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Redefining human-animal relationships: an evaluation of methods to allow their empirical measurement in zoos

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Abstract

Scientific studies of human-animal interactions (HAIs) and how these develop into human-animal relationships (HARs) now represent some of the most significant contributions to animal welfare science. However, due to the current definition of HAR, studies have only been able to measure HAIs and infer its impact on HARs and animal welfare. Here, we redefine HARs as a series of repeated HAIs between two individuals known to each other, the nature of which is influenced by their historical HAIs and where consideration to the content, quality and the pattern of the interactions is also vital. With a new definition, it is now feasible to empirically measure HARs, however, first, it is important to evaluate current methods utilised in animal industries to allow standardisation across HAR research in zoos. Here, we review the current methods that have been used to assess HAIs in animals and determine their overall suitability for measuring HARs and their use in a zoo environment. Literature searches were conducted using the search terms 'human-animal' AND 'interaction', 'human-animal' AND 'relationship', 'human-animal' AND 'bond'. Subsequently, 'zoo', 'companion', 'agriculture', 'laboratory' and 'wild' were added to each combination yielding five potential methods to evaluate. These methods were assessed according to a panel of indicators including reliability, robustness, practical application and feasibility for use in a zoo environment. Results indicated that the methods utilising 'latency', 'qualitative behaviour assessment' and the 'voluntary approach test' were potentially viable to assess HARs in a zoo environment and could subsequently contribute to the assessment of welfare implications of these HARs for the animals involved. These methods now require empirical testing and comparisons within a zoo environment.

Keywords: *animal welfare, behaviour, human-animal-interactions, human-animal-relationships, method, zoo*

Introduction

The scientific study of human-animal interactions (HAI) is a multi-disciplinary field of interest to biologists, sociologists, psychologists, and conservationists, amongst others. An *interaction* has been traditionally defined in respect of inter-human interactions as a sequence in which an individual performs a behaviour towards another, which is subsequently responded to with a specific reaction (Hinde 1976; p 3). According to Hinde (1976), a relationship is a succession of interactions between two individuals known to each other and influenced by a history of past interactions. Previous definitions of human-animal relationships (HARs) suggest that each participant within the relationship is able to make predictions about the other's responses (Estep & Hetts 1992; Hemsworth *et al* 1993). However, if this definition is applied *verbatim*, it is not feasible to measure the ability of an animal to predict a human's behaviour. With this in mind, it appears necessary to devise a new definition that would enable the measurement of HARs. Therefore, in this context and throughout this paper, an HAR is defined as a series of repeated HAIs between two individuals known to each other, the nature of which is influenced by their historical HAIs. Aspects to consider involve the content, quality and pattern of the interactions. For example, an HAR within a zoo setting can occur because of daily routine interactions with familiar zoo personnel (including keepers, education providers, maintenance and gardens teams, etc). We propose that the HAR should be measured over a period of time, recording the content of interactions (ie the behaviours performed to create the interaction), the quality of interactions (ie positive, negative and/or neutral) and the order in which these interactions occur (ie the consistency of the interactions, eg human A calls over animal B, B comes over to A, A strokes B or A calls over B, B slowly comes over to B with no further interactions). An important consideration is that due to this definition, interactions between unfamiliar humans (eg visitors) cannot develop into HARs.

Within the agricultural animal sciences, methods to assess HAIs have been studied extensively and tested for validity and reliability. Similarly, zoo-based HAIs between animals and unfamiliar visitors have been relatively well investigated, albeit with varied outcomes. In contrast, empirical studies of HAIs with familiar humans in zoos (ie regular keepers), which have the potential to develop into HARs, have only recently begun to attract significant scientific attention (Hosey & Melfi 2014). Moreover, the fundamental processes of method evaluation and standardisation are yet to be performed for HAI and HAR studies in zoos. This study addresses this knowledge gap by performing a systematic review of existing literature utilising methods designed to measure either HAI or HAR in zoo settings.

Human animal interactions and animal welfare

Based on findings in companion (rabbits [*Oryctolagus cuniculus*]: Podberscek *et al* 1991), livestock (dairy calves [*Bos taurus*]: Ellingsen *et al* 2014), and laboratory (primates: Baker 2004) animals, HAIs and HARs represent a significant influencing factor in animal health and welfare, as well as having important roles to play in human health and well-being (O'Haire 2010). Likewise, HAIs and HARs have been recorded and investigated in zoos and are considered to have implications for the health and welfare of the animals involved (HAR: Carlstead 2009; Smith 2014; Martin & Melfi 2016; Carlstead *et al* 2019, HAI: Chelluri *et al* 2013; Ward & Melfi 2013, 2015). However, most studies are reliant primarily on one method alone, ie studies of animal behaviour, and as welfare is multi-faceted, additional measures would be needed to measure this specifically. According to our definition, the features of any interaction between an animal and a human will influence how an HAR develops. Interactions can be perceived by the animal and the human as negative, neutral or positive and, consequently, result in the development of a negative, neutral or positive HAR categorisation (Waiblinger *et al* 2006; Hosey 2008; Carlstead 2009; Smith 2014).

Previous research into the implications of HAIs for farm animal welfare have suggested that whilst some animals may become accustomed to human contact, the majority of observed reactions involve some level of fear (Jones 1997). Many of the HAIs which occur in a farm environment are of a negative nature, such as exposure to rough handling, restraint and veterinary treatments, with positive or neutral HAIs generally only associated with feeding (Hemsworth & Coleman 1998). It follows that assessments of HAIs and HARs based on the measurement of an animal's level of fear (or confidence) in humans have been well tested and commonly used in on-farm welfare assessments with the use of both familiar and unfamiliar humans (de Passillé & Rushen 2005; Battini *et al* 2016). However, fear-induced responses from these negative HAIs can include attempts to escape which, in turn, can result in injuries or deaths (ie from animals running into obstacles or other individuals) which raises welfare concerns and has detrimental health and safety implications in zoos. The use of an unfamiliar human within these tests focuses primarily on assessing an animal's general fear of humans. However, based on the HAR definition where an HAR can only occur between two individuals known to each other, a familiar human would be used to explore this specific aspect.

The behaviour and attitude of stockpersons have been shown to be major variables that can determine an animal's fear or confidence in a human, therefore stockmanship has the potential to influence the quality of HAIs and HARs as well as animal welfare. Positive or neutral HAIs and HARs can be beneficial to animal welfare, for example, gentle handling or the presence of a familiar human may calm the animals in potentially negative situations, reducing the risk of injuries and therefore improving welfare (eg dairy cows; Waiblinger *et al* 2004). Ellingsen *et al* (2014) studied stock-person handling styles at 100 Norwegian dairy farms (100 stockpersons, mean number of 31 calves per farm) and identified differing styles which were termed 'positive interactions', 'calm/patient', 'dominating/aggressive', and 'insecure/nervous.' Results suggested that stockpersons who handled calves patiently and calmly were associated with animals exhibiting a higher level of positive mood, whereas a nervous or dominating style of handling was associated with calves of a more negative mood. Positive moods in animals can be interpreted as pleasant emotions; this state is predicted to influence the nature of the HAI in a positive manner through a positive feedback cycle (see Waiblinger *et al* 2006). The emotional state of an animal during an HAI will likely influence its perception and reaction to humans and subsequently impact on the nature of the HAI itself; a range of influential factors contribute to this emotional dimension and must be considered during HAI studies, particularly when welfare is a measured outcome. In particular, fear and nervousness in animals has been associated with stress and reduced animal welfare (Rushen *et al* 1999). Waiblinger *et al* (2006) described the establishment of a negative feedback cycle between the handlers and animals, whereby worsening stockperson behaviour and attitude of stockperson sees a subsequent increase in fear for the animal, resulting in continued or increased negativity of the stockperson's attitude. For example, in Ellingsen's (2014) study, handling situations involving calves with a negative mood led, potentially, to animals which were more difficult to handle and unco-operative, resulting in more dominating or aggressive behaviour and attitude of handlers, and consequently a negative HAI and low welfare. In contrast, positive interactions that lead to pleasant emotions and a neutral or positive HAI, through the positive attitude of handlers, promotes good welfare. This is represented in one of the few zoo HAI studies relating to welfare; Ward and Melfi's (2013) zoo stockmanship cycle. This study highlighted the concept that a positive response from the animal following a positive keeper-animal interaction, promotes further positive responses from the animal and so forth, developing a positive HAR and potentially positive animal welfare. Positive HAIs have also been found to improve the welfare of laboratory animals; increased periods of positive HAIs have reduced fearful reactions in rabbits (Podberscek *et al* 1991), and lowered levels of abnormal behaviours in laboratory chimpanzees (*Pan troglodytes*) (Baker 2004).

Within companion animal HAR research, the typical focus is on how the relationship and/or an animal-assisted therapy benefits human health and well-being (Walsh 2009), with only a few studies investigating the influence on animal welfare (Vitztum & Urbanik 2016). However, factors including attachment level, anthropomorphism, and owner empathy and attitude towards their pets are elements which influence HAIs and consequently the animals' welfare (Marinelli *et al* 2007; Ellingsen *et al* 2010) or benefit to the human participant.

The importance of HAIs in animal welfare is an area of active research and highlighted as being one of the most significant recent contributions to zoo animal welfare science (Meehan *et al* 2016). Moreover, the connections among animal welfare, zoo visitor experience, and wildlife conservation are clear and notable in the revised vision of the World Association of Zoos and Aquaria (Barongi *et al* 2015). However, the role HAIs and HARs have in modulating animals' behavioural repertoire, their social interactions, or life history events and outcomes have not been adequately explored, with only a handful of studies published to-date (Mellen 1991; Wielebnowski 1999; Wielebnowski *et al* 2002; Carlstead 2009; Carrasco *et al* 2009; Smith 2014; Carlstead *et al* 2018). It is possible that the modulating effect of HAIs and HARs on these factors could exert significant influence on the welfare, management, and conservation consequences of zoo-housed animals. Whilst the extent of HAI influence is yet to be quantified in zoos, the potential exists for HAIs to impact on the welfare status of hundreds of thousands of animals, many of which are involved in captive breeding programmes of international significance to the *ex situ* conservation of their species. For example, data retrieved on 31st May 2018 from the 'Zoological Information Management System' (an animal records subscription-based database [Species360 2018]) revealed there to be 280,762 mammalia, 290,792 aves and 99,872 reptilia held in member facilities. These numbers are highly under-representative of zoos since it was estimated that within the > 10,000 zoos worldwide (Fravel 2003), just over 10% contribute to this global database. The welfare implications of zoo HAIs and HARs therefore potentially affect a vast number of individual animals.

Despite this potential impact on animal welfare at the individual and population level, we have yet to establish a basic understanding of how HAIs and HARs function in zoos, or what the consequences of such HAIs and HARs may be for the diverse range of species in zoos. In addition, the results and discussion of published work on zoo HAR refer mainly to welfare as being related to HAIs or HARs rather than measuring welfare implications specifically. Therefore, it is important to firstly determine an appropriate method to measure HARs that can be applied in a standardised manner across a range of zoological settings and to a variety of species. Given the behavioural variation between species, let alone among different taxa, this is a difficult task. However, as the HAR is a product of HAIs, the response of the animal to a specific human stimulus can be measured as a fundamentally common starting point. As a first step towards this goal, this evaluation provides a comprehensive review of the current methods available to study HAIs and HARs from the perspective of the animal, the majority deriving from agricultural contexts (see Waiblinger *et al* 2006; Table 1 in the supplementary material to papers published in *Animal Welfare*: https://www.ufaw.org.uk/the-ufaw-journal/supplementary-material). This will determine methods predicted to be suitable for testing in zoo environments, in order to establish a robust, reliable and feasible method (or panel of methods) for future zoo HAI and HAR studies. Equally important is the human dimension of the HAI (ie the response and/or perception of the HAI by the human involved), necessitating the utilisation of proven, reliable methods from the social sciences. However, the measurement of the human perspective, or the implications of the HAI or HAR for animal welfare or human well-being are beyond the scope of this review. Similarly, the important but under-investigated field of multi-zoo comparisons of husbandry factors involved in determining HARs warrants further investigation and will likely benefit from the application of standardised methodology to assess HARs.

The evaluation process

Methods currently used to assess HAIs and HARs were determined through literature searches on Google Scholar, Proquest and Web of Science, prior to November 2017. The search criteria included 'human animal', 'keeper animal' or 'caretaker animal' AND 'interaction', 'relationship' or 'bond'. Subsequently, 'zoo', 'captive', 'companion', 'domestic', 'farm', 'agriculture', 'laboratory' and 'wild' were added to each combination. Relevant studies from the dataset generated by the search engines were then identified from their keywords, paper titles, and abstract contents. Criteria for inclusion also required that papers specifically measured HAI or HAR, rather than it being a subsequent or potential finding of a larger study. Additionally, only original research articles were included; review papers were excluded. Since the purpose of this study was to determine and evaluate the scientific methods used to assess HAI and HARs in zoos from the perspective of the animals, only reports that presented empirically determined data and analyses of HAI or HAR studies were included. Data pertaining to the assessment of HAIs or HARs using social science methods, or investigated welfare outcomes of an HAI or HAR without measuring the HAI itself, were excluded in order to focus on animal-directed measures of HAI and HAR. Unpublished research, theoretical discussions, or manuscripts written in a language other than English were excluded. No further searches followed the initial search.

The HAI or HAR methods used in the studies were firstly categorised by the animal environment (zoo, companion, agricultural, laboratory) and then by the most commonly used methods, as shown in Table 1 (https://www.ufaw.org.uk/the-ufaw-journal/supplementary-material). Factors used to evaluate the zoo HAI or HAR methods (Table 2) were adapted from Waiblinger *et al* (2006) and could be of use within the zoo industry. The important aspect here is that the majority of the studies

Table 2 Factors used for evaluation of methods to measure human-animal relationships in zoos.

Evaluation factor	Variables	Additional considerations
Reliability	Measures are free from random errors	The amount of background 'noise'
	Repeatability, ie same results with the same animals	
Robustness	Application to same species?	Application for other species?
	A stand-alone measure?	
Practical application	Time requirements; total study daily time, keeper/zoo staff	Zoological institutions vary extensively in regards to the species, housing, keeper regimes, animal accessibility and the collection/facility type - need to suit all zoos
	The training or skills for observers (time involved and cost)	
	Analytical requirements (software, training, constraints)	
Validity	Relationship between the measured variable and what is supposed to be predicted	
Accuracy	Measures are free from systematic errors	Test inter- and intra-observer reliability, if there is disagreement between observers this may lead to low accuracy
Feasibility		
Safety	The risk posed to humans; categorised according to legislation or zoo policy	What type of contact is allowed with the animal?
	Nature of the equipment	Is the experimental procedure safe for both animals and human observers?
Financial	Staffing and travel expenses	
	External laboratory fees	
	Equipment required	
Long-term study	Practical application, financial and safety	

included in Tables 1 and 2 use the assessment of HAI to determine the HAR rather than measuring HAR empirically and so it is difficult to explain how these methods differ when measuring HAI or HAR. Employing the new definition of HAR means we are now able to measure HAR distinctly from HAI and have devised a scoring system to evaluate methods, based on our evaluation criteria (Table 3; see supplementary material to papers published in *Animal Welfare*: https://www.ufaw.org.uk/the-ufaw-journal/supplementary-material).

Response-based tests

Units of measurement

Latency (time taken) to respond and distance parameters are an *ex situ* mechanism to determine how animals respond to each other, or in the case of HAIs to measure their response to a human (McBride 1963; Stricklin 1979; Keeling 1995; Waiblinger *et al* 2006). For application to HAR assessment, latency and distance parameters could be used to compare responses by an animal towards different people, as well as being used in longitudinal studies to evaluate the nature of an HAR. Human-animal interaction tests, such as 'avoidance distance' and 'voluntary approach' tests use latencies to measure an animal's reaction to a human, by recording the time taken for an animal to approach or avoid a human (Waiblinger *et al* 2006). However, latency to specific responses, such as performing a requested behaviour (Ward & Melfi 2015), latency to feed (Boissy & Bouissou 1988), or latency to move to a desired area (Breuer *et al* 2003) can also be used as measures. Measuring distance parameters, most commonly the proximity to a human, is accomplished through using a laser distance meter, or distance estimations (Sherwen *et al* 2014; Smith 2014; Martin & Melfi 2016). Animals which initiate or accept close contact with conspecifics may cluster in groups, whereas aversion (or displacement) from conspecifics will be exhibited as greater spacing of animals (Keeling 1995). These spatial patterns can indicate the choices an animal has made, taking fixed environmental barriers to proximity into account. It follows that proximity to humans, and an animal's decision to approach or move away from a human, will provide an indication of the animal's perception of the HAI, or HAR. Although, due to the knowledge that individual keeper-animal dyads can be established (Ward & Melfi 2015), in order to truly understand the HAR, comparisons between different humans must be incorporated into methods using latency and distance parameters. Therefore, highlighting the need for a method that can incorporate this aspect with a degree of standardisation for use of these measures within the zoo environment.

Response to cues

Latencies can be used during routine HAIs (eg husbandry tasks) to investigate the nature of the HAI: this involves measuring the time an animal takes to respond to a specific cue from a keeper which can be monitored and recorded from areas within close proximity, ie as if the researcher were a zoo visitor. Ward and Melfi (2015) described how a shorter latency was indicative of an animal's enhanced co-operation and representative of a positive HAI. However, it is possible that the animal could be responding out of fear; this emphasises the importance of measuring other parameters in order to differentiate affective states, or potential motivation for behaviours. Depending on the cue provided, recording the latency to respond requires no formal training routine, especially where behaviours have been linked to current husbandry practices (Ward & Melfi 2013). Latency tests can also fit easily into an animal management routine and be recorded from a distance, therefore being safe for the observer and reducing the potential observer effect on the animal. However, the motivation with which an animal responds may affect its latency, and this may be influenced by either the HAR or unrelated factors (eg appetite, presence of conspecifics). Longitudinal testing, appropriate replication of tests, and/or comparisons between human-animal dyads is therefore necessary to determine HAR using these methods. In addition, the manner in which the observer first appears to the animal (eg suddenly, or from a specific direction; or the way that the human is dressed) may have an impact on latency, which may be difficult to quantify without repeated testing and longitudinal study designs.

Spatial parameters and latency to respond to a cue have also been used to assess HAIs and HARs between zoo-keepers and zoo-housed Chapman's zebra (*Equus quagga chapmani*), Sulawesi macaque (*Macaca nigra*) and black rhinoceros (*Diceros bicornis*) (Carlstead 2009; Ward & Melfi 2013, 2015). A significant difference in animals' latency to respond appropriately (ie perform the required/requested behaviour) to cues and signals from different zookeepers was interpreted to indicate that unique zookeeper-animal dyads had been formed (Carlstead 2009; Ward & Melfi 2013, 2015). In addition to measuring latency, Ward and Melfi (2013, 2015) also measured the keepers' escalation (positive or negative advances) of the original cue to try and quantify any differences between the keepers, as well as performance from the animals. However, it could also be that the animal's latency varies according to the time of day, the mood of the animal on the day, or some form of environmental impact; therefore, these potential factors would require either standardisation or at least measurement. Whilst this method has been utilised within the zoo context with a variety of species and within a multi-zoo set-up (Ward & Melfi 2013, 2015), it may be difficult to compare across species and zoos. This challenge arises because of differing animal management routines, different cues provided by keepers and different enclosure designs; each distinct request would likely elicit different periods in latencies and responses.

The influence of animal sociality must also be considered when using latencies to assess HAIs and HARs. For example, Ward and Melfi (2013) showed that socially housed animals (Chapmans zebra and Sulawesi black crested macaques) responded to keeper cues and commands by performing the requested behaviours significantly quicker than solitary animals (black rhinoceros). This could be dependent on the individual or a result of social facilitation, ie once one individual has performed the required behaviour others follow (Zentall 2006). It was predicted that the animal most likely to initiate a response within a group will demonstrate personality traits associated with confidence and boldness (Ward & Melfi 2013). Battini *et al* (2016) investigated the validity and feasibility of multiple HAR tests in dairy goats (*Capra aegagrus hircus*). During HAI approach tests with dairy goats within a social housing system, the male bucks were generally the first to approach, and inhibited the approach behaviour of the female goats (Battini *et al* 2016). Whilst this social influence could compromise the HAI tests by reducing validity and feasibility, this still requires testing in a zoo setting, and highlights a potentially novel aspect of HAIs and HARs to elucidate in non-domestic species. In this instance, when investigating HAIs, it would be beneficial to perform qualitative behaviour assessments (see *Reaction to handling*) and personality profiling (Carlstead *et al* 1999b; Wemelsfelder & Lawrence 2001) for each individual in order to investigate the potential effect of hierarchy or personality on HAI and HAR. However, the impact that social facilitation and hierarchy are likely to have within a group renders the investigation of specific keeper-animal dyads (ie HAR) difficult to accomplish in socially housed zoo species. Moreover, separating individual animals for research purposes is also unlikely to be feasible, or ethical.

Martin and Melfi (2016) compared zoo animal behavioural responses during HAIs to a familiar and an unfamiliar keeper ('keeper for the day'), to discover whether animals were able to distinguish between the two, and the influence on the animals' behavioural response. During routine HAI events, such as feeding and cleaning, observations of measurable animal behaviours were recorded including interactions and avoidance behaviours. In addition, estimations of the proximity of the animal to the keeper were recorded (< 1 m, 1 m, > 1 m) using scan sampling. This method allowed differences between responses with familiar and unfamiliar keepers to be detected, through a decrease in avoidance behaviour towards familiar keepers. Authors did not distinguish between the routine HAIs. For example, cleaning and feeding may have quite different effects on the animal with cleaning potentially perceived as negative/neutral, being associated with increased noise, smells of disinfectant and removal of their bedding, and feeding perceived as positive/neutral. In addition, other variables, such as the clothing worn, and the presence of the unfamiliar keeper in combination with the familiar keeper need to be standardised in future studies to ensure that they are not influencing the results. There were significantly more positive HAIs (reported as 'physical contact') between animals and unfamiliar keepers than familiar keepers. This method was able to identify HAIs that could then be linked to the development of possible HARs for unfamiliar keepers. However, interpretation of findings with this method could be challenging due to the multiple potential mechanisms involved, such as curiosity towards an unfamiliar keeper, which could therefore influence the interpretation of the HAI and consequently the HAR. Additionally, an HAR between the familiar keepers and animals may already have been established and therefore less need existed to reinforce the interaction, again suggesting that HARs could be objectively quantified using this method.

When using distance-based measures of an HAI, the method of estimating distance, or the use of broad distance categories varies between studies (eg Dalla Costa *et al* 2015; Battini *et al* 2016). This is likely to introduce inconsistencies and lower validity and accuracy between and even within studies if different observers are used to estimate distance, ie depending on the manner in which distance is estimated, or the size of the distance categories used. In order to generate accurate data for analysis, recording the positional parameter will require using appropriate apparatus to measure the distance between the animal and human during different events. These measurements could be potentially marked out onto the enclosure floor prior to behavioural observations. However, in turn, there is potential that a changed, novel addition to an area may influence animal behavioural responses. In addition, prior marking may not always be practical depending on enclosure design, substrate, accessibility and other variables. Some zoo HAI studies have successfully used remote distance measuring devices (eg Sherwen *et al* 2014, 2015), which use a laser to record distance from the meter to a solid surface. However, the handling of distance meters can potentially increase the risk of observers missing subtle cues or movements from the animals, compared to studies without this technology. Moreover, using a distance meter may not be feasible depending on zoo enclosure design and accessibility. Again, strict implementation of methods is needed to enable a full comparison of the animals' distance and therefore analysis of HAI.

Measuring the response to cues through latency and distance parameters can provide information on multiple features of HAIs that could contribute to the understanding of HARs within a zoo environment. The use of distance meters and the conductance of behavioural observations alongside latencies during routine HAI events will increase validity and accuracy as well as the practical application of this method. However, this method could prove difficult for multi-zoo comparisons due to different behavioural requests and enclosure accessibility between institutions.

Voluntary animal approach

The voluntary approach test was developed for horses by Søndergaard and Halekoh (2003) and has been referred to as the 'reaction to a stationary human' test (Waiblinger *et al* 2006). In this regard, it differs from the avoidance test in which the human approaches or attempts to touch an animal. An approach behaviour is defined as the animal approaching a stationary human; this can also be interpreted as the level of fear of humans an animal may have (Hemsworth & Coleman 1998; de Passillé & Rushen 2005). There are variations in terms of the experimental procedure and variables measured in order to utilise this method, however the basic concept is the same. A test person enters an area and stands stationary, the latency of an animal's approach can then be recorded, or when observing a group of animals, the percentage of animals observed to approach the human within a fixed time is recorded. Consequently, the level of fear of humans can be interpreted from the variables measured and used to establish the nature of the HAI, or HAR if assessed longitudinally.

Battini *et al* (2016) found that measuring latency during a voluntary approach test (defined, in this case, as the time interval between the stimulus and response) was the most feasible indicator to measure quality of the HAI in dairy goats, when evaluated against avoidance distance test and sneezing, ie the number of alert sounds. However, the definition of an approach varies between studies; it may be defined as the first contact (goats: Battini *et al* 2016), the animal moving within a specific distance radius (dairy cows: Rousing & Waiblinger 2004) or the first display of a species-specific approach behaviour (piglets [*Sus scrofa domesticus*]; front leg and head in the zone where the person is sitting (De Oliveira *et al* 2015). It may be that different definitions and implementation strategies are required for different species, however, this makes it increasingly challenging to evaluate the most appropriate way of utilising and replicating this particular method in a standardised manner. Battini *et al* (2016) also used distance parameters to record the percentage of dairy goats that entered within a 1.5-m radius around the test person at 1-min intervals, subsequent to the test person entering and standing stationary. The test person created a 1.5-m radius outline on the floor of the test area in order to easily record the number of individuals. However, the authors state that the feasibility of recording the distance parameter was reduced due to the time required to measure and mark out the semi-circumference on the test floor (Battini *et al* 2016). Other methods of demarcating the zone of interest may therefore be more appropriate. Nonetheless, the reaction to a stationary human is easily performed and frequently used for on-farm assessment (Waiblinger *et al* 2006). However, curiosity of a novel event, such as a human's presence, may increase the motivation to approach (Marchant *et al* 1997).

A study of the response of 12 ungulate species to a stationary human keeper, under two conditions (inside and then outside the enclosure) was conducted by Thompson (1989). Behaviours categorised as either interactive or non-interactive were observed and recorded; the recipient (either another animal or human) of an interactive behaviour, visual orientation, and physical contact towards the recipient were also scored. Behavioural observations were made outside of normal feeding times, with a keeper who was not the animals' normal keeper, and all had *ad libitum* access to food, with the aim of avoiding the potential confounder of keeper-food provisioning association existing for the animals (Thompson 1989).

Within this study there were instances of aggressive behaviour from some animals which resulted in the procedure and position of the stationary keeper needing to be altered to include a physical barrier and, in some cases, the cessation of data collection. This highlights the risks to both animal and human safety which will require consideration prior to using this test procedure to assess HAI and HARs in zoos. To ensure safety for both the keepers and animals involved, preliminary behavioural observations can be made, as well as the provision of a physical barrier. Smith (2014) included the use of approach behaviours as one of several prosocial human-directed behaviours by great apes towards both visitors and keepers in a zoo environment with a physical barrier. These affiliative behaviours were classified collectively as 'close' (< 3 m) or 'distant' (> 3 m), with positive interactions expected to be characterised by high levels of close affiliative behaviours. The degree of familiarity and close affiliative behaviours, including approach, were much greater in orangutans (*Pongo abelii*) compared with gorillas (*Gorilla gorilla gorilla*), which may suggest this method was sensitive enough to detect a difference between species. However, other zoo environmental factors, such as enclosure design and quality, group size and the availability of conspecifics within a group could also be influential, and therefore need to be considered.

Similarly, using quantitative measures in avoidance and approach tests to investigate an emotional state, such as fear, may be inappropriate. These tests are likely to elicit different behavioural reactions, which can be misinterpreted. For example, Zebu cattle (*Bos taurus indicus*) fear responses can range from intense avoidance, active defence, or inhibition of movement ('freezing') (Burrow & Corbet 2000). As such, the freezing behaviour of Zebu cattle may be misinterpreted using quantitative measures of distance to a human (ie as an animal having a good temperament and/or low fear). However, using qualitative and species-specific methods would better enable the identification of the fear response in this species (Burrow & Corbet 2000) and would likely be of benefit to a wide range of zoo-housed species (see *Qualitative behavioural assessment*).

Some agricultural HAI studies using the voluntary approach test involve the movement of the animal into a test area in order to minimise confounding variables and ensure safety. However, this in itself could elicit a behavioural response from the animal prior to the test (Søndergaard & Halekoh 2003; Waiblinger *et al* 2006; De Oliveira *et al* 2015). In order to avoid strong fear reactions to a human entering the test area, the test procedure should include a period of habituation (Battini *et al* 2016). This method requires minimal financial cost and training, however safety inside the enclosure is the primary concern with this method. The practical application of the voluntary approach test would be highly dependent on the accessibility to animals, enclosure design, training and the time taken to perform the test.

Avoidance tests

The avoidance test was developed in an agricultural context, initially for cows (Waiblinger *et al* 2003), and has since been used and validated for a number of species (dairy goats: Battini *et al* 2016; horses [*Equus ferus*] and donkeys [*Equus africanus*]: Dalla Costa *et al* 2015). This test involves a person approaching an animal, with an attempt made to touch or handle the animal. The latency of the animal to avoid (eg walk away from) the human is recorded in addition to the animal's behavioural responses. The test ends when the animal withdraws and moves away from the human. The avoidance distance from a human can be defined as the minimum distance to which an animal will allow a moving human to approach. This is thought to reflect the previous experience of the animal, under the assumption that animals which are most fearful will maintain a greater distance (de Passillé & Rushen 2005). However, it could be possible that the manner in which the animal retreats could indicate more about the HAI than just the distance, however this has not been evaluated. In order to assess HARs using this method, the animal response would need to be compared using different humans and will most likely necessitate multiple repeat testing to confirm findings.

In zoos, avoidance behaviours have been investigated in terms of response to conspecifics, obstacles, and visitors (eg gorillas: Collins & Marples 2016, penguins (*Eudyptula minor*): Sherwen *et al* 2015, polar bears (*Ursus maritimus*): Renner & Kelly 2006). However, these studies did not include empirical testing regarding the animal response to a specific HAI. There are currently no examples of using the avoidance test method in a zoo setting. This may be due to the ethical implications of creating a situation predicted to potentially elicit a fear response, or the safety risks involved with certain species. Nonetheless, this method has been successfully used in monitoring HAIs between humans and horses whilst utilising a physical barrier to ensure safety (Dalla Costa *et al* 2015) and could therefore be used for species that are housed in a protected contact management system (ie management of animals from behind barriers). Dalla Costa *et al* (2015) estimated the distance between a horse's head and assessor's hand in 'arm lengths.' Within a zoo setting, when considering HAIs with potentially dangerous species, the human may not be permitted to get within an arm's length of the animal, meaning if the animal does not move away from the physical barrier and the human does not approach further for safety reasons, the precise avoidance distance could not be assessed.

Battini *et al* (2016) assessed the feasibility of the avoidance method to determine its suitability for use as a farm welfare monitoring tool for dairy goats. Notably, the method was found to be time consuming on a large farm scale; it also required specific training by the observer to properly move into an area, recognise a first avoidance reaction, and assess the correct distance (Battini *et al* 2016). The interpretation of animal response can also be difficult if the animal did not move and neither approached nor avoided the human (Rousing & Waiblinger 2004; Battini *et al* 2016). In light of the limitations identified for this method, it may not be feasible within a zoo setting due to the lack of standardisation.

Reaction to handling

Experimental procedures have been developed for certain species to allow the observation and evaluation of an animal's reaction to handling (Waiblinger *et al* 2006). Within domestic animal studies, these methods usually involve responses to leading or moving, capture, restraint, and specific handling events, such as veterinary procedures (eg dairy cows: Waiblinger *et al* 2004; horses: Jezierski *et al* 1999; piglets: Brajon *et al* 2015; lambs [*Ovis aries*]: Caroprese *et al* 2012, eg poultry: Korte *et al* 1999). Both behavioural and physiological parameters can be measured during handling tests, such as time taken for a handling or restraint procedure, vocalisations, heart rate and circulating cortisol concentrations (eg in cattle: Lensick *et al* 2001; Waiblinger *et al* 2004). These tests rely on the animal being suitable for handling by humans in a safe manner (for both animal and humans). However, the requirement to include animal handling in the assessment also has ethical implications when conducted for research purposes, and opportunistic sampling may be limited for many zoo species due to the rarity of handling events.

In contrast, handling events, including leading, moving, or capture are generally common practice in livestock husbandry, albeit with varying styles, frequency or intensity among farms. Given the variation in the degree of handling that animals will experience in zoos, typically depending on the species and the safety implications of human contact with them, the reaction to handling test may not be suitable in all zoo species. However, particular species are regularly handled during educational programmes within some zoological institutions; in these instances, investigating the nature of HAI would be a beneficial addition to welfare assessments of these animals (Baird *et al* 2016). Through measuring the response of animals that are subjected to routine handling and therefore repeated interactions, information about the HAR can be determined.

Due to varying species and management routines, the lack of standardised handling procedures for the 'reaction to handling test' can affect the animal's response to humans therefore reducing reliability. The reaction to handling test has been used to directly assess the HAR through measuring response to humans, behavioural and physiological variables, and following different previous HAI treatments. Lensink *et al* (2001) measured heart rate, number of pushes from human, time to load the animal and number of buck-kicks during transportation loading in calves which had previously been subjected to either minimal human contact or daily human contact. Heart rate was a sensitive parameter which showed differences between calves which received additional previous human contact and calves subjected to minimal contact, however, it must be noted that heart rate could also have been influenced by human presence and degree of locomotion. Measuring heart rate in zoo species could be accomplished through on-animal monitors or stethoscope measurements, however, this is likely to prove challenging and not possible for some zoo species due to safety and ethical concerns. The study also found that housing systems influenced how calves reacted to humans during handling, suggesting that other factors can also influence an animal's response to humans during a handling event.

In some zoos, animals undergo positive reinforcement training whereby the animal receives a reward in order to increase the frequency of a desired behaviour (Heidenreich 2007). Zoo professionals are then able to cue the animal to participate in medical or husbandry procedures. Assessing responses during training, including handling, may not be a true representation or measurement of the 'reaction to handling' but more the reaction to the training and/or a learned response. Positive reinforcement training does, however, increase the opportunity for positive HAIs and is therefore likely to increase positive HAR (Ward & Melfi 2013) but would not be suitable as a method to measure the HAI or HAR.

The robustness and practical application of the response to handling test is species-dependant, meaning this test cannot be used for all species within a zoological institution due to the safety implications of contact with certain animals. It also requires some standardisation, in regards to how the animal is handled; the variation between handling style and skills of the handlers could potentially affect the results of the test (de Passillé & Rushen 2005), thereby decreasing reliability. Likewise, if this test is used with animals that are not handled as part of their daily routine, this method has the potential for negative ethical/welfare implications. Lastly, the additional time required of zoo staff to participate in this method would deem it unsuitable for long-term monitoring. Therefore, the response to handling test is considered unsuitable for use within zoo settings as a measure of HAIs or HARs.

Qualitative behaviour assessment

Qualitative behaviour assessment (QBA) is a 'whole-animal' assessment of an animal, based on evaluating body language and posture; it is used effectively to determine the animals' affective state, their personality, temperament and individual behaviour profiles (Wemelsfelder *et al* 2000, 2001; Wemelsfelder & Lawrence 2001). However, it may be possible to adapt this method to assess HAIs and HARs with specific familiar or unfamiliar humans. Qualitative behaviour assessments involve using free-choice profiling in which observers are asked to generate their own descriptive vocabularies of how an animal behaves, based on observing the whole animal's body language from numerous video clips (known as phase one). Subsequently, using these adjectives, observers score the animal from these and additional video clips (phase two). However, due to the requirement for multiple observers to analyse video clips during the two phases of free-choice profiling, the practical application of the QBA method can be challenging, time consuming, and even costly (eg observer expenses, IT equipment). Alternatively, a validated fixed-list of terms can be determined and used during phase two. Clarke *et al* (2016) directly compared the use of a fixed list and free-choice profiling using the same videos of group-housed sows and concluded that there was little difference. For application to HAR research, video clips which depict HAIs between animals and humans in a variety of settings or situations can be scored through free-choice profiling or using a fixed-list of descriptors. However, the fixed-list would remove the process of qualitatively interpreting the animals' expressions (Wemelsfelder *et al* 2009; Napolitano *et al* 2012), therefore free choice profiling would be preferred when measuring HAIs and HARs in the zoo environment and would represent a novel application of this method. Whilst it may be possible to conduct QBA with live observations of animals (Wemelsfelder & Lawrence 2001) in order to reduce recording logistics, ensuring that the number of observers required (around 20) does not impact on the behaviour and/or response of the animals and keepers involved will be difficult (if not impossible). Previous published studies utilising QBA have all used video footage and this is also likely to be the most efficient form of observation in a zoo setting.

Qualitative behaviour assessments have been applied to agricultural species as a reliable and cost-effective approach to monitoring animal welfare (dairy cattle: Wemelsfelder *et al* 2009; horses and ponies; McMillan 2000; Morton 2000; Wemelsfelder & Lawrence 2001; Napolitano *et al* 2008). The method includes the incorporation of subtle movements, posture and aspects of the context in which the behaviour occurs into an animal's overall style of behaviour; thereby evaluating the 'animal-as-a-whole' (eg bold, shy, hostile) (Wemelsfelder *et al* 2000, 2001; Napolitano *et al* 2008). There are few HAI studies which use QBA, although this approach has been used to determine the nature of HAIs in regard to stockperson handling style on dairy calves and has also demonstrated the ability of stockpersons to predict animal behaviour (Ellingsen *et al* 2014; Ebinghaus 2017). These assessments have also been used to determine individual traits in zoo species (eg snow leopards [*Panthera uncia*]: Gartner & Powel 2011), such as scores on 'friendly to keeper', which can then be correlated with other factors, such as breeding success and welfare (eg black rhinos: Carlstead *et al* 1999a,b). Applying QBA as HAI or HAR assessment method within zoos will elicit a more sensitive, integrative, 'whole-body' assessment of how an animal interacts with humans in their environment, incorporating responses which may not be captured during quantitative assessments. This method comes the closest to being able to measure an HAR from the animal's perspective so long as it incorporates long-term monitoring and the video footage enables observers to monitor the pattern of the interactions. Daily interactions, such as routine tasks for a particular species that occur with multiple keepers and animals, can be observed using QBA to investigate HARs in terms of how the animal responds holistically to these repeated interactions. For example, the authors have observed footage during a daily husbandry routine whereby a giraffe (*Giraffa camelopardalis*) was provided food by a keeper, the keeper attempted to touch the giraffe, the giraffe then pulled away and removed itself from the interaction and the food. Through using QBA, and therefore capturing the 'whole body' response and affective state of the animal, this scenario could be more comprehensively documented and evaluated in accordance with our new definition, including the content, quality and pattern of interactions.

Some studies have combined the use of QBA and quantitative methods, such as behaviour frequencies (eg Napolitano *et al* 2012; Rutherford *et al* 2012). This suggests QBA could be used alongside quantitative data obtained from an HAI test. By combining QBA with ethogram-derived data, it may be possible to gain a better understanding of an animal's affective state during particular HAI events or assess the existence or character of an HAR. This is advantageous when assessing the nature of HARs at an individual level, which consequently may aid in understanding the potential impact personality, social facilitation and hierarchy have on HARs.

The practical application and feasibility of QBA is challenging. Recording the initial videos, especially if this requires specific HAI events to be observed, and the requirement to capture varying aspects of an animal's behavioural repertoire are key logistical factors to consider when implementing QBA. Nonetheless, logistical challenges can be overcome. Importantly, this method can be performed without contact or interference with the animal, and videos can typically be easily obtained for all species within a zoo environment, dependant on enclosure design and accessibility. High agreement among observer groups with varying backgrounds has been demonstrated in agricultural studies, and among keepers in the limited zoo studies that exist, proving QBA to be a reliable method to investigate HAIs as well as HARs.

Within the zoo environment where routine HAI events occur daily among multiple keepers and animals, QBA will enable subtle movements, posture and aspects of the context in which the behaviour occurs (which may otherwise be overlooked in quantitative methods) to be incorporated into HAI evaluations. Although means to validate QBA exist and have been used in the few QBA zoo studies published to date, further testing is required to determine the validity and reliability of QBA for use in studies investigating the presence or characteristics of HAI and HARs. Therefore, applying QBA could elicit a better understanding and interpretation of how HAIs can determine and influence HARs and warrants further investigation.

Common themes

Throughout this evaluation, common constraints and limitations have become apparent when considering the application of these methods to a zoo environment with a wide variety of species and accessibility. External factors, such as housing, social groupings, husbandry and environmental aspects may influence the results of the described tests. For example, varying responses to HAIs were elicited at different times of the year for lactating cows, which could be associated with altered husbandry practices, namely the variation in quality and quantity of HAIs during indoor and outdoor housing periods (Battini *et al* 2011). Seasonal husbandry practices, breeding or group dynamics have the potential to influence differences in avoidance distance; these factors may also be difficult to control within the zoo environment (Thompson 1989; Waiblinger *et al* 2006; Battini *et al* 2011) but would be worthwhile investigating.

Latency and distance parameters used in response to cues, voluntary approach and avoidance tests all rely on the assumption that how an animal responds through performing a behaviour or moving represents how that animal perceives human presence or interaction. From this, the features of the HAI can consequently be used to determine the HAR. However, animal responses could also be the response to a different interaction or movement. It will also be difficult to know specifically whether the animal is responding to the human or coincidentally moving towards or facing a given direction for an unrelated reason. Curiosity of a novel event could also increase the motivation to approach or perform a requested behaviour in the presence of a human (Marchant *et al* 1997). In a zoo setting, this could suggest that this test is less suitable for animals that rarely have human contact, as it may be measuring animal curiosity rather than an HAI or an indicator of fear or personality (Marchant *et al* 1997; Waiblinger *et al* 2003; Chelluri *et al* 2013). Smith (2014) discussed the findings that apes tended to seek proximity to certain staff members, such as waste disposal and education staff, even though the staff behaviours were not necessarily rewarding to the ape compared to zoo professionals that may, for example, feed them. This suggested that an approach behaviour might indicate an interest or curiosity instead of familiarity or the anticipation of a reward. In a zoo setting, this could suggest that the voluntary approach and avoidance test is less suitable for animals that rarely have human contact, as it may be measuring animal curiosity rather than an HAI or an indicator of fear (Marchant *et al* 1997; Waiblinger *et al* 2003; Chelluri *et al* 2013).

The safety risks for participants will prohibit the use of the voluntary approach, avoidance and reaction to handling tests for some zoo-housed species. Although there are studies within zoo settings in which the response of animals is observed in the presence of relatively stationary humans, such as zoo visitors, these situations are far from ideal since visitors are not stationary for consistent times, or may be part of a group with mixed activity, and are most often separated from the animal by some form of barrier (Chamove *et al* 1988; Sherwen *et al* 2014, 2015). The voluntary approach and avoidance test have been used with the presence of a physical barrier to ensure safety with some agricultural and zoo species (ungulates: Thompson 1989; horses: Dalla Costa *et al* 2015). However, this still may not be feasible for some zoo species that are potentially dangerous and are managed via protected contact, therefore suggesting these tests may not be applicable to all zoo species.

Animal welfare implications

In zoos, research has identified that positive HAIs can lead to positive HARs, however no previous studies have empirically measured HARs due to the difficulties associated with the previous definition. The influence that these diverse HAIs have on an animal's welfare state has only recently started to be quantified. As such, practical and evidence-based recommendations are not available to ensure high animal welfare during HAIs. Data exist to demonstrate the overwhelming potential for HAI and HARs to exert significant impact on zoo animal welfare status, however these are derived primarily from preliminary pilot studies (in zoos) or extrapolation from more comprehensively conducted animal welfare studies in agricultural settings. Along with the new definition of HARs, this evaluation, based on a subjective assessment using defined criteria, has highlighted three potential methods (qualitative behaviour assessment, latency to respond, and voluntary approach tests) that could be used to assess HARs within a zoo environment in order to empirically determine the impact that these may have on animal welfare.

Conclusion

In agricultural HAI and HAR research, specific tests have been investigated extensively in terms of reliability, validity, feasibility and effectiveness. However, although the current research on HAI and HARs in zoos is a growing area of scientific interest, methods of assessing these in zoos have not yet been standardised. Previous studies have inferred the HAR from the animal's perspective based on measuring HAIs alone. An extension of measuring HAI to HAR is not automatic, therefore emphasising the need for standard methods to measure HAI and HAR specifically across species, rather than erroneously using the terms interchangeably. Of the methods available and evaluated, three have been identified as having potential for successful application to measuring HAI in zoos. Following our evaluation criteria, measuring latency to respond (eg to a cue), QBA and the voluntary approach test are methods that are considered reliable and feasible tests to assess HAIs within a zoo environment. Perhaps more importantly, under the new HAR definition, these methods are considered likely to be of particular value when empirically measuring HARs. With some modifications, such as the use of physical barriers, these tests do not induce fear in the animals or risk the safety of the staff or animals involved. Due to the variation in species, husbandry practices and enclosures within the zoo environment, we recommend that each of the three methods identified here should be subjected to further testing in a zoo environment using the evaluation scoring factors adopted in this review. Our identification of three potential methods enables the progression of the study of HARs within a zoo environment, ultimately ensuring that the implications of HARs for animal welfare can be reliably investigated and compared.

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