn or winter. Calves belonging to those batches also showed more coughing at the end of fattening, more severe pneumonia at slaughter and performed more manipulation of substrates. These factors should, however, be taken into consideration when interpreting the welfare results of farms or for farm comparisons.

Remedial measures could be taken at farm level (quality of housing and management), but also at the level of the integration (supply of feed) or even at the level of the supply of 2-weeks old calves for the veal sector (improving quality of calves at arrival through a good care of newborn calves in dairy farms providing them) (Figure 3).

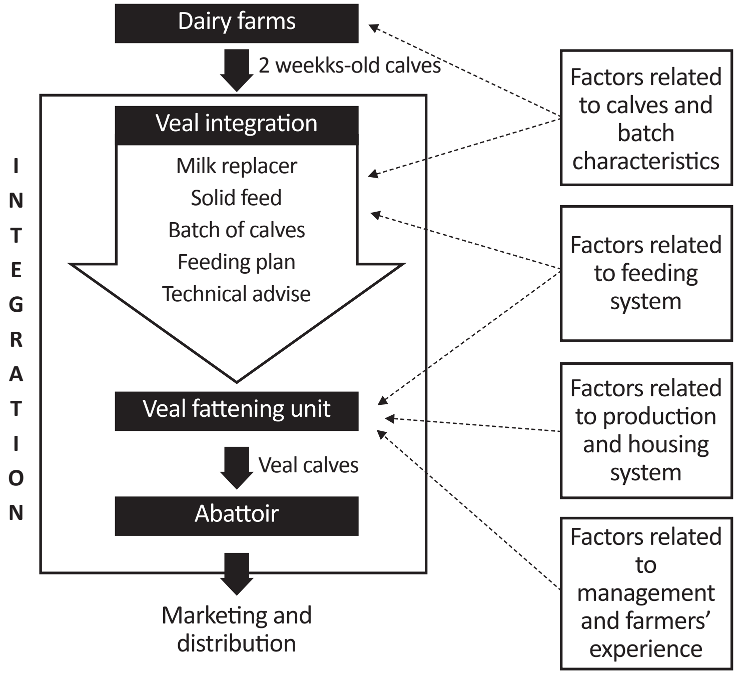

Figure 3
Position on the veal production chain of the different categories of potential factors for which remedial measures for the welfare of calves could be taken.

Advice should be given considering the full sets of measures as the factors presented here also impact other aspects of the welfare of calves that were not presented in the present thesis such as locomotion problems or gastrointestinal disorders (see Brscic et al. 2011).

SOME PRACTICAL ISSUES FOR THE ASSESSMENT OF WELFARE ON COMMERCIAL VEAL FARMS

Welfare assessment tools are now available for most farm animal species. The measures that were developed and validated for veal production systems are included in a welfare assessment protocol for cattle (Welfare Quality® 2009a). For a correct comparison of farms against each other ('benchmarking'), the on-farm assessment of welfare should be performed under similar conditions to avoid bias. In this last section, first some potential bias in the assessment of the welfare of veal calves related to the findings of this thesis will be further discussed. Thereafter, the question of the timing and frequency of the assessments will be discussed for farm monitoring and refining of the welfare assessment tool.

In this thesis, three issues were identified that should be considered carefully in welfare assessment, because they might affect and possibly bias the outcome.

1) The age of calves observed influences the results of different measures especially clinical signs of respiratory disorders and abnormal oral behaviours and should be similar between farms for several reasons. For safety reasons, a young age might be chosen as some measures

(for instance human-animal relationship tests) require that the observer enters the pens. These observations could be better performed when calves are not too old (during the first half of the fattening) as calves are playful animals and direct contact can be dangerous when they reach a certain size and weight. Another aspect is related to the fact that behaviour of calves and some clinical measures are strongly related to age of calves.

2) Unfortunately, when the age of calves is set for observations, a season effect is introduced because calves in different farms reach a specific age (for instance 13 weeks of fattening) in different seasons. Different seasons of observation can give different results. The effect of season can be dealt with in two ways. Season of observation can be set, e.g., observations are performed in autumn and results are reflecting the situation for farms in autumn. Another option is to perform observations any time of the year and to apply a corrective factor to be able to compare farms. Given the complex effects (each season having a different impact for each welfare measure) it would be difficult to determine such a corrective factor. In addition, there is a lack of knowledge in what sense season affects the outcomes of the welfare assessment in different farms (farm x season interaction). For instance, farms with better climate control might be less subjected to seasonal effects.

3) Despite standardized protocols and careful training of observers there is always room for different interpretations and differences in recording between observers. A good protocol for training observers is crucial because of the need for multiple observers to visit many farms. The training of observers should also be repeated to ensure that there is no drift in time (Martin and Bateson 1993). In addition to the precise protocols that were developed during the Welfare Quality project (Welfare Quality® 2009a), some on-going projects are focused on the development of training material.

In addition to the above mentioned factors, an important question is how many times and at what moment a veal farm should be assessed for welfare within a certain time frame? Different options are possible that should be carefully selected according to the production system. In broilers, the age of observation was set at the end of fattening, a few days before slaughter, when most welfare problems occur (Welfare Quality® 2009b). Given the duration of fattening (approximately 35 to 90 days depending on the strain) welfare assessment in broilers can be performed at one farm at different seasons. For dairy cows, the welfare assessment protocol is developed to be performed indoors and therefore the winter season is most convenient to take the measures. Such options are not applicable on veal fattening units due to the all in-all out procedure and the relatively long duration of fattening (5-6 months). The present assessment tool for welfare was designed for performing most of the observations in the middle of the fattening period (around 13-15 weeks). Therefore, one farm cannot be assessed at all seasons when the age of the calves at the time of observation is set.

When the welfare of calves should be assessed once at the middle of fattening, 1 to 2 visit(s) per farm per year should be envisaged. If the number of visits needs to be reduced, several options could be considered. The farms could be visited once every 2 to 4 batches which would reduce the visits to once a year or once every two years. In order to reduce the number of farm visits, and to concentrate the effort on those farms that need it most, De Vries (2012) suggested using routine herd data for a pre-screening of dairy farms to identify those farms with a potentially low welfare level. To implement such an approach in veal production, open and accessible databases are needed containing essential information about, for example, production and health parameters at farm level over years. In veal calves, farms could be visited for every batch but, as suggested by de Vries (2012), with the use of a lighter/partial assessment tool. For instance, the first batch could be assessed at the middle of the fattening period with observations related to behaviour and health criteria. Then the following batch could be assessed at the end of fattening with observations focused on measures for which the farms did not achieve a good level during previous visits, or on measures that are the most relevant at this age of calves (such as mortality rate, locomotion problems and enteric disorders for health criteria and abnormal oral behaviours for behavioural criteria). Finally, some batches could be assessed only at slaughter for post-mortem measures of respiratory and gastrointestinal disorders. That option would permit to get a full picture of the welfare of calves at different stages of the fattening period without leading to too many farm visits. Another option would be to perform a full monitoring at a specific frequency on farms (for instance every 5 or 6 years, but the optimal frequency should be determined) and a partial monitoring each year. With repeated assessments of welfare on farm in time all the different situations (e.g. age of calves, season) can occur. On the long run this would give the best estimate of the global welfare level of a farm.

Benchmarking of farms, or advice on remedial measures to optimize welfare should be based on repeated measurements rather than on the outcome of one visit. For this thesis, a total of 174 farms were visited in the three main veal producing countries in Europe, representing a cross section of veal farms in Europe. This sample was used not only to validate the application of these measures on a large scale, but also to calculate averages and variation of these measures. This permitted the comparison of the results of individual farms with those of the other farms in the sample (benchmarking). This approach also provided input data for the construction of thresholds for welfare criteria and the definition of overall welfare scores which were performed by Veissier et al. (2010) and are described in the assessment protocol for cattle (Welfare Quality® 2009). A farm will be considered as performing good or bad when it reaches a level that is below a specific threshold based on the information on the sample of farms (for methodology see Veissier et al. 2010). When a farm reaches a level for a welfare measure that is above an 'intervention threshold', remedial measures - based on the risk factor assessment (Chapter 7) will be taken. These thresholds for farm evaluation should be refined when the data collection on the welfare status of farms increases (Figure 4). Welfare monitoring on farms consists of regular visits within a given time frame. These repeated welfare assessments on a large number of farms will permit to monitor the welfare on farms. They will also permit to provide advice for farmers and the veal integration based on the comparison of up–to-date farm results from other farms (benchmarking) and with the inputs from the risk factor analysis. Finally the assessment tool could be refined by adapting the protocols and thresholds to the actual situation on farms (i.e. by defining thresholds according to welfare levels recorded on farms, by adapting sampling strategies to the evolution of farm size etc.) (Figure 4).

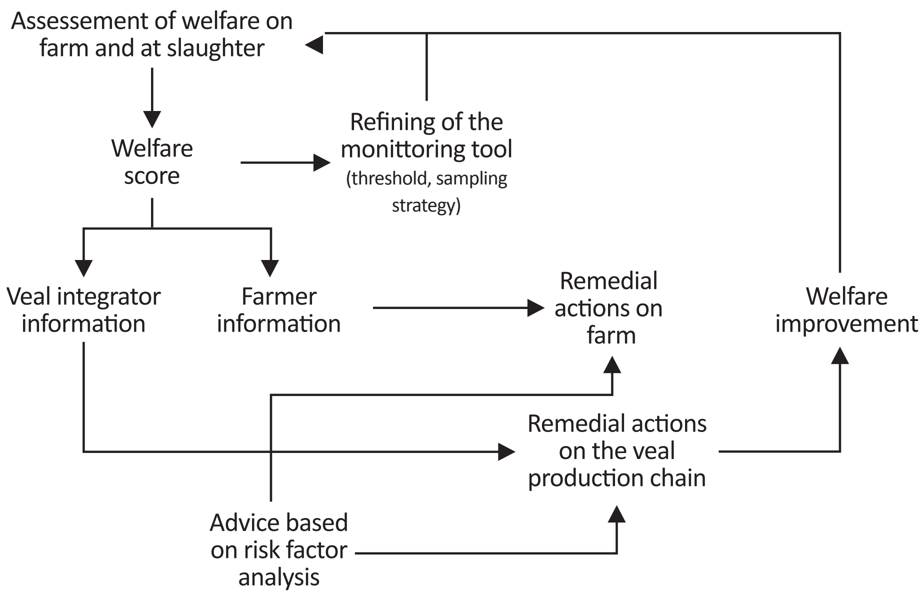

Figure 4
Potential use of the welfare assessment tool for farm benchmarking, welfare improvement and refining of the monitoring tool

Conclusions

The animal-based measures on human animal relationship, behaviour and respiratory disorders are valid and contribute reliably to an animal welfare assessment tool for veal calves. Some issues were identified that should be considered carefully in welfare assessment:

The assessment of the welfare of calves should be performed at a fixed age around the middle of the fattening period for feasibility reasons and to avoid an effect of the age of calves on the outcome of the welfare assessment
Season has an effect on the outcome of the welfare assessment that should be taken into account for farm evaluation and when comparing results between farms
Observers should be trained carefully prior to conducting observations on farms and should be checked at regular intervals for potential drift in time

Factors implying a potential risk and factors that are potentially beneficial for welfare were identified for human-animal relationship tests, level of abnormal oral behaviour and prevalence of clinical and post-mortem signs of respiratory disorders in veal calves. These factors are related to several characteristics of the farm such as characteristics of production and housing system, characteristics of the batch of calves, management of calves and farmer's experience, characteristics of the feed and feed distribution system. Knowing these factors and understanding their effect on the outcome of welfare assessment can help in advising farmers in their effort to improve welfare of veal calves.

Recommendations

Some effort still has to be made to improve the veal calf welfare assessment tool on farms. First, the measurement of some of the welfare criteria can be improved. For instance, the evaluation of respiratory disorders in veal calves using clinical signs and post-mortem measures of lung lesions should be further investigated, because the current measures do not give a full picture of the health status of calves through the fattening period. Second, the practical organisation of the monitoring of farms should be set: timing and frequency for farm visits, training of observers and feedback to farmers. Some on-going projects are currently focusing on the development of training material for the observers and on the development of comprehensive feedback material for farmers. The monitoring of veal farms should be performed on a large scale in European veal fattening units to benchmark farms against other farms and to refine thresholds for the different welfare measures. Finally, risk factors for the welfare of calves could be further investigated in two possible ways. First, once larger data sets become available from future welfare assessments in practice, risk factor analyses as performed in the present thesis could be systematically repeated. This will allow for the reliable confirmation of the results of the risk factor analyses of this thesis, and for the possible identification of additional risk factors. Second, more research in specific experimental set ups could be performed, to determine causality between risk factors and welfare measures related to human-animal relationship, abnormal oral behaviours and respiratory disorders in veal calves. Collectively, this will promote the provision of scientifically-sound advice to veal farmers, and the improvement of veal calf welfare in practice.

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SUMMARY

Veal calves are young bovines slaughtered before 8 months of age to produce soft, pale coloured meat. Veal calves are raised indoors in specialised fattening units and receive a diet controlled for iron content consisting mainly of milk replacer with additional solid feed. In Europe, minimal standards for the protection of veal calves are defined in EU directives. These standards however do not necessarily guarantee a sufficient level of animal welfare as each farm has specific conditions for housing and management that may cause welfare issues. There is a demand from part of the consumers to be informed about the level of welfare of farm animals and therefore a need for a scientifically-approved on-farm assessment tool of the welfare state of the animals. The European Union supported a research program called Welfare Quality® from 2004 to 2009, which aimed at "developing a standardized monitoring system of animal welfare" and "identifying practical solutions to improve animal welfare". In veal calves, a number of measures had to be developed and validated on farms during this Welfare Quality® project including measures related to the assessment of human-animal relationship, abnormal oral behaviours, and respiratory disorders. In order to contribute to the development of a reliable on-farm welfare assessment tool for veal calves, the specific objectives of this thesis were to study the quality in terms of validity, reliability and feasibility of different types of measures for assessing veal calf welfare on farm and to identify specific risk factors for impaired welfare at farm level. For this project, data were collected on 174 farms in the three main veal producing countries in Europe (France, the Netherlands and Italy).

The human–animal relationship is an important component of the welfare of farm animals as its quality might influence fear reactions of animals and for this reason animal responsiveness tests to humans are included in on-farm welfare assessment schemes. Several tests were developed and the inter-observer and test-retest reliability of these tests were analysed in **Chapter 2** to select the best tests to include in an on-farm animal welfare monitoring system for veal calves. 23 Farms were visited twice with two observers simultaneously. Behavioural tests measuring the response of calves to a human entering the barn, a novel object, a passive unfamiliar person, disturbance in the pen, and an active approach by an unfamiliar and a familiar person were conducted. For all behavioural tests, inter-observer reliability was very high. Except for the response on disturbance, all behavioural tests also showed a high test-retest reliability. Although the active approach test with the familiar person was reliable, it was not feasible in practice due to availability of the farmer. The active approach test with the unfamiliar person called Calf Escape Tests (CET) gave similar results and was recommended for an on-farm animal welfare assessment tool. The response of calves to a passive human called Human Approach test (HAT) was less feasible on all farms but was kept for further analysis. Apart from the behaviour of stockpersons towards their animals, other factors may also influence animals' reactivity to humans as observed through behavioural tests, which can add a further layer of complexity to the interpretation of test results. An analysis of factors that could influence the outcome of the two human–animal relationship tests selected (CET and HAT) was performed in **Chapter 3**. Tests were performed on 148 farms when calves were aged around 15 weeks. The latency to touch an unfamiliar human (HAT) was significantly correlated

with the CET scores. Total number of calves on the farm, space allowance, breed, environmental enrichment, stockperson's experience and season of observation influenced the percentage of calves that scored 0 in CET (i.e. calves that could not be approached). The type of milk distribution, type of breed and number of calves per stockperson influenced the percentage of calves that scored 4 in CET (i.e. calves could be touched). For both calves that could be touched and calves that could not be approached the level of self-reported contacts by the stockperson (analysed on a French subset of 36 farms) did not influence the results. According to the tests conducted, factors such as milk distribution method, breed of the calves or the level of experience of stockpersons with veal farming can have an impact on the results of tests focusing on human-animal relationships.

Veal calves raised under intensive conditions may express abnormal oral behaviours as signs of mental suffering and reduced welfare due to a mismatch between environment and the animals' needs. On-farm observations of these behaviours should be performed in a way that they permit to detect behaviours that differ from the norm. There are two main possible ways to record the behaviour of calves on farms: direct observations and observations from video recordings. The effect of the observation method (direct or from video) and the effect of the presence of an observer on the behavioural results in veal calves were evaluated in **Chapter 4**. A study was performed on one farm, where 24 Pens of 4-5 calves per pen were observed by a trained observer for 60 min on one day, meanwhile video recording were made for four consecutive days. The two observation methods gave similar results for the time calves spent standing, but different results for all other behaviours. The observer did not affect the behaviour of calves at day level, but affected their behaviour when actually present in front of the pens. A higher percentage of calves were standing and were manipulating substrates in presence of the observer, but there was no effect on the prevalence of abnormal oral behaviour. It was concluded that direct observations are a more suitable observation method than observations from video recordings for detailed behaviours in veal calves even though the presence of an observer had a short term effect on abnormal oral behaviours of calves. The prevalence of abnormal oral behaviours at farm level was recorded by direct observations and potential influencing factors for this behaviour were investigated in **Chapter 5**. The identification of the factors influencing each abnormal oral behaviour is helpful to define potential actions that could be taken on farms to improve the welfare of calves and to reduce the prevalence of these unwished behaviours. Observations of the three selected abnormal oral behaviours (manipulating substrates, tongue playing and manipulating a penmate) were performed on 157 farms when calves were aged 14 weeks. Results showed that calves spent on average 11.0 ± 0.46% of the observation time on manipulating substrates, 2.8 ± 0.18% on performing tongue playing, and 2.7 ± 0.09% on manipulating a penmate; all three variables showed a high variation between farms. Allowing more space to calves than the legal requirement of 1.8 m^2 and housing them in groups of >10 calves/pen reduced manipulating substrates and tongue playing. Occurrence of manipulating substrates was lower for calves fed maize silage compared to calves fed cereal grain, pellets or muesli. A higher risk of tongue playing was found when babyboxes were not used. Risk of calves manipulating a penmate was higher for calves of milk or meat type breeds compared to dual purpose breeds, and for calves fed 280 to 308 kg compared to calves fed over 380 kg of milk powder over the total fattening period.

The presence and severity of lung lesions recorded post-mortem is commonly used as an indicator to assess the prevalence of respiratory problems in batches of bovines. In the context of welfare assessment based on on-farm measures, the recording of clinical signs on calves at the farm would be more convenient than the recording of lung lesions at slaughter, because adding observation at slaughterhouse would increase costs. The aim of **Chapter 6** was to investigate the relationship between clinical respiratory signs at farm and post-mortem analyses of lung lesions observed at slaughter in veal calves. If clinical signs were a good predictor of lung lesions it could be possible to integrate only those measures in a welfare assessment tool. For 174 farms, trained veterinarians visually evaluated calves at 3 and 13 weeks after arrival of the calves at the farm and at 2 weeks before slaughter. For each batch a maximum of 300 calves was observed and the proportions of calves showing abnormal breathing, nasal discharge and coughing were recorded. Post-mortem inspection was carried out at the slaughterhouse on a random sample of lungs belonging to calves from the observed batches to evaluate mild/moderate, or severe signs of pneumonia, and presence of pleuritis. The clinical signs recorded were significantly correlated with moderate and severe lung lesions for observations at 13 weeks and 2 weeks before slaughter. Nevertheless, batches of calves with a high proportion of lungs with moderate or severe lesions could not be accurately detected by the three clinical signs of respiratory disorders. These results suggested that both clinical signs and post-mortem inspection of lung lesions must be included in a welfare assessment tool for veal calves. The in vivo and postmortem prevalence of respiratory disorders in veal calves and risk factors associated with them can be found in **Chapter 7**. In a study on 174 farms, Regardless of the stage of fattening, the prevalence of in vivo signs of respiratory disorders in calves was always <7%. This low prevalence was likely the outcome of the general implementation by veal producers of standardized practices such as prophylaxis, all-in/all-out, and individual daily checks of the calves, which are recognized tools for effective disease prevention and management. However, at postmortem inspection, 13.9% and 7.7% of lungs showed mild/moderate, and severe signs of pneumonia, respectively, and 21.4% of the inspected lungs showed signs of pleuritis. Thus, even mild clinical signs of respiratory disorders in calves at specific time points during the fattening period may be associated with high prevalence of lungs with lesions at slaughter. Alternatively, clinical symptoms recorded during routine visual inspections of veal calves on-farm may be poor predictors of the true prevalence of respiratory disorders in calves. Among all potential risk factors considered, those concerning the characteristics of the (quality of the) batch were predominant but factors related to housing, management and feeding equipment were also relevant. Different risk factors were involved at different stages of the fattening period.

This thesis showed that animal based measures on human animal relationship, abnormal oral behaviours and respiratory disorders are valid and contribute reliably to assess the welfare of veal calves. In addition, some conditions were identified that should be considered carefully in welfare assessment. The study allowed assessing multiple factors across farms that influenced the outcome of human-animal relationship tests, the level of abnormal oral behaviour and the prevalence of clinical and post-mortem signs of respiratory disorders in veal calves. Although part of the variability remains unexplained, a significant part of the variation was explained by factors related to characteristics of production and housing system, batch of calves, management and farmer experience, feed and feed distribution system. These factors imply a potential negative or positive impact on welfare. Knowing these factors can help in advising farmers in their effort to improve welfare of veal calves although the biological causality between some factors and the welfare measures is not always known. The next steps in the development of the welfare assessment tool will consist in improving the protocols used on farms by determining the best frequency of visits and to investigate further the risk factors in order to be able to provide farmers with practical advices.

SAMENVATTING

Vleeskalveren zijn jonge runderen die geslacht worden op een leeftijd van maximaal 8 maanden. Vleeskalveren worden gehouden om mals en lichtroze vlees te produceren. Hiertoe worden ze op gespecialiseerde bedrijven gehouden waar ze gevoerd worden met hoofdzakelijk kunstmelk en vast voer (i.e. krachtvoer, maïssilage, stro).

In de Europese Unie (EU), minimumnormen voor welzijn en huisvestingcondities voor vleeskalveren zijn gereguleerd via de EU richtlijnen. Deze minimumnormen garanderen niet noodzakelijkerwijze dat het welzijnsniveau van de kalveren altijd optimaal is, aangezien huisvesting- en managementcondities tussen bedrijven kunnen verschillen. Burgers willen geïnformeerd worden over het werkelijke welzijnsniveau van dieren op boerderijen en daardoor ook een noodzaak voor een wetenschappelijk onderbouwde welzijnsmaatlat. De EU heeft van 2004 to 2009 een onderzoeksproject gefinancierd genaamd *Welfare Quality*® met als doelstelling “het ontwikkelen van een gestandaardiseerd dierenwelzijnsmonitor” en “het identificeren van praktische oplossingen voor het verbeteren van dierenwelzijn”.

Dit proefschrift heeft bijgedragen aan het ontwerpen van een welzijnsmonitor op bedrijfsniveau voor vleeskalveren met als specifieke doelstelling de kwaliteit van de verschillende waarnemingen te toetsen betreffende hun validiteit, betrouwbaarheid en uitvoerbaarheid. Verder is voor elke waarneming gekeken naar specifieke risicofactoren voor een verlaagd dierenwelzijn op in totaal 174 kalverbedrijven in de drie belangrijkste vleeskalverproductielanden in Europa (Frankrijk, Nederland en Italië).

Een aantal waarnemingen moesten worden ontworpen en gevalideerd op praktijkbedrijven, waaronder de waarnemingen aan de mens-dierrelatie, abnormaal oraal gedrag en luchtwegaandoeningen. De mens-dierrelatie is een belangrijk component van dierenwelzijn omdat de kwaliteit van die relatie de mate van angstgedrag bij dieren mede bepaald. Om deze reden worden testen die de angstrespons van dieren ten opzichte van mensen meten in dierenwelzijnsmonitors opgenomen. In **hoofdstuk 2** van dit proefschrift worden verschillende testen beschreven die zijn ontwikkeld en geanalyseerd op onder andere hun herhaalbaarheid (tussen waarnemingen en waarnemers). Drieëntwintig bedrijven werden twee keer bezocht door twee waarnemers op hetzelfde moment. De gedragsrespons van kalveren ten opzichte van een persoon die de stal binnenkomt, een onbekend object, een passief onbekend persoon, verstoring in het hok, en een actieve toenadering van een onbekend en bekend persoon zijn gemeten. Voor alle waarnemingen was de herhaalbaarheid tussen waarnemers hoog. Behalve voor verstoring in het hok vertoonden alle gedragsobservaties een hoge herhaalbaarheid tussen waarnemingen. Hoewel de actieve toenaderingstest met een bekend persoon erg betrouwbaar was, is deze test in de praktijk niet echt toe te passen omdat de aanwezigheid van de kalverhouder nodig is. Dezelfde test maar met een onbekend persoon (*Calf Escape Test – CET*) gaf dezelfde resultaten en is daarom voorgesteld voor integratie in de welzijnsmonitor. Een tweede test met de reactie van kalveren op een passief, niet bewegend persoon (*Human Approach Test – HAT*) was iets minder betrouwbaar, maar is wel meegenomen voor grootschalige testen op bedrijven.

Naast het gedrag van de kalverhouder (en andere personen die met de kalveren werken) kunnen ook andere variabelen mogelijk invloed hebben op het uiteindelijke resultaat van gedragstesten. In **hoofdstuk 3** van dit proefschrift wordt de uitkomst besproken van de twee geselecteerde gedragstesten (CET en HAT) die op 15 weken oude kalveren zijn uitgevoerd op in totaal 148 bedrijven. De latentietijd voor het aanraken van een onbekend persoon (HAT) was gecorreleerd met de resultaten van de actieve toenadering (CET scores). Het totaal aantal kalveren op het bedrijf, beschikbare oppervlakte per dier, ras, omgevingsverrijking, ervaring van de kalverhouder en het seizoen van observatie beïnvloedden de score 0 van de CET test (d.w.z. dieren die niet aangeraakt konden worden). Het voersysteem voor kunstmelk, ras, en aantal kalveren per dierverzorger waren van invloed op de score 4 van de CET test (d.w.z. kalveren konden worden aangeraakt). Echter, op de 36 Franse bedrijven, de dagelijkse hoeveelheid contact met kalveren (zoals aangegeven door de kalverhouders) beïnvloedde niet de resultaten van de twee scores 0 en 4 van de CET test. Hieruit kan worden geconcludeerd dat factoren anders dan menselijk gedrag het resultaat van mens-dier gedragstesten beïnvloeden.

Vleeskalveren die in intensieve condities opgroeien, kunnen mogelijk abnormaal oraal gedrag vertonen als teken van een niet optimale leefomgeving of management. Abnormaal gedrag is een teken van verminderd welzijn. Het uitvoeren van waarnemingen op bedrijven kan op twee manieren gebeuren: direct of via videoregistratie. In **hoofdstuk 4** worden de resultaten van de waarnemingsmethodes (direct of via video) met elkaar vergeleken en is het effect van de aanwezigheid van een waarnemer tijdens waarnemingen op het gedrag van de kalveren onderzocht. Deze studie werd op één bedrijf uitgevoerd waar 24 hokken met 4-5 kalveren per hok werden geobserveerd door een getraind persoon gedurende 60 minuten op één dag. Tegelijkertijd werden video-opnames gemaakt op dezelfde dag maar ook op de vier volgende dagen. Beide waarnemingsmethodes gaven dezelfde resultaten voor het stagedrag van kalveren, maar verschilde voor alle andere gedragingen. De aanwezigheid van een waarnemer beïnvloedde niet het kalvergedrag gemiddeld over een dag, maar beïnvloedde wel het kalvergedrag op het moment dat de waarnemer aanwezig was. In dat geval vertoonde een hoger percentage van de kalveren stagedrag en meer manipulatie van substraten. In dit hoofdstuk werd de conclusie getrokken dat directe waarnemingen op bedrijven beter zijn dan video-opnames voor het registreren van abnormaal gedrag ondanks het feit dat het gedrag van de dieren kortstondig werd verstoord door de aanwezigheid van de waarnemer.

In **hoofdstuk 5** wordt de prevalentie van abnormaal oraal bedrag op bedrijfsniveau beschreven via directe waarnemingen als wel de factoren die het niveau van dit gedrag mogelijk kunnen beïnvloeden. De specifieke gedragingen waren "manipulatie van substraten", "tongspelen" en "manipulatie van een hokgenoot" bij 14-weken oude kalveren op 157 bedrijven. Kalveren toonden gemiddeld 11.0 ± 0.46% van hun tijd manipulatie van substraten, 2.8 ± 0.18% tongspelen en 2.7 ± 0.09% manipuleren van een hokgenoot. Een groter oppervlakte per dier en huisvesting in groepen van meer dan 10 kalveren per hok waren geassocieerd met lagere niveaus van substraat manipulatie en tongspelen. Verder was er een verlaagd risico van substraatmanipulatie wanneer maïssilage werd bijgevoerd in vergelijking tot het voeren van graan, pellets of muesli. Een hoger risico voor tongspelen werd gevonden wanneer geen individuele huisvesting werd gebruikt bij het opstarten van de mestperiode. Het risico voor manipulatie van een hokgenoot was hoger voor kalveren van melk- of vleesrassen ten opzichte van dubbeldoelrassen en voor kalveren die 280 tot 380 kg kunstmelkpoeder werden gevoerd over de hele opfokperiode in vergelijking tot dieren die meer dan 380 kg kunstmelkpoeder werden gevoerd.

Het post-mortem registreren van longbeschadigingen kan worden gebruikt als indicator voor de prevalentie van longproblemen voor groepen runderen. Echter aangezien de welzijnsmonitor gebaseerd is op waarnemingen voor het slachten, dus op de veehouderijbedrijven zelf, is het wenselijk klinische waarnemingen tijdens het opfokken uit te voeren. Bovendien houdt het registreren van longbeschadigingen in slachthuizen in dat een extra waarneming nodig is met bijbehorende extra kosten. De doelstelling van **hoofdstuk 6** was het bestuderen van de relatie tussen klinische longproblemen tijdens opfok en post-mortem longbeschadigingen bij vleeskalveren. Mocht er een goede correlatie bestaan tussen beide waarnemingen, dan zou kunnen worden voorgesteld om alleen klinische waarnemingen in de welzijnsmonitor op te nemen. Getrainde dierenartsen hebben klinische observaties uitgevoerd op 3 en 13 weken na opstarten, en 2 weken voor de geplande slachtdatum op in totaal 174 vleeskalverbedrijven. Op elk bedrijf werden maximaal 300 kalveren geobserveerd betreffende de volgende aandoeningen: abnormaal ademhalen, neusuitvloeiing en hoesten. Post-mortem waarnemingen werden uitgevoerd op het slachthuis op een random aantal longen (van kalveren die tijdens opfok geobserveerd waren) waarbij vastgelegd werd de mate van milde, matige en ernstige aandoeningen van pneumonie en pleuritis. De klinische waarnemingen op 13 weken van opfok en 2 weken voor slachten waren significant gecorreleerd met matige en ernstige symptomen van pneumonie en pleuritis post-mortem. Desondanks konden groepen kalveren met een hoge frequentie qua longbeschadigingen tijdens slachten niet voorspeld worden door de drie klinische waarnemingen gedurende opfok. De resultaten suggereren dan ook dat zowel klinische als post-mortem observaties in de welzijnsmonitor moeten worden meegenomen om de juiste evaluatie voor dit thema binnen kalverwelzijn te verkrijgen.

In **hoofdstuk 7** worden de prevalenties van klinische en post-mortem longproblemen besproken als wel de risicofactoren. Op de 174 kalverbedrijven kwam de prevalentie van klinische problemen nooit boven 7% uit, op wat voor moment dan ook. Echter, tijdens de post-mortem waarnemingen vertoonden 13.9% van de longen matige en 7.7% ernstige symptomen van pneumonie, terwijl 21.4% van de longen pleuritis hadden. De conclusie zou kunnen worden getrokken dat matige klinische indicaties van longproblemen op bepaalde momenten tijdens opfokken geassocieerd zijn met hoge prevalenties qua longbeschadigingen waargenomen tijdens slachten. Ook zou het kunnen dat visuele inspectie van vleeskalveren op bedrijfsniveau een zwakke indicator is voor wat de werkelijke prevalentie van longproblemen is. Wat betreffende de analyse van risicofactoren blijkt dat de karakteristieken van de groep dieren tijdens aankomst op het bedrijf, maar ook huisvesting, de algemene bedrijfsvoering en voersysteem op verschillende momenten hun invloed hebben op de klinische en post-mortem longaandoeningen.

Dit proefschrift laat zien dat metingen gebaseerd op dierniveau zoals mens-dierrelatie en abnormaal oraal gedrag maar ook longproblemen goed geïntegreerd kunnen worden in een welzijnsmonitor voor vleeskalveren. Verder worden in de discussie van dit werk een aantal suggesties geformuleerd betreffende de manier waarop de welzijnsmonitor gebruikt zou moeten worden. De verschillende studies hebben er toe bijgedragen om verschillende potentiele risicofactoren voor slecht mens-dier gedrag, verhoogd abnormaal oraal gedrag en verhoogde longproblemen te ontdekken of te bevestigen. Hoewel een gedeelte van de variatie onverklaard blijft, blijkt dat een flink aantal variabelen gerelateerd aan bedrijfsvoering, huisvesting, voersysteem en karakteristieken van de kalverhouder een relevant aandeel hebben in het verklaren van het niveau van de genoemde waarnemingen. De betreffende variabelen kunnen worden gebruikt voor praktisch advies aan kalverhouders om het welzijnsniveau van hun kalveren te verbeteren. Echter, in een aantal gevallen is de biologische relatie tussen variabelen en welzijnsmetingen niet echt duidelijk en is extra onderzoek nodig om een aantal verbanden te bevestigen. De volgende stappen in de ontwikkeling van de uiteindelijke welzijnsmonitor voor vleeskalveren zijn het verbeteren van de waarnemingsprotocollen op bedrijfsniveau en verdere analyses van risicofactoren voor een verminderd welzijn om vleeskalverhouders beter te kunnen adviseren.

CURRICULUM VITAE AND LIST OF PUBLICATIONS

Hélène Leruste was born in 1981 in Lille, France. She completed a master's level study in agriculture science at ISA Group and a Master in applied ethology at the University of Paris XIII in 2004. Since then, she worked as a researcher and lecturer at ISA. Her research activities are focused on the study of farm animal behaviour in intensive systems and assessment of animal welfare at farm level. She started her research activity in collaboration with a pig breeding company, with the main question being whether early behaviour of gilts could be predictive of later behaviour of sows around farrowing and open the possibility to select animals that would represent a lower risk of piglet crushing. Since 2005 she has been working on the assessment of the welfare of veal calves on commercial farms, through the European program Welfare Quality®. She teaches animal production, ethology and animal welfare at ISA, partner schools and universities. Next to research, she also performs extension activity mainly for dairy farmers and is co-author of a large audience book on the observation of bovine herds.

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Lensink, B.J. and H. Leruste. 2006. L'observation du troupeau bovin : voir, interpréter, agir. Editions France Agricole, 256 pages.

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