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

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13 Abstract

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

33

34 Keywords: Animal welfare, behaviour, human-animal-interactions, method, zoo

35

36 1.0 Introduction

37 The scientific study of human-animal interactions (HAI) is a multi-disciplinary field that is of interest
38 to biologists, sociologists, psychologists, and conservationists, amongst others. An *interaction* has
39 traditionally been defined in respect of inter-human interactions as a sequence in which an individual

40 performs a behaviour towards another, which is subsequently responded to with a specific reaction
41 (Hinde 1976, p3). According to Hinde (1976) a relationship is a succession of interactions between two
42 individuals known to each other and influenced by a history of past interactions. Previous definitions
43 of human-animal relationships (HARs) suggest that each participant within the relationship are able to
44 make predictions about the other's responses (Estep & Hetts 1992; Hemsworth et al 1993). However,
45 if this definition is applied verbatim, it is not feasible to measure the ability of an animal to predict a
46 human's behaviour. With this in mind, it appears necessary to devise a new definition that would enable
47 the measurement of HARs. Therefore in this context and throughout this paper, a HAR is defined as a
48 series of repeated HAIs between two individuals known to each other, the nature of which is influenced
49 by their historical HAIs. Aspects to consider involve the content, quality and pattern of the interactions.
50 For example, a HAR within a zoo setting can occur because of daily routine interactions with familiar
51 zoo personnel (including keepers, education providers, maintenance and gardens teams, etc.). We
52 propose that the HAR should be measured over a period of time, recording the content of interactions
53 (i.e. the behaviours performed to create the interaction), the quality of interactions (i.e. positive,
54 negative and/or neutral) and the order in which these interactions occur (i.e. the consistency of the
55 interactions e.g. human A calls over animal B, B comes over to A, A strokes B or A calls over B, B
56 slowly comes over to B with no further interactions). What is important to consider is that due to this
57 definition, interactions between unfamiliar humans (e.g. visitors) cannot develop into HARs.

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

66 1.1 Human animal interactions and animal welfare

67 Based on findings in companion (rabbits: Podberscek et al 1991), livestock (dairy calves: Ellingsen et
68 al 2014), and laboratory (primates: Baker 2004) animals, HAIs and HARs represent a significant
69 influencing factor in animal health and welfare, as well as having important roles to play in human
70 health and wellbeing (O’Haire 2010). Likewise, HAIs and HARs have been recorded and investigated
71 in zoos, and are considered to have implications for the health and welfare of the animals involved
72 (HAR; Carlstead, 2009, Smith 2014, Martin & Melfi 2016, Carlstead et al 2018, HAI; Carrasco et al
73 2009, Chelluri et al 2013, Ward & Melfi 2013, 2015). However, most studies are primarily reliant on
74 one method alone i.e. studies of animal behaviour and as welfare is multifaceted, additional measures
75 would be needed to measure this specifically. According to our definition, the features of any interaction
76 between an animal and a human will influence the way in which a HAR develops. Interactions can be
77 perceived by the animal and the human as negative, neutral or positive, and consequently result in the
78 development of a negative, neutral or positive HAR categorisation, respectively (Carlstead 2009, Hosey
79 2008; Smith 2014, Waiblinger et al 2006).

80
81 Previous research into the implications of HAIs for farm animal welfare have suggested that, whilst
82 some animals may become accustomed to human contact, the majority of observed reactions involve
83 some level of fear (Jones 1997). Many of the HAIs which occur in a farm environment are of a negative
84 nature, such as exposure to rough handling, restraint and veterinary treatments, with positive or neutral
85 HAIs generally only associated with feeding (Hemsworth & Coleman 1998). It follows that assessments
86 of HAIs and HARs based on the measurement of an animal’s level of fear (or confidence) in humans
87 have been well tested and are commonly used as part of on-farm welfare assessments with the use of
88 both familiar and unfamiliar humans (Battini et al 2016; de Passillé & Rushen 2005). However, fear
89 induced responses from these negative HAIs can include attempts to escape, which in turn can result in
90 injuries or deaths (i.e. from animals running into obstacles or other individuals) which raises welfare
91 concerns and have detrimental health and safety implications in zoos. The use of an unfamiliar human
92 within these tests primarily focusses on assessing an animal’s general fear of humans, however based

93 on the HAR definition in which a HAR can occur between two individuals known to each other, a
94 familiar human would be used to explore this specific aspect.

95

96 The behaviour and attitude of stockpersons have shown to be major variables that can determine an
97 animal's fear or confidence in a human, therefore stockmanship has the potential to influence the quality
98 of HAIs and HARs as well as animal welfare. Positive or neutral HAIs and HARs can be beneficial to
99 animal welfare, for example gentle handling or the presence of a familiar human may calm the animals
100 in potentially negative situations, reducing the risk of injuries and therefore improving welfare (e.g.
101 dairy cows; Waiblinger et al 2004). Ellingsen et al (2014) studied stockperson handling styles at 100
102 Norwegian dairy farms (100 stockpersons, mean number of 31 calves per farm) and identified differing
103 styles which were termed 'positive interactions', 'calm/patient', 'dominating/aggressive', and
104 'insecure/nervous'. Results suggested that stockpersons who handled calves patiently and calmly were
105 associated with animals exhibiting a higher level of positive mood, whereas those who used a nervous
106 or dominating style of handling were associated with calves of a more negative mood. Positive moods
107 in animals can be interpreted as pleasant emotions; this state is predicted to influence the nature of the
108 HAI in a positive manner through a positive feedback cycle (see Waiblinger et al 2006). The emotional
109 state of an animal during a HAI will likely influence its perception and reaction to humans and
110 subsequently impact on the nature of the HAI itself; a range of influential factors contribute to this
111 emotional dimension and must be considered during HAI studies, particularly when welfare is a
112 measured outcome. In particular, fear and nervousness in animals has been associated with stress and
113 reduced animal welfare (Rushen et al 1999). Waiblinger et al (2006) described the establishment of a
114 negative feedback cycle between the handlers and animals, in which as the behaviour and attitude of
115 stockperson worsens, fear subsequently increases for the animal, resulting in continued or increased
116 negativity of the stockperson's attitude. For example, in Ellingsen's (2014) study, handling situations
117 involving calves with a negative mood potentially led to animals which were more difficult to handle
118 and uncooperative, resulting in more dominating or aggressive behaviour and attitude of handlers, and
119 consequently a negative HAI and low welfare. In contrast, positive interactions that lead to pleasant
120 emotions and a neutral or positive HAI, through the positive attitude of handlers, promotes good

121 welfare. This is represented in one of the few zoo HAI studies relating to welfare; Ward and Melfi's
122 (2013) zoo stockmanship cycle. This study highlighted the concept that a positive response from the
123 animal following a positive keeper-animal interaction, promotes further positive responses from the
124 animal and so forth, developing a positive HAR and potentially positive animal welfare. Positive HAIs
125 have also been found to improve the welfare of laboratory animals; increased periods of positive HAIs
126 have been shown to result in a reduction of fearful reactions in rabbits (Podberscek et al 1991), and to
127 lower levels of abnormal behaviours in laboratory chimpanzees (Baker 2004).

128

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

135

136 The importance of HAIs in animal welfare is an area of active research, and was highlighted as being
137 one of the most significant recent contributions to zoo animal welfare science (Meehan et al 2016).
138 Moreover, the connections among animal welfare, zoo visitor experience, and wildlife conservation are
139 clear and notable in the revised vision of the World Association of Zoos and Aquaria (Barongi et al
140 2015). However, the role HAIs and HARs have in modulating an animals' behavioural repertoire, their
141 social interactions, or life history events and outcomes has been inadequately explored, with only a few
142 studies published to date (Mellen 1991, Wielebnowski 1999; Wielebnowski et al 2002, Carlstead 2009,
143 Carrasco et al 2009, Smith 2014, Carlstead et al 2018). It is possible that the modulating effect of HAIs
144 and HARs on these factors could exert significant influence on the welfare, management, and
145 conservation consequences of zoo-housed animals. Whilst the extent of HAI influence has not yet been
146 quantified in zoos, the potential exists for HAIs to impact on the welfare status of hundreds of thousands

147 of animals, many of which are involved in captive breeding programmes of international significance
148 to the *ex-situ* conservation of their species. For example, data retrieved on 31st May 2018 from the
149 ‘Zoological Information Management System’ (an animal records subscription-based database
150 (Species360, 2018) revealed that there were 280,762 mammalia, 290,792 aves and 99,872 reptilia held
151 in member facilities. These numbers are highly under-representative of zoos since it was estimated that
152 within the > 10,000 zoos worldwide (Fravel 2003), just over 10% of these zoos contribute to this global
153 database. The welfare implications of zoo HAIs and HARs therefore potentially affect a vast number
154 of individual animals.

155
156 Despite this potential impact on animal welfare at the individual and population level, we have yet to
157 establish a basic understanding of how HAIs and HARs function in zoos, or what the consequences of
158 such HAIs and HARs may be for the diverse range of species in zoos. In addition, the results and
159 discussion of published work on zoo HAR mainly refer to welfare as being related to HAIs or HARs
160 rather than measuring welfare implications specifically. It is therefore important to firstly determine an
161 appropriate method to measure HARs that can be applied in a standardised manner across a range of
162 zoological settings and to a variety of species. Given the behavioural variation between species, let
163 alone among different taxa, this is a difficult task. However, as the HAR is a product of HAIs, the
164 response of the animal to a specific human stimuli can be measured as a fundamentally common starting
165 point. As a first step towards this goal, this evaluation provides a comprehensive review of the current
166 methods available to study HAIs and HARs from the perspective of the animal, the majority deriving
167 from agricultural contexts (see Waiblinger et al 2006; Table 1). This will determine methods predicted
168 to be suitable for testing in zoo environments, in order to establish a robust, reliable and feasible method
169 (or panel of methods) for future zoo HAI and HAR studies. Equally important is the human dimension
170 of the HAI (i.e. the response and/or perception of the HAI by the human involved), necessitating the
171 utilisation of proven, reliable methods from the social sciences. However, the measurement of the
172 human perspective, or the implications of the HAI or HAR for animal welfare or human well-being are
173 beyond the scope of this review. Similarly, the important but under-investigated field of multi-zoo

174 comparisons of husbandry factors involved in determining HARs warrants further investigation, and
175 will likely benefit from the application of standardised methodology to assess HARs.

176 2.0 The evaluation process

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

193
194 The HAI or HAR methods used in the studies were categorised by the animal environment (zoo,
195 companion, agricultural, laboratory) and additionally categorised into the most commonly used
196 methods, as shown in Table 1. Factors used to evaluate the zoo HAI or HAR methods (Table 2) were
197 adapted from Waiblinger et al (2006) and could be of use within the zoo industry. The important aspect
198 here is that the majority of the studies included in Tables 1 and 2 use the assessment of HAI to determine
199 the HAR rather than measuring HAR empirically and so it is difficult to explain how these methods
200 differ when measuring HAI or HAR. Employing the new definition of HAR, we are now able to measure

201 HAR distinctly from HAI and have devised a scoring system to evaluate methods, based on our
202 evaluation criteria (Table 3).

203 3.0 Response-based tests

204 3.1 Units of measurement

205 Latency (time taken) to respond and distance parameters are an *ex situ* mechanism to determine how
206 animals respond to each other, or in the case of HAIs to measure their response to a human (Keeling
207 1995; McBride 1963; Stricklin 1979; Waiblinger et al 2006). For application to HAR assessment,
208 latency and distance parameters could be used to compare responses by an animal towards different
209 people, as well as being used in longitudinal studies to evaluate the nature of a HAR. Human-animal
210 interaction tests such as ‘avoidance distance’ and ‘voluntary approach’ tests use latencies to measure
211 an animal’s reaction to a human, by recording the time taken for an animal to approach or avoid a human
212 (Waiblinger et al 2006). However, latency to specific responses such as performing a requested
213 behaviour (Ward and Melfi, 2015), latency to feed (Boissy and Bouissou, 1988), or latency to move to
214 desired area (Breuer et al., 2003) can also be used as measures. Measuring distance parameters, most
215 commonly the proximity to a human is accomplished through using a laser distance meter, or distance
216 estimations (Martin and Melfi 2016, Sherwen et al 2014, Smith 2014). Animals which initiate or accept
217 close contact with conspecifics may cluster in groups, whereas aversion (or displacement) from
218 conspecifics will be exhibited as greater spacing of animals (Keeling 1995). These spatial patterns can
219 indicate the choices an animal has made, taking fixed environmental barriers to proximity into account.
220 It follows that proximity to humans, and an animal’s decision to approach or move away from a human,
221 will provide an indication of the animal’s perception of the HAI, or HAR. Though, due to the knowledge
222 that individual keeper-animal dyads can be established (Ward and Melfi, 2015), in order to truly
223 understand the HAR, comparisons between different humans must be incorporated into methods using
224 latency and distance parameters. Therefore, highlighting the need for a method that can incorporate this
225 aspect with a degree of standardisation for use of these measures within the zoo environment.

226

227 3.2 Response to cues

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

245
246 Spatial parameters and latency to respond to a cue have also been used to assess HAIs and HARs
247 between zookeepers and zoo-housed Chapmans zebra (*Equus quagga chapmani*), Sulawesi macaque
248 (*Macaca nigra*) and black rhinoceros (*Diceros bicornis*) (Carlstead 2009; Ward & Melfi 2013; 2015).
249 A significant difference in animals' latency to respond appropriately (i.e. perform the
250 required/requested behaviour) to cues and signals from different zookeepers was interpreted to indicate
251 that unique zookeeper-animal dyads had been formed (Carlstead 2009; Ward & Melfi 2013, 2015). In
252 addition to measuring latency, Ward and Melfi (2013; 2015) also measured the keepers' escalation
253 (positive or negative advances) of the original cue to try and quantify any differences between the
254 keepers, as well as performance from the animals. However, it could also be that the animal's latency

255 varies according to the time of day, the mood of the animal on the day, or some form of environmental
256 impact; therefore these potential factors would require either standardisation or at least measurement.
257 Whilst this method has been utilised within the zoo context with a variety of species and within a multi-
258 zoo set up (Ward & Melfi 2013; 2015), it may be difficult to compare across species and zoos. This
259 challenge arises because of differing animal management routines, different cues provided by keepers
260 and different enclosure designs; each distinct request would likely elicit different periods in latencies
261 and responses.

262

263 The influence of animal sociality must also be considered when using latencies to assess HAIs and
264 HARs. For example, Ward and Melfi (2013) showed that socially housed animals (Chapmans zebra and
265 Sulawesi black crested macaques) responded to keeper cues and commands by performing the requested
266 behaviours significantly quicker than solitary animals (black rhinoceros). This could be dependent on
267 the individual or a result of social facilitation, i.e. once one individual has performed the required
268 behaviour others follow (Zentall 2006). It was predicted that the animal most likely to initiate a response
269 within a group will demonstrate personality traits associated with confidence and boldness (Ward &
270 Melfi 2013). Battini et al (2016) investigated the validity and feasibility of multiple HAR tests in dairy
271 goats. During HAI approach tests with dairy goats within a social housing system, the male bucks were
272 generally the first to approach, and inhibited the approach behaviour of the female goats (Battini et al
273 2016). Whilst this social influence could compromise the HAI tests by reducing validity and feasibility,
274 this still requires testing in a zoo setting, and highlights a potentially novel aspect of HAIs and HARs
275 to elucidate in non-domestic species. In this instance, when investigating HAIs it would be beneficial
276 to perform qualitative behaviour assessments (see below, section 3.5) and personality profiling
277 (Wemelsfelder & Lawrence 2001; Carlstead et al 1999b) for each individual in order to investigate the
278 potential effect of hierarchy or personality on HAI and HAR. However, the impact that social
279 facilitation and hierarchy are likely to have within a group renders the investigation of specific keeper-
280 animal dyads (i.e. HAR) difficult to accomplish in socially housed zoo species. Moreover, separating
281 individual animals for research purposes is also unlikely to be feasible, or ethical.

282

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

304

305 When using distance-based measures of a HAI, the method of estimating distance, or the use of broad
306 distance categories varies between studies (e.g. Battini et al 2016; Dalla Costa et al 2015). This is likely
307 to introduce inconsistencies and lower validity and accuracy between and even within studies if
308 different observers are used to estimate distance, i.e. depending on the manner in which distance is
309 estimated, or the size of the distance categories used. In order to generate accurate data for analysis,
310 recording the positional parameter will require using appropriate apparatus to measure the distance

311 between the animal and human during different events. These measurements could potentially be
312 marked out onto the enclosure floor prior to behavioural observations. However, in turn there is
313 potential that a changed, novel addition to an area may influence animal behavioural responses. In
314 addition, prior marking may not always be practical depending on enclosure design, substrate,
315 accessibility and other variables. Some zoo HAI studies have successfully used remote distance
316 measuring devices (e.g. Sherwen et al 2014; 2015), which use a laser to record distance from the meter
317 to a solid surface. However, the handling required of distance meters can potentially increase the risk
318 of observers missing subtle cues or movements from the animals, compared to studies without this
319 technology. Moreover, using a distance meter may not be feasible depending on zoo enclosure design
320 and accessibility. Again, strict implementation of methods is needed to enable a full comparison of the
321 animals' distance and therefore analysis of HAI.

322

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

329

330 *3.3 Voluntary Animal Approach*

331 The voluntary approach test was developed for horses by Søndergaard & Halekoh (2003), and has been
332 referred to as the “reaction to a stationary human” test (Waiblinger et al 2006). In this regard, it differs
333 from the avoidance test in which the human approaches or attempts to touch an animal. An approach
334 behaviour is defined as the animal approaching a stationary human; this can also be interpreted as the
335 level of fear of humans an animal may have (Hemsworth & Coleman 1998; de Passille & Rushen 2005).
336 There are variations in terms of the experimental procedure and variables measured in order to utilise
337 this method, however the basic concept is the same. A test person enters an area and stands stationary,

338 the latency of an animal's approach can then be recorded, or when observing a group of animals, the
339 percentage of animals observed to approach the human within a fixed time is recorded. Consequently,
340 the level of fear of humans can be interpreted from the variables measured and used to establish the
341 nature of the HAI, or HAR if assessed longitudinally.

342

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

362

363 A study of the response of 12 ungulate species to a stationary human keeper, under two conditions
364 (inside the enclosure and then outside the enclosure) was conducted by Thompson (1989). Behaviours
365 categorised as either interactive or non-interactive were observed and recorded; the recipient (either

366 another animal or human) of an interactive behaviour, visual orientation, and physical contact towards
367 the recipient were also scored. Behavioural observations were made outside of normal feeding times,
368 with a keeper who was not the animals' normal keeper, and all had access to food *ad libitum*, with the
369 aim of avoiding the potential confounder of keeper-food provisioning association existing for the
370 animals (Thompson 1989).

371

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

386

387 Similarly, using quantitative measures in avoidance and approach tests to investigate an emotional state
388 such as fear may be inappropriate. These tests are likely to elicit different behavioural reactions, which
389 can be misinterpreted. For example, Zebu cattle fear responses can range from intense avoidance, active
390 defence, or inhibition of movement ("freezing") (Burrow & Corbet 2000). As such, the freezing
391 behaviour of Zebu cattle may be misinterpreted using quantitative measures of distance to a human (i.e.
392 as an animal having a good temperament and/or low fear). However, using qualitative and species-
393 specific methods would better enable the identification of the fear response in this species (Burrow &

394 Corbet 2000) and would likely be of benefit to a wide range of zoo-housed species (see Qualitative
395 Behavioural Assessment section below).

396

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

405 *3.4 Avoidance tests*

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

419

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

434
435 Battini et al (2016) assessed the feasibility of the avoidance method to determine its suitability for use
436 as a farm welfare monitoring tool for dairy goats. Notably, the method was found to be time consuming
437 on a large farm scale; it also required specific training by the observer to properly move into an area,
438 recognise a first avoidance reaction, and assess the correct distance (Battini et al 2016). The
439 interpretation of animal response can also be difficult if the animal did not move and neither approached
440 nor avoided the human (Battini et al 2016, Rousing & Waiblinger 2004). In light of the limitations
441 identified for this method, it may not be feasible within a zoo setting due to the lack of standardisation.

442

443 *3.5 Reaction to handling*

444 Experimental procedures have been developed for certain species to allow the observation and
445 evaluation of an animal's reaction to handling (Waiblinger et al 2006). Within domestic animal studies,
446 these methods usually involve responses to leading or moving, capture, restraint, and specific handling

447 events such as veterinary procedures (e.g. dairy cows: Waiblinger et al 2004; horses: Jeziarski et al
448 1999; piglets: Brajon et al 2015; lambs: Caroprese et al 2012, e.g. poultry: Korte et al 1999). Both
449 behavioural and physiological parameters can be measured during handling tests, such as time taken for
450 a handling or restraint procedure, vocalisations, heart rate and circulating cortisol concentrations (e.g.
451 in cattle, Waiblinger et al 2004; Lensick et al 2001). These tests rely on the animal being suitable for
452 handling by humans in a safe manner (for both animal and humans). However, the requirement to
453 include animal handling in the assessment also has ethical implications when conducted for research
454 purposes, and opportunistic sampling may be limited for many zoo species due to the rarity of handling
455 events.

456

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

466

467 The lack of standardised handling procedures for the reaction to handling test due to varying species
468 and management can result in additional influencing factors affecting an animal's response to humans
469 therefore reducing reliability. The reaction to handling test has been used to directly assess the HAR
470 through measuring response to humans, behavioural and physiological variables, and following
471 different previous HAI treatments. Lensink et al (2001) measured heart rate, number of pushes from
472 human, time to load the animal and number of buck-kicks during transportation loading in calves which
473 had previously been subjected to either minimal human contact or daily human contact. Heart rate was
474 a sensitive parameter which showed differences between calves which received additional previous

475 human contact and calves subjected to minimal contact, however it must be noted that heart rate could
476 have also been influenced by human presence and degree of locomotion. Measuring heart rate in zoo
477 species could be accomplished through on-animal monitors or stethoscope measurements, however this
478 is likely to prove challenging and not possible for some zoo species due to safety and ethical concerns.
479 The study also found that housing systems influenced how calves reacted to humans during handling
480 suggesting that other factors can also influence an animal's response to humans during a handling event.

481

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

489

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

499

500 4.0 Qualitative Behaviour Assessment

501 Qualitative Behaviour Assessment (QBA) is a “whole-animal” assessment of an animal, based on
502 evaluating body language and posture; it is used effectively to determine the animals’ affective state,
503 their personality, temperament and individual behaviour profiles (Wemelsfelder & Lawrence 2001,
504 Wemelsfelder 2000, 2001). However, it may be possible to adapt this method to assess HAIs and HARs
505 with specific familiar or unfamiliar humans. Qualitative behaviour assessments involve using free-
506 choice profiling in which observers are asked to generate their own descriptive vocabularies of how an
507 animal behaves, based on observing the whole animal’s body language from numerous video clips
508 (known as phase one). Subsequently, using these adjectives observers score the animal from these and
509 additional video clips (phase two). However, due to the requirement for multiple observers to analyse
510 video clips during the two phases of free-choice profiling, the practical application of the QBA method
511 can be challenging, time consuming, and even costly (e.g. observer expenses, IT equipment).
512 Alternatively, a validated fixed-list of terms can be determined and used during phase two. Clarke et al
513 (2016) directly compared the use of a fixed list and free-choice profiling using the same videos of group-
514 housed sows and concluded that there was little difference. For application to HAR research, video clips
515 which depict HAIs between animals and humans in a variety of settings or situations can be scored
516 through free-choice profiling or using a fixed-list of descriptors. However, the fixed-list would remove
517 the process of qualitatively interpreting the animals’ expressions (Wemelsfelder 2009; Napolitano et al
518 2012), therefore free choice profiling would be preferred when measuring HAIs and HARs in the zoo
519 environment, and would represent a novel application of this method. Whilst it may be possible to
520 conduct QBA with live observations of animals (Wemelsfelder and Lawrence, 2001) in order to reduce
521 recording logistics, ensuring that the number of observers required (around 20) does not impact on the
522 behaviour and/or response of the animals and keepers involved will be difficult (if not impossible).
523 Previous published studies utilising QBA have all used video footage and is also likely to be the most
524 efficient form of observation in a zoo setting.

525 Qualitative behaviour assessments have been applied to agricultural species as a cost-effective and
526 reliable approach to monitoring animal welfare (dairy cattle: Wemelsfelder et al 2009; horse and ponies;
527 Napolitano et al 2008; Wemelsfelder and Lawrence 2001; McMillan 2000; Morton 2000). The method
528 includes the incorporation of subtle movements, posture and aspects of the context in which the
529 behaviour occurs into an animal's overall style of behaviour; thereby evaluating the "animal-as-a-
530 whole" (e.g. bold, shy, hostile) (Napolitano et al 2008; Wemelsfelder et al 2001; 2000). There are few
531 HAI studies which use QBA, although this approach has been used to determine the nature of HAIs in
532 regard to stockperson handling style on dairy calves, and has also demonstrated the ability of
533 stockpersons to predict animal behaviour (Ellingsen et al 2014, Ebinghaus 2017). These assessments
534 have also been used to determine individual traits in zoo species (e.g. snow leopards; Gartner and Powel
535 2011), such as scores on "friendly to keeper", which can then be correlated with other factors such as
536 breeding success and welfare (e.g. black rhinos: Carlstead et al 1999a; 1999b). Applying QBA as an
537 HAI or HAR assessment method within zoos will elicit a more sensitive, integrative, "whole-body"
538 assessment of how an animal interacts with humans in their environment, incorporating responses which
539 may not be captured during quantitative assessments. This method comes the closest to being able to
540 measure a HAR from the animal's perspective so long as it incorporates long term monitoring and the
541 video footage enables observers to monitor the pattern of the interactions. Daily interactions, such as
542 routine tasks for a particular species that occur with multiple keepers and animals can be observed using
543 QBA to investigate HARs in terms of how the animal responds holistically to these repeated
544 interactions. For example, the authors have observed footage during a daily husbandry routine whereby
545 a giraffe was provided food by a keeper, the keeper attempted to touch the giraffe, the giraffe then
546 pulled away and removed itself from the interaction and the food. Through using QBA, and therefore
547 capturing the "whole body" response and affective state of the animal, this scenario could be more
548 comprehensively documented and evaluated in accordance with our new definition including the
549 content, quality and pattern of interactions.

550

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

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

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

575

576 5.0 Common themes

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

587 Latency and distance parameters used in response to cues, voluntary approach and avoidance tests all
588 measure the assumption that how an animal responds through performing a behaviour or moving
589 represents how that animal perceives human presence or interaction. From this, the features of the HAI
590 can be consequently used to determine the HAR. However, animal responses could also be the response
591 to a different interaction or movement. It will also be difficult to know specifically whether the animal
592 is responding to the human or coincidentally moving towards or facing a given direction for an unrelated
593 reason. Curiosity of a novel event could also increase the motivation to approach or perform a requested
594 behaviour in the presence of a human (Merchant et al 1997). In a zoo setting, this could suggest that
595 this test is less suitable for animals that rarely have human contact, as it may be measuring animal
596 curiosity rather than a HAI or an indicator of fear or personality (Chelluri et al 2013; Waiblinger et al
597 2003; Marchant et al 1997). Smith (2014) discussed the findings that apes tended to seek proximity to
598 certain staff members such as waste disposal and education staff, even though the staff behaviours were
599 not necessarily rewarding to the ape compared to zoo professionals that may feed them, for example.
600 This suggested that an approach behaviour might indicate an interest or curiosity instead of familiarity
601 or the anticipation of a reward. In a zoo setting, this could suggest that the voluntary approach and
602 avoidance test is less suitable for animals that rarely have human contact, as it may be measuring animal

603 curiosity rather than a HAI or an indicator of fear (Chelluri et al 2013; Waiblinger et al 2003; Marchant
604 et al 1997).

605

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

616

617 6.0 Conclusion

618 In agricultural HAI and HAR research, specific tests have been extensively investigated in terms of
619 reliability, validity, feasibility and effectiveness. However, although the current research on HAI and
620 HARs in zoos is a growing area of scientific interest, methods of assessing these in zoos have not yet
621 been standardised. Previous studies have inferred the HAR from the animal's perspective based on
622 measuring HAIs alone. An extension of measuring HAI to HAR is not automatic, therefore emphasising
623 the need for standard methods to measure HAI and HAR specifically across species, rather than
624 erroneously using the terms interchangeably. Of the methods available and evaluated, three have been
625 identified as having potential for successful application to measuring HAI in zoos. Following our
626 evaluation criteria, measuring latency to respond (e.g. cue or command), QBA and the voluntary
627 approach test are methods that are considered reliable and feasible tests to assess HAIs within a zoo
628 environment. Perhaps more importantly, under the new HAR definition, these methods are considered
629 likely to be of particular value when empirically measuring HARs. With some modifications, such as

630 the use of physical barriers, these tests do not induce fear in the animals or risk the safety of the staff or
631 animals involved. Due to the variation in species, husbandry practices and enclosures within the zoo
632 environment, we recommend that each of the three methods identified here should be subjected to
633 further testing in a zoo environment using the evaluation scoring factors adopted in this review. Our
634 identification of three potential methods enables the progression of the study of HARs within a zoo
635 environment, ultimately ensuring that the implications of HARs for animal welfare can be reliably
636 investigated and compared.

637

638 *6.1 Animal welfare implications*

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

651

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657

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950 Table Captions:

951 Table 1. Current studies and methods to assess human-animal-relationships in various animal groups

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

953 Table 3. Evaluation factor scoring for each HAR method evaluated

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