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Abstract

Fear is an important welfare problem for farm animals including cattle. A variety of methods of assessing fear have been proposed, but the reliability and validity of these methods, and ways of improving these characteristics, have received little study. We conducted a series of experiments to assess the consistency of dairy calves' responses of novel objects and to humans, and to investigate factors that might improve reliability. In the first experiment, latency to touch a novel object had moderate reliability (r_s=0.54), and latency to touch a stationary, familiar human had negligible reliability (r_s=0.26). Experiment 2a used the same test protocols, but with a shorter interval between repeat testing and using different stimuli in the two novel object tests; this change did not improve reliability (e.g. r_s=0.29 for the novel-object test). Reliability for this test was improved (r_s=0.58) in Experiment 2b, when the same object was used in both tests rather than a truly novel object being used the second time. Experiment 2a found ceiling effects in the response to human test associated with the short period during which approach responses were recorded. High reliability was found in Experiment 2b, where the maximum test duration was doubled, but this effect not due to the extended duration. Experiment 3 assessed reliability of a response to human approach at the farm rather than individual level, in this case assessing responses to an unfamiliar person. The proportion of calves making contact with the person was not reliable (r_s=0.22), but the proportion retreating from the person had moderate reliability (r_s=0.52). Reliability was improved by excluding data from calves that had coughs on the day of testing. Conducting multiple tests per individual using different stimuli and reporting health status of the animals are recommended for future research and animal welfare assessment schemes that include measures of fear.

Keywords

Fearfulness; neophobia; human-animal relationship; well-being; reliability; validity

1. Introduction

Fear is widely recognized as a welfare concerns for cattle and other farm animals (e.g. Farm Animal Welfare Council 2009; Hemsworth et al. 2000; Jones and Boissy 2011). Fearful animals can also cause production and management challenges, including decreased productivity (e.g. Barnett et al. 1992; Hemsworth et al. 2000) and animals that are afraid of humans may be more dangerous to handle (Boivin et al. 1992; Hemsworth et al. 1989). Unfortunately, methods of assessing fear (a negative emotional state resulting from a perceived threat [Gray 1987; Ennaceur 2014]) and fearfulness (a personality trait characterized by a tendency to express fear when exposed to potentially threatening stimuli or situations) appear not to be well-validated and have uncertain reliability (Forkman et al. 2007). Of 112 papers published in this journal over a five-year period ending in August 2015 with fear* or anx* in the keywords, abstract or title, only 65 papers (or 58%) contained any form of the words reliable or repeatable anywhere in the text, and of these, only 15 actually estimated reliability. Measures also vary considerably across studies, making it difficult to extrapolate results from one approach to the next (Forkman et al. 2007).

The need for valid, reliable ways of assessing welfare in farm animals is widely recognized, to be used for example in assurance schemes for commercial farms (see Scott et al. 2001). Currently, fear is often assessed in farm animals through response to novelty (neophobia, although other factors such as exploratory motivation also influence the response), most commonly using a novel object test. Another common type of fear-related test is in response to humans (e.g. Forkman and Keeling 2009), as fear of handlers may have a major impact on the lives of intensively farmed animals. Research published to date indicates that responses are not closely associated in these two contexts (e.g. Hegelund and Sorensen 2007), and that separate measures may be needed. From the perspective of animal welfare, fearfulness and long-lasting states of fear are of special interest, meaning that we are especially interested in fear responses that are consistent over time. Unfortunately, test-retest reliability (also called repeatability) is often weak making it difficult to draw strong inferences from a single test.

In cattle, for example, the novel object test was reported to be reliable within individuals between tests in at least two calf studies (using measures derived from factor analysis in Van Reenen et al. 2004, and approach latency in Bokkers et al. 2009), but was unreliable in older heifers and adult cows when tested using avoidance (Van Reenen et al. 2013), reactivity (Gibbons et al. 2009), number of interactions and time in proximity (Kilgour et al. 2006). Results have been mixed across a range of measures and ages in other studies (Graunke et al. 2013; MacKay et al. 2014). Even the methods of assessing 'repeatability' vary: while most studies replicate the test exactly using the same stimulus, others (e.g. Gibbons et al. 2009) instead assess consistency of response across different novel stimuli because there is no way to repeat a test and have it be truly novel (see e.g. Forkman et al. 2007 for a discussion of this problem). Nonetheless, the novel object test has face validity, meaning that it appears sensible based on our understanding of fear and comparisons with human behaviour, as judged by experts (e.g. Scott et al. 2001; Whay et al. 2003). It is also one of the few tests that has undergone some successful validation for cattle, suggesting it may be a true indicator of fear (based on correlation with other fear- and stress-related measures and pharmacological validation using anxiolytic drugs; e.g. Van Reenen et al. 2005; Van Reenen et al. 2009). Confirming or finding ways to improve its reliability would thus be valuable.

Responses to humans (typically measured as approach or avoidance by the animal) are more consistently reported to be reliable (at the individual level in calves [Rousing et al. 2005] and cows [Gibbons et al. 2009; Turner et al. 2011]). However, some papers found moderate to high repeatability only for some measures and time periods (Haskell et al. 2012; Mazurek et al. 2011; Windschnurer et al. 2008; Windschnurer et al. 2009; see also review of responses to humans by de Passillé and Rushen 2005), and other studies have found no repeatability (Battini et al. 2011), although all of these studies depended on some measure of avoidance or retreat from a human. Fina and colleagues (2006) reported that reliability of responses to restraint differed depending upon the calves' initial responses, with calm individuals remaining calm across tests but fearful ones showing reduced fear over time.

Farm-level repeatability is also important for measures of approach or avoidance of humans, because this type of measure has been proposed for use in on-farm welfare assessments (e.g. Winckler et al. 2003; Winckler et al. 2007), focussing on herd-level differences. Only a few papers have investigated farm-level repeatability of responses to humans, all in adult cows, and studies have sometimes confounded test-retest reliability with inter-observer reliability (e.g. Windschnurer et al. 2009). In these tests (based upon avoidance of an approaching human) low to moderate reliability has been reported (De Rosa et al. 2003; Winckler et al. 2007). Reliability can also be estimated at the level of the pen or group (intermediate between individual and farm levels), and indeed some farm level estimates are based upon observations of a single pen. Only one study on calves has assessed the reliability of approach responses measured at the pen level, and this study reported high reliability (Bokkers et al. 2009, with similar results for an avoidance measure).

Even among papers that claim repeatability, correlations are sometimes low. For example, Turner and colleagues (2011) assessed repeatability across and within tests of fear of humans in beef cattle and found the proportion of variance explained by individual consistency ranged from 0.17 to 0.54. In fact, a meta-analysis of the personality literature in wild animals found an average repeatability (intraclass correlation coefficient) of only 0.37 (Bell 2009), which is considerably below the level generally deemed acceptable (0.6 being a traditional standard in the human literature (e.g. Bruton et al. 2000, Mroczek 2007). In humans, typical correlations over long intervals (years) are often over 0.7 in adults (Mroczek 2007). Conversely, correlation coefficients for children and college students were only 0.31 and 0.54 respectively, for major personality traits in one meta-analysis (Roberts and DelVecchio 2000). It therefore seems likely that other juvenile animals, such as calves, may also show limited correlations in their fear responses over time.

The aims of the current study were to assess the individual-level test-retest reliability of versions of novel object and response to human tests, and the farm-level test-retest reliability of a response to human test. An additional aim was to identify factors that influence reliability, enabling refinements in protocols used in future research and on-farm welfare assessments. The factors investigated included consistency of the object used in the novel object test, test duration, and calf health. We also assessed inter- and intra-observer reliability (i.e. consistency between and within people recording the data) of the measures, as these are essential to obtaining test-retest reliability.

2. Materials and methods

2.1. Experiment 1

All of the research presented in this paper was approved by the University of British Columbia Animal Care Committee. In this experiment we used 32 Holstein bull calves, housed at the University of British Columbia Dairy Education and Research Centre. These calves also served in a concurrent study on the effects of early social housing, comparing individually housed calves (n=10), pair-housed calves (n=12), and calves kept in a complex social group with access to their dams (n=10). More detail regarding these treatments is available in Meagher et al. (2015). Pens were cleaned once per week. Calves were offered 8 L of milk per day for the first 28 d, at which time the milk ration was reduced to 6 L over 3 d, always split between two daily feedings. This reduction was intended to stimulate solid feed intake. At approximately 58 d, calves were weaned over a 3-day period. Calves had ad libitum access to water throughout the experimental period, and access to grain (Hi-Pro Medicated Calf Starter) and a mixed ration beginning at day 5 ± 2. Health checks were performed weekly throughout the experimental period to assess symptoms of common illnesses, including respiratory and enteric disease. Calves were treated when appropriate according to standard farm protocols.

Two tests for fearfulness were used: novel object and response to human (in this case approach to a stationary, familiar person). These tests were conducted on consecutive days at approximately 41 d of age and repeated at approximately 62 d of age. The response to human test was also conducted at 25 d of age. Tests were conducted between the two daily feedings, but never within 30 min of either feeding time. Novel object tests took place in a test pen that the calves had visited twice daily (for cognitive training; see Meagher et al. 2015) for several weeks. After 2 min of habituation to the pen, the novel object (in this case, a brightly coloured ball) was lowered into the pen using a length of twine. The test lasted 10 min, and latency to make contact with the ball was recorded. The response to human tests were conducted during weekly weighing of the animals, following a similar procedure to Duve and colleagues (2012) in which calves were allowed to approach a human and then their response to weighing was assessed. In brief, the calf was released from its pen into the alley, and given up to 90 s to make contact with the stationary person. The stationary person (one person per experiment) was familiar to the calves and stood 2.4 m away. The first author (RKM, who was also familiar to the calf) stood inside the pen and recorded the latencies to touch the person. Wooden dividers blocked the view of calves on the other side of the aisle, leaving an alley approximately 1.2 m wide for the individual and pair treatments; however, calves could see into neighbouring pens on the same side of the alley as they approached the person. For the group-housed calves, the distance to the person was equivalent, but the space was wider and no other calves were in sight. The calf was then encouraged or pushed onto the scale (by the previously stationary person), and the difficulty of pushing was scored by the handler on a scale of 0 to 4, with 0 indicating the calf walked onto the scale with no physical guidance, and 4 that a single handler could not get them on the scale alone.

Test-retest reliability was assessed using Spearman rank correlations due to non-normality of the data. Weighted sums of Spearman correlations are presented to control for effects of housing treatment (Taylor 1987). Correlation coefficients and not p-values are reported, because p-values are too dependent on sample size to be very useful measures of reliability (Martin and Bateson 2007). Throughout the paper, we categorize reliability as negligible (correlation <0.30), low (0.30-0.49), moderate (0.50-0.69), high (0.70-0.89) or very high (≥0.90) following Hinkle and colleagues (2003). For the ordinal data from scores of difficulty of handling during weighing, we used two types of analysis: kappa scores for agreement on the ordinal data (categorized according to Dohoo et al. 2002), and kappas combined with percent agreement when converted to a binary analysis for some force needed (scores 2 to 4) versus no force needed (scores 0 or 1).

2.2. Experiment 2

2.2.1. General methods

In Experiment 1, the testing schedule was partially determined by the other experiment running simultaneously, and the calves had some experiences between tests that might have caused changes in behaviour, including weaning from milk onto solid feed. Thus, in Experiment 2 we assessed the reliability of the handling and novel object responses using a shorter inter-test interval and during a period of consistent management.

The subjects were two cohorts of Holstein calves. In Experiment 2a we used 27 calves (18 male, 9 female), and in Experiment 2b we used 13 calves (all female). Calves were individually housed until the end of the experiment and cared for in the same way as described above, except for the following differences in feeding: no total mixed ration was provided during this experiment, and calves were stepped down to 4 L of milk rather than to 6 L beginning at d 26. Also, for the purposes of a related experiment, 13 of the calves in the first group were given a nutritional supplement with their milk, alpha S1 casein hydrosylate (Zylkène®, distributed by Vétoquinol, Princeville, QC), beginning 7 d before the start of fear testing and ending on the day they were moved to the group pens. This treatment did not affect any of the response measures except latency in the first novel object test.

2.2.2. Experiment 2a

At age 36 ± 3 d, a novel object test was conducted in the home pen. This test was repeated 7 d later (d 43 ± 3). Two different objects were used to maintain the novelty of the test rather than conducting an exact replicate: a red and white ball, and a blue plastic basket. Half of the calves received the ball in the first test and the basket in the second, and the other half received the objects in the reverse order. The tests were conducted in the same way as Experiment 1, but in addition to latency to make contact, total time in contact with the object was recorded. All measures were assessed from video recordings by trained observers (one per variable) who were blind to the study aims, and intra-observer reliability was tested by having these observers score a subset of the videos a second time to ensure that they were consistent in their scoring; latency to make contact was also recorded live for all calves by the first author, who also assessed the other measures from a subset of videos for inter-observer reliability testing. Responses to a human handler were also assessed as in Experiment 1. These tests were conducted on the day following each novel object test.

Normality of the data was assessed using Shapiro-Wilk tests. Latency data were non-normally distributed, so repeatability was assessed using Spearman rank correlations. Difficulty of handling scores were analysed as in Experiment 1. Six calves showed symptoms of illness at some time during the testing period, primarily with enteric illness, which may have affected reaction speed and likelihood of approaching the object; these calves were excluded from the analyses.

2.2.3. Experiment 2b

The second cohort of calves was used to assess whether modifying the protocols used in Experiment 2a would improve reliability. Housing, care and testing protocols were the same as in Experiment 2a, except for one change in each test. For the novel object tests, the same object was used in both tests (each calf being assigned to either the ball or the basket) rather than calves getting a different object in Test 1 and 2. For the response to human tests, the duration of the test was extended from 90 s to 180 s to reduce potential ceiling effects. The data were analysed for test-retest reliability as above, again excluding calves that were ill.

2.3. Experiment 3

This experiment was conducted on 15 dairy farms in the Fraser Valley of British Columbia, Canada, with the aim of assessing farm-level repeatability in response to humans. Unweaned calves between 7 and 70 d of age were tested. Because each farm was visited twice, 6 to 8 wk apart, the individual calves tested on the second visit were a completely separate cohort, but represented the full range of ages where possible (average age in test 1: 34; test 2: 37 d). All calves were Holstein or Holstein crosses. Data were collected from a total of 677 calves, with an average of 21 calves per farm on each visit. Tests were conducted between morning and afternoon feedings and never within an hour of feeding time.

Fear of humans was assessed using an approaching human test, which could be conducted without opening the calf pens. Unlike in the previous experiments, the human (RKM) was unfamiliar to the calves. The person walked along the row of pens, parallel to them and approximately 1 m from the front of each pen or hutch (space permitting). Once directly in front of a pen, she then turned to face the calf and said “hello” to attract their attention (cf. Bokkers et al. 2009). After pausing for 5 s to record any locomotor response, she approached the calf at a pace of approximately 1 step per second (as in e.g. Windschnurer et al. 2008), and then extended her arm to where the calf could reach it, with the hand flat and oriented sideways. Direct eye contact was avoided (Bokkers et al. 2009). Retreats were scored on an ordinal scale according to Table 1. We also recorded whether the calf touched the experimenter, and the latency to do so, within 2 min. The experimenter then repeated the procedure at the next pen in the row, following the same route through the pens on both visits to a farm, and never passing directly in front of a calf prior to its test if at all possible. For socially-housed calves, latencies and retreats for each calf in the pen were recorded.

Calf health was visually assessed after each test. The presence of a spontaneous cough, or faecal consistency scoring greater than 2 (following McGuirk, 2013) were considered indicators of illness.

Repeatability of the test was assessed at the farm level for the proportion of calves making contact with the experimenter, since calves within a farm were non-independent, using a Spearman rank correlation. Repeatability of retreats in this test was also assessed with Spearman rank correlations, using three different ways of summarizing the behaviour: proportion of calves retreating by the time the experimenter was at the pen with hand extended (score 2 or above) or prior to extending the hand (score 3 or above), and the average score for each farm.

One farm was excluded because a major housing change occurred between tests. On the remaining 14 farms, individual calves were excluded if they showed signs of diarrhoea or respiratory illness or both, based on the criteria above. The reliability analyses were then repeated to check for an effect of these illnesses on the results.

3. Results

3.1. Experiment 1

Latencies to approach the novel object were moderately correlated between tests at 42 and 60 d of age, with a correlation coefficient (r_s) of 0.54 (n=24; Figure 1). There was little evidence of any relationship in approach latencies to the human handler between tests at 25 and 42 d of age (r_s = 0.26, n=23), nor at 42 versus 60 d of age (r_s = 0.21, n=26).

Difficulty of handling scores showed low reliability using the ordinal scale. Kappa values were 0.33 for day 25 vs. 42 and 0.22 for day 42 vs. 60 (indicating “fair agreement”: Dohoo et al. 2003). However, 22 of 31 calves (71%) were consistent from days 25 to 42 in terms of whether any force was needed (kappa 0.44, indicating moderate reliability). For day 42 vs. 60, percent agreement was similar: 23 of 34 calves (68%; kappa 0.35).

3.2. Experiment 2a

3.2.1. Test-retest reliability

The correlation between Tests 1 and 2 for latency to touch the novel object was negligible (r_s=0.29, n=20; Figure 2a). Excluding calves that failed to touch the object in at least one test, which often happened if calves were resting immediately before the test, perhaps reflecting drowsiness rather than increased fear or lack of interest, improved the correlation between tests (r_s=0.70, n=15). The reliability of time in contact with the object was low when considering all calves (r_s=0.30, n=20), and negligible when excluding those that did not make contact (r_s=0.02, n=16).

For the response to humans, a correlation between latencies in the two tests could not be meaningfully assessed because only 6 of 27 calves ever made contact with the handler on the first test, and of these only three also made contact during the second test. Agreement in difficulty of handling scores was very low whether data were analysed as ordinal or binary (kappa 0.07 and 0.03, respectively), although there was 50% agreement in the latter (10 of 20 calves).

3.2.2. Intra- and inter-observer reliability

Inter-observer reliability for latency to touch the novel object was very high (r_s=0.93, n=27), and intra-observer reliability was also high for the subset of videos that were re-assessed (r_s=0.81, n=15). Total time in contact also had high inter-observer reliability (r_s=0.70, n=10) and very high intra-observer reliability (r_s=0.94, n=15).

3.2.3. Experiment 2b

Test-retest reliability for latency to approach the novel object was higher in this Experiment (r_s=0.58, n=11; Figure 2b), but excluding non-contacts did not improve reliability (r_s=0.32, n=10). Reliability of the response to human was high in this experiment, (r_s=0.76, n=10; Figure 3). However, this improvement was not the result of using the extended maximum test duration of 180 s; only 1 calf made contact with the handler between 90 and 180 s on both tests, and artificially imposing a 90 s ceiling produced a high reliability coefficient (r_s=0.83, n=10). The high reliability was partially due to the fact that failure to make contact within 90 s was consistent among individuals: 5 of the 6 who did not make contact on the first test also failed to make contact in the second test. Agreement in difficulty of handling scores was fair for this group (kappa 0.26), and this value was similar (0.27) for whether any force was needed to get the calf on the scale, with 7 of 11 (64%) calves in agreement.

3.3. Experiment 3

Repeatability depended on the response measure and exclusions for illness, as presented in Table 2. In brief, the proportion of calves making contact with the person showed low or negligible repeatability; indeed, the slope of the relationship was negative. Retreats were moderately repeatable for the full data set. Using yes/no data for whether a calf retreated at all, before the person's arm was extended (score 3 or above) was slightly more reliable than including retreats at the time the arm was extended (score 2). The most reliable measure was the average retreat score for the farm.

Signs of illness were recorded for 68 of 599 calves on the 14 farms analysed. For three of the four response variables, excluding calves with coughs improved repeatability. Excluding calves with diarrhoea only improved repeatability for two response measures, and excluding both groups reduced repeatability for all measures relative to excluding coughs alone.

4. Discussion

4.1. Factors influencing repeatability

The results show varying levels of repeatability in both novel object tests and those assessing response to humans. We speculated that the low reliability in Experiment 1 was due to a long test-retest interval (approx. 20 d), combined with important management changes (including weaning from milk). Consistent with this idea, we found some improvement in reliability estimates for the novel object test in some groups when we switched to shorter intervals (7 d) with more consistent management (pre-weaning only) in Experiment 2, and for the response to human test in Experiment 2b. Agreement in scores of difficulty of handling was typically low to fair across the experiments, although it was higher for the binary (some force vs. no force needed) scale than the ordinal scale in Experiment 1.

In Experiment 2a, the improvement in novel object reliability occurred only when including animals that were alert during testing. Unfortunately, the results of this inclusion criteria differed between Experiments 2a and b, which may reflect some instability in the correlation estimates due to the small sample sizes available (see e.g. Goodwin and Leach 2006). Based on the human literature, the sample sizes needed for stability of personality correlation estimates would be very difficult to achieve (e.g. n=250: Schönbrodt and Perugini 2013); we suggest instead the use of multi-study replication, ideally with meta-analyses, to confirm the reported effects. However, the result from 2a suggests that it would be worthwhile to investigate the benefit of a further refinement that could be used for both the novel object test and human approach tests conducted in the home pen: imposing a procedure or criteria to ensure that animals were attending to the test situation. For example, in Experiment 1 calves were moved to a testing pen and the test began shortly afterwards. This ensured that no calves were asleep or resting at the time the stimulus was presented, as well as removing possible distractions such as the presence of food. Home pen tests are desirable for practical reasons and because they avoid introducing handling effects and social isolation for group housed animals (see Forkman et al. 2007; Tecott and Nestler 2004), but in this case it seems that the costs may outweigh the benefits (the reverse may be true when measuring exploration rather than fear; see Carter et al. 2013).

In Experiment 2, we considered two additional factors thought to improve repeatability: increasing the test duration when latencies are measured, and the consistency of the novel object. Repeatability of the latency to touch humans could not be assessed in Experiment 2a due in part to ceiling effects associated with a short test; we thus hypothesised that increasing the time allowed would improve reliability for the latency measures. The latencies in Experiment 2b did show high reliability, but this was not due to the longer tests. That said, given that ceiling effects prevented discrimination among individuals in Experiment 2a, we still contend that longer test durations improve the validity and usefulness of the test by avoiding an artificial upper limit in measures of latency. Others have similarly argued that extending test durations improves test validity (e.g. in tests of chronic anxiety in rodents; Fonio et al. 2012).

The improved repeatability of the novel object in Experiment 2b versus 2a was likely due to using a second presentation of the same object. In Experiment 2a we had used a different novel object for each test (to retain the novelty), but a disadvantage of this approach is that animals may find some objects inherently more fear-inducing than others thus making responses more variable. Although we found that using the same 'novel' object for multiple tests improved the repeatability of the test, we do not recommend this practice in future tests. Instead we argue that there is much to be gained from examining a range of objects; if individual rankings differ between arbitrarily chosen objects with no apparent biological significance, it is likely not valid to draw broad conclusions regarding 'fear of novelty' from tests with a single object.

Experiment 3 identified the role of sickness, particularly respiratory illness, in reducing reliability of responses to humans. Sickness behaviour is widely accepted to include lethargy and decreased exploratory behaviour (e.g. Millman 2007; Swiergiel and Dunn 2007). A recent study in calves found that respiratory illness and fever decreased probability of calves approaching novel objects and stationary humans; diarrhoea did not immediately have this effect, although during recovery from this ailment calves were less likely to approach people (Cramer and Stanton 2015). Changes in health status could thus reduce repeatability of the results for both types of test. Cramer and Stanton (2015)'s findings mirror the current results, in that excluding calves with signs of respiratory illness most consistently improved the correlation between tests across variables. Excluding calves with signs of diarrhoea or both illnesses was less helpful, although this may have been due to the reduced sample size (Goodwin and Leech 2006), and this should therefore be retested in a larger sample of calves. While the differences in reliability estimates in this experiment were relatively small, collectively, these findings support our choice to exclude animals that were sick around the day of testing in Experiment 2. Unfortunately, health checks were not conducted on test days in Experiment 1. In future, health status should be addressed when reporting responses to these tests.

Another lesson from Experiment 3 was that the proportion of calves making contact with an unfamiliar human has low repeatability relative to other response measures. This is surprising since this measure, and the related measure of latency to contact, are commonly used (e.g. Bokkers et al. 2009; Forkman and Keeling 2009). We found that the most reliable response measure was the retreat score. For retreat as a yes/no variable, which is simpler to record, particularly when calves are group-housed, the correlation between visits was slightly higher when counting retreats before the researcher's arm was extended versus retreats at the time the arm was extended. Although the difference was small, it may reflect inconsistency in the behaviour of the test person, such as speed of arm extension or positioning of the hand relative to the calf. Repeatability of the retreat measures at farm level was comparable to the individual-level results using latencies in Experiment 1.

Several factors that could influence repeatability of tests of fear were not investigated here. As described by Waiblinger and colleagues (2006), human-animal relationship tests in farm animals can be influenced by many factors, including interference by neighbouring animals, exploratory, social, feeding and lying motivations, and social isolation. Feeding motivation was relatively constant within each of the experiments in the current study (as tests were held outside of regular feeding times, although this was not a perfect control since the testing window was relatively large for practical reasons, likely increasing variation between days), and social motivation and responses to isolation were not relevant in most cases.

Interference by neighbouring animals was not an issue during the novel object tests, since the calves were alone during testing, and were minimal throughout Experiment 3 since most calves were housed alone and vocalizations were not common. However, it may have been an issue in the response to human tests of Experiments 1 and 2, as calves could walk past the pens of neighbours. In Experiment 3, there may also have been fluctuations in farm practice such as staff members providing most care to the calves, or feeding times. However, this will be the reality for any on-farm work and such variation must be accepted except where changes are predictable (e.g. due to season) and can thus be accounted for in the study design.

One effect that has not been directly investigated in this context, but which is known to play a role in animals' responses to potentially threatening stimuli, is laterality. Vertebrates, including cows, typically prefer to view threatening stimuli from the left eye (Robins and Phillips 2010), and the eye that first sees a stimulus can influence escape responses (e.g. Austin and Rogers 2007). It would be of interest to test whether inconsistency in the orientation of cattle relative to fear-inducing stimuli can explain differences in responses on repeated tests. Testing this idea will require a test environment that allows control of presentation side.

4.2. Strategies for using tests with limited repeatability

Even if protocols are refined to reduce noise, there are likely limitations in the level of repeatability that can be achieved. As discussed in the Introduction, the average repeatability reported for personality traits of wild animals is only 0.37 (Bell et al. 2009). How consistency of behaviour in farm animals will compare is difficult to predict. As de Passillé and Rushen (2005) point out, even where there are moderate, statistically significant correlations, a large number of animals will be "misclassified" by a single test. These limitations do not necessarily prevent the tests from being useful; despite their typically low reliability, personality tests in wild animals can still predict ecologically or practically important outcomes (e.g. Smith and Blumstein 2008). In the experiments described here, despite low to moderate reliability, the tests conducted at 41 d of age in Experiment 1 were able to detect some effects of treatment that correspond with theory: fear of novelty was higher in calves reared in simpler, more socially restricted housing (Meagher et al. 2015). Human personality studies typically report repeatability estimates averaging 0.7 to 0.8 for the Big Five factors of personality (e.g. Gnambs 2014, Mroczek 2007), but these factors are typically derived from multi-item scales. Having only one or two measures, which is the norm in animal studies, is expected to increase measurement error (Credé et al. 2012).

A common recommendation when assessing traits is to conduct repeated tests and sum or average responses. However, in the case of novelty, repeated testing is logically problematic (see Forkman et al. 2007), as the object is no longer novel when presented a second time; even if the object is changed, the test procedure becomes less novel. One approach to circumvent this difficulty is to consider decreases in fear as an acceptable result when assessing reliability (e.g. Meagher et al. 2011); repeated testing can then still be used to draw inferences, because differences in habituation or sensitization rates may also be consistent, welfare-relevant individual traits (Jones and Boissy 2011).

The results from the current study suggest that multiple tests might be needed, but using a range of objects or other stimuli, given the differences in individual rankings depending on the objects used. Similarly, Ramos (2008) argues that for measuring trait anxiety (and/or modelling human anxiety disorders), conducting multiple types of tests is necessary. He argues that these should be conducted simultaneously if the alternative is placing the animal in the same test chamber or apparatus multiple times, but this would not allow assessment of how much of the response is due to temporary states present at the time of testing. One difficulty with recommending multiple tests is the time and expense, for example, of conducting multiple visits to farms for on-farm welfare audits. Current protocols sometimes focus on ensuring inter-observer reliability (e.g. Wemelsfelder and Lawrence 2001), but our results indicate that this is not sufficient for producing repeatability.

Farm-level repeatability could conceivably be attained without individual-level repeatability, if problems with the latter are due only to the inherent problems in repeating a test involving novelty. As long as the results are repeatable within farm using new groups of animals, this would not be a major concern for farm-level investigations. Understanding why results change within individuals is nonetheless important, since differences due to age or season should be taken into account when selecting samples and testing times (see Haskell et al. 2012).

4.3. Outstanding concerns regarding test validity

The discussion above was focused primarily on practical issues regarding reliability of fear tests, but even if these issues can be resolved questions remain about test validity. Very little validation testing has been done for response to human tests in calves, including the voluntary approach-type tests used here, although some studies indicate that both voluntary approach to humans and avoidance distance are influenced by rough or gentle handling (Lensink et al. 2000; Schuetz et al. 2012; Windschnurer et al. 2009**).** The tests of neophobia used in farm animals, such as novel object (e.g. Misslin and Ropartz 1981) and open field tests (Hall 1936; Archer 1973), are largely adapted from tests originally developed and validated for laboratory species. In some cases, the rationale for the test was based on the behavioural ecology of the rodent species, and applicability to other species is questionable. For example, the open field test makes sense for rats and mice that fear open areas (presumably because these are associated with increased predation risk; e.g. Lister 1987; Ohl 2003; Rodgers 1997), but cattle are too large to be at risk of overhead predators and are adapted to life in open habitats. The novel object test is expected to apply more broadly (e.g. Russell 1973), but in some cases species-specific responses, such as burying, need to be taken into account (e.g. Misslin and Ropartz 1981). As noted above, the object-specificity of the test results in Experiment 2 also raises some concerns about its validity as a general measure of neophobia. A second potential problem is that, even in laboratory animals where these tests have been better validated and sometimes proved useful in drug screening, the validity of some tests (e.g. the open field) has also been called into question (e.g. Ennaceur 2014). Known issues from the laboratory animal literature include sensitivity to environmental variables unrelated to the intended treatment, reducing external validity (Garner 2005) and preventing accurate measures of trait anxiety because they are overshadowed by the effects of temporary states (e.g. Ohl 2003; Sylvers et al. 2011). This is likely one reason for failures to replicate results in different laboratories (e.g. Dawson and Tricklebank 1995; Sousa et al. 2006; Wurbel, 2002).

Although careful attention to the methodological factors described above will likely reduce problems of poor reliability and aid in the interpretation of data, the use of short-term tests may be inherently problematic if the aim is to assess consistent traits in animals. Temperament ratings by people who can integrate behaviour over time are one suggested alternative (see Carlstead et al. 1999; Meagher 2009), but the relationship between these measures and standard tests is not well understood (e.g. de Passillé and Rushen 2005). Finally, the same underlying motivation can be expressed very differently depending on the testing situation (e.g. approaching to bury an object when possible versus retreating from it if not), potentially leading to misinterpretations regarding fearfulness (Franks et al. 2012). More species-specific validations of the different types of fear test, taking into account natural behaviour, are thus needed.

5. Conclusions

Moderate test-retest reliability seems achievable for both novel object and response to human tests in dairy calves. It is, however, contingent on allowing sufficient time for the behavioural response, and excluding calves with respiratory illness and perhaps other forms of illness if replications of this work can confirm that they decrease reliability. In the case of novel object tests, moving subjects to a testing pen or otherwise assuring that calves are alert at the beginning of the test and not distracted by competing motivations will also help. For tests using an unfamiliar human as the stimulus, moderate repeatability was only achieved for retreat scores and not for likelihood of making contact with the person. None of the protocols assessed provided consistently high repeatability, and results of neophobia tests seem to be dependent on the specific stimuli chosen. For these reasons, we suggest that future research use multiple tests to assess fearfulness or anxiety, using different stimuli.

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Tables

Table 1

Fear scoring system in Experiment 3 based on stage at which the calf retreated from the approaching experimenter. The experimenter approached the calf or calves in the home pen, in a standardized way each time, and the calf was given a total of 2 min to approach or retreat.

Score	Description
9	Retreat before arrive at pen
8	Retreat when face pen
7	Retreat when speak
4-6	Retreat during approach (each step the experimenter took towards the pen before a retreat reducing the score by 1)
3	Retreat when reached front of pen
2	Retreat when extend arm
1	Retreat during remainder of test
0	No retreat

Table 2

Spearman correlation coefficients from Experiment 3. Coefficients describe the repeatability of responses to the approaching human test on commercial farms, depending on the response variable and exclusion criteria**.** Each of 14 farms was tested on two occasions. The highest coefficient for each response variable is indicated in bold.

	Exclusion criteria			
Response variable	Sick calves included	Calves with diarrhoea excluded	Calves with coughs excluded	Both excluded
Proportion of calves that made contact	0.222	0.279	**0.332**	0.253
Proportion retreating when arm extended or before	**0.508**	0.450	0.494	0.486
Proportion retreating before arm extended	0.516	0.477	**0.521**	0.486
Average retreat score	0.538	**0.587**	0.582	0.560

Figures

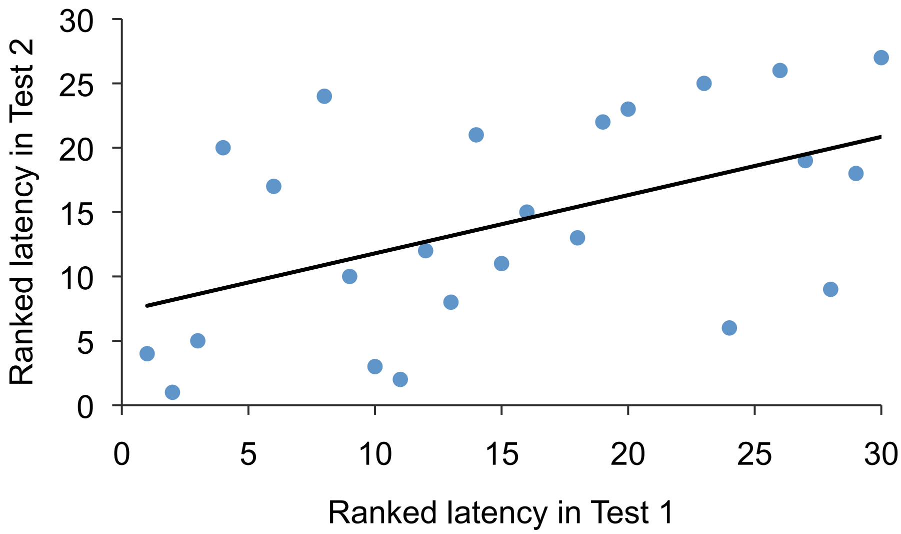
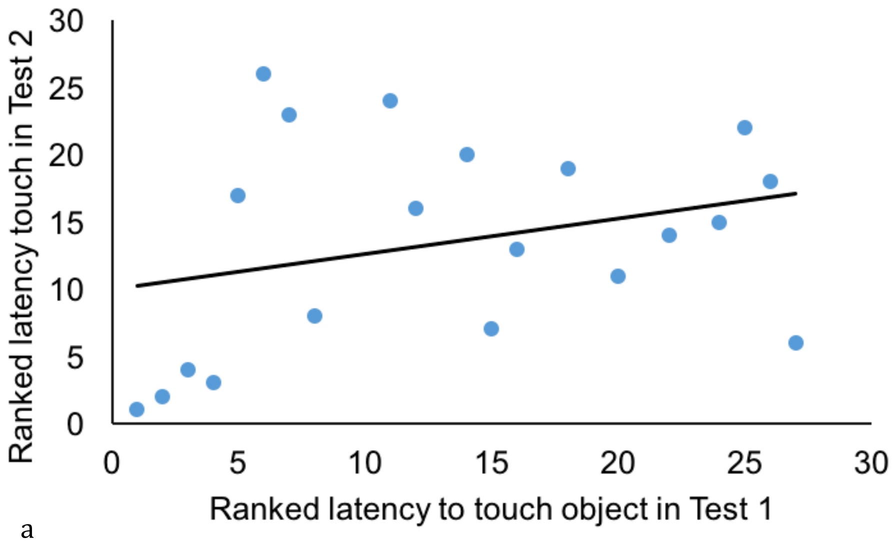
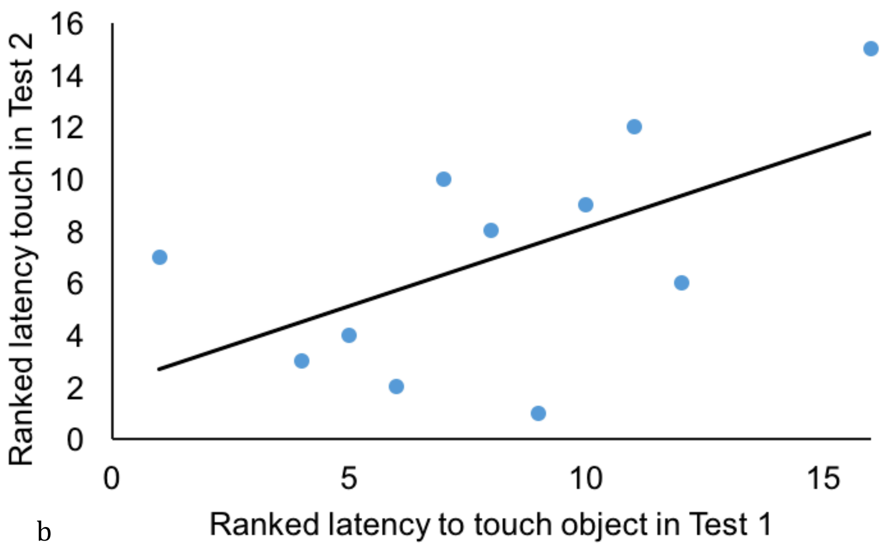


Fig. 1. Rank correlation between dairy calves' latencies to touch a novel object across two tests in Experiment 1. Calves were tested at approximately 41 and 62 d of age, with the object being a colourful ball.



a



b

Fig. 2. Rank correlation between calves' latencies to touch a novel object across two tests in Experiments 2a and b. Calves were tested at approximately 36 and 43 d of age, using a different object each time in a) versus the same object in b).

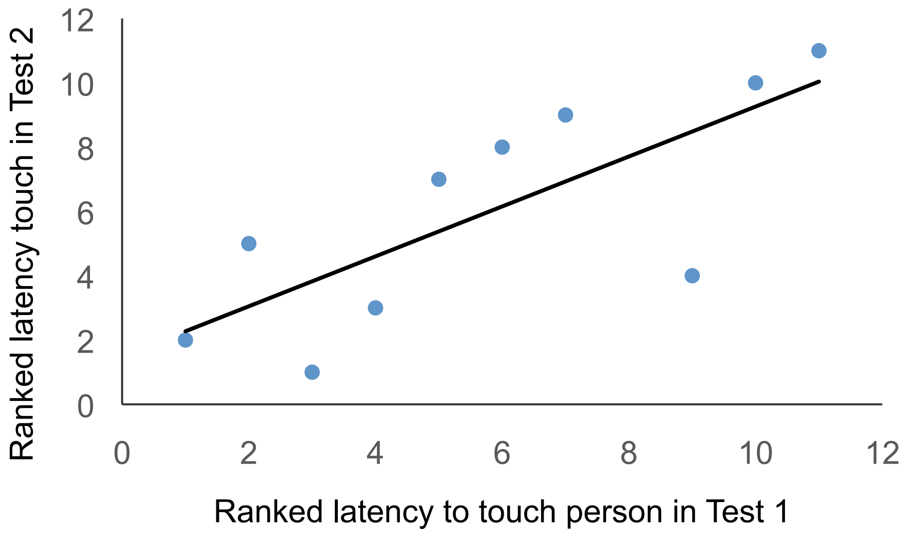


Fig. 3. Rank correlation between calves' latencies to touch a familiar handler across two tests in Experiment 2b. Calves were let out of their pens and given up to 3 min to touch a stationary person.