

Validation of a Radio frequency identification system for tracking location of laying hens in a quasi-commercial aviary system

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ABSTRACT

Cage-free housing is increasingly chosen in Europe, North America, and Australia as an animal-welfare friendly farm system for laying hens. However, hens are kept in large numbers in those systems which makes controllingchecking for health and welfare difficult and individuals cannot be identified. Tracking systems like radio frequency identification allow researchers to monitor these individuals almost continuously. Individual tracking data has reve revealed substantial individual variation in movement patterns, however, in recent studies, only a subset of animals per flock was-were tracked. We applied an RFID tracking system to monitor ~~for~~ all 1115 laying hens of a flock, which were divided into 5 pens of ~~à~~ 225 birds each in a barn with an aviary system. In each pen, 26 antennas were placed on the edges of three tiers and in the litter. For validation purposes, 3 hens in 2 connected pens were fitted with colored backpacks. They were recorded on video and their location throughout the pen was taken from the video and compared with registrations from the RFID system. For 93% of compared transitions, the RFID data matched the observational data regarding the tier or litter whereas the value fell to 39% for specific antennae. When the antennae on the litter were excluded for the validation, the match on tier-level was at least 98% but on antenna-level it remained lower than 50%. The sensitivity of the detection of tiers/litter but not antennae differed among the three hens. We conclude that the RFID tracking system was suitable for studying the movement pattern of individual hens among tiers in an aviary system in a reliable way but tracking birds on the litter needs to be improved.

Keywords: RFID, laying hen, poultry, validation, tracking, aviary, accuracy

Introduction

43 Cage-free housing systems for laying hens may contain tens of thousands of animals. Although
44 considered welfare-friendly, cage-free housing systems including aviaries are known to entail risks
45 concerning health (e.g. parasites, infections) and animal welfare (e.g. damaging behaviours like feather-
46 pecking and cannibalism) (Platz, et al., 2009; Blatchford, et al., 2015; Louton, et al., 2017; Li, et al., 2019;
47 Ali, et al., 2020). In principle, aviaries are designed to offer essential functional areas to the hens like aerial
48 perches for (nighttime) roosting, secluded nest areas for laying, and a litter area for exploratory behavior
49 and dust-bathing. However, individual birds access these areas to a different extent (Rufener, et al., 2018)
50 which is known to correlate to various health risks (Rufener, et al., 2019; Ali, et al., 2020).

51 Tracking individuals in large groups of identically looking laying hens is a challenge that can either be
52 attempted by visually marking the animals or by an electronic tracking system (for reviews see Li et al.,
53 2020; Neethirajan (2022)). Visually tracking hens in a three-dimensional aviary system where birds can
54 move to places where they are invisible due to equipment of conspecifics is difficult, time consuming, and
55 limited. Various technologies including Infrared (Rufener, et al., 2018), Radio Frequency Identification
56 (RFID) (Zhang, et al., 2016; Sibanda, et al., 2019), and other (reviews by Siegford et al., 2016; Brown-Brandl
57 et al., 2019) types of systems have been successfully used to track individuals within the aviary in order to
58 measure individual movement patterns and the amount of time spent in the functional areas. Despite these
59 efforts, tracking is typically limited to a subset of the flock or for a limited time which might not suffice in
60 certain research projects, e.g. in genetics heritability estimates for breeding programs. In any case, all
61 automated tracking devices should be validated with video observations (Iserbyt et al., 2018) using the
62 instances when hens are tracked and visible. Therefore, the aim of this study was to validate an RFID system
63 with the capacity to track a much larger number of individual laying hens in an aviary by assessing the
64 accuracy of registrations. For this purpose, we tracked three focal animals within a larger flock of 450 hens
65 within a commercial aviary.

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Methods

67 Ethical note

68 The use of animals was approved by the Veterinary Office of the Kanton of Bern (BE136/2020) on 10-
69 FEB-2021 and met all Cantonal and Federal regulations for the use of animals in scientific research.

70 Barn-setup and RFID system

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72 Twenty six 12-field SPEED ~~antennas~~ antennae (length: 75 cm) of a passive 125 kHz RFID System
73 (Gantner Pigeon Systems GmbH, Schruns, Austria) were placed at different locations in a Bolegg Terrace
74 aviary system (Vencomatic Group, Eersel, NL) (Fig. 1). The antennae were encased in plastic, connected to
75 reading devices which were connected by multiplexers (Moxa, New Taipei City 242, Taiwan) to a computer.
76 A similar system was described in Gebhardt-Henrich et al. (2014). On each tier at each side of the aviary
77 structure (upper, nestbox, lower) as well as in the litter, three antennae were put side-by-side joining at
78 the short end. Additional antennae were placed on each side of the wintergarden although not evaluated
79 in this effort. As a test trial for future experiments on a large number of birds, All birds in 5 pens of a barn
80 with 20 pens ~~à~~ with 225 birds per pen were fitted with a glass tag (HITAGS 4x22mm, 125KHz, HTS256) in a
81 custom-developed leg band (Fig. 2). If a tag was detected by an antenna a time stamp and the identities of
82 tag and antenna were written into a .csv file every 0.1 s. However, if a tag remained on the same antenna
83 for a 10 s period, the registration was not repeated in order to limit the size of the generated files. The
84 maximal vertical reading distance of all antennae was about 15 cm and the horizontal reading distance was
85 close to 0 cm. Three hens in a pen that was connected at the level of the litter to a neighboring pen for free
86 movement between the two pens wore color-coded back-packs that were visible on video recordings. One
87 observer watched videos recorded between April 21st and 29th, 2021 until a total of 10 hours of video were
88 scored, on which at least one hen with a custom-made backpack (Fig. 3) for identification was visible. Based
89 on the recorded video (30 fps), the location of those hens walking, standing, or sitting on the antennae and
90 the pen at each change of location with the respective video time stamps was entered into a spreadsheet.

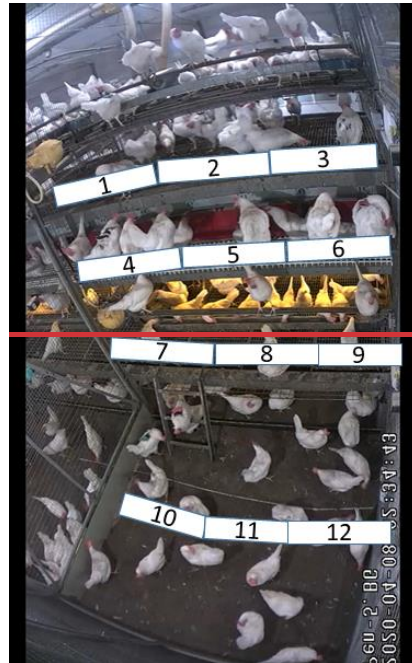
91 Additionally, the observer noted whether the identification of the hen was certain or uncertain due to poor
92 visibility.

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94 **Analyses**

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96 Initial data processing of the registrations of the RFID data (date and time stamp to the closest tenth of
97 a second, ID of the bird, ID of the antenna) were done in R (version 4.2.0). For each observation from the
98 video it was noted whether the RFID system had recorded the bird on the same antenna, tier, and side





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Figure 1: View of ~~one~~both sides of the aviary with the location of ~~12-24~~ of the ~~14-26~~ antennae. Two antennae were located in front and behind a pophole leading to a wintergarden available on one side of the aviary only (left). These 2 antennae were not used in the validation trial.



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Figure 2: The blue legbands on the right legs contain the RFID tag.

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of the aviary, and in the correct pen. In particular, several variables were extracted for the closest RFID registrations in time that matched the hen (see Table 1), and tests were performed to compare the RFID data and coded observations (see Table 2).

111 The results were entered in a confusion matrix to calculate the sensitivity of the RFID system (true
112 positive cases / sum of true positive and false negative cases) and the time differences between the time
113 stamp of the video and the time stamp of the RFID system were analyzed using *PROC FREQ* and *PROC*
114 *UNIVARIATE*, (SAS Institute Inc., 2016).
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117 [Fig. 3. The back of a hen wearing a backpack.](#)

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Table 1: Variables that were extracted from the RFID data for events from coded video observations.

Variable	Meaning
Time difference of the closest event	Time difference [s] between the closest (by time) RFID registration for the observed hen and the observation by the observer.
Closest antenna	The antenna code recorded in the closest RFID event as stated above.
Closest tier	The tier corresponding to the closest RFID event as stated above.
Closest side	The side of the aviary corresponding to closest RFID event as stated above.
Closest pen	The pen recorded in the closest RFID event as stated above.
Time difference of the exact antenna match	Time difference [s] between the closest (by time) RFID registration that matches the antenna and the observation by the observer.
Time difference of the tier + side + pen match	Time difference [s] between the closest (by time) RFID registration that matches the triple (tier, side, pen) and the observation by the observer.
Time difference of the tier match	Time difference [s] between the closest (by time) RFID registration that matches the tier and the observation by the observer.

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Table 2: ~~Tests performed on~~ Categories of agreement between coded video observation events and corresponding RFID data variables

Variable	Meaning
Side correction needed	The side as recorded by the observer had to be corrected <u>by a second person</u> because there was an obvious error in coding by the observer (antenna coding for the wrong side was used, based on the antenna and side mismatch). This did not involve RFID data.
Closest antenna matches	The antenna code recorded in the RFID event for the observed hen with closest timestamp to the coded video observation time is the same as observed.
Same antenna within 1 min.	Same as above but the RFID data matches the observed antenna within a 1 min. window. It is not necessarily the match closest in time.
Closest tier + side + pen match	The tier recorded by the observer matches the tier (and side) of the antenna code of the RFID registration closest in time for the respective bird.
Same tier + side + pen within 1 min.	Same as above but the observer matches the tier (and side) within a 1 min. cutoff. It is not necessarily the match closest in time.
Closest pen matches	The pen recorded by the observer matches the pen of the antenna code of the RFID registration closest in time for the respective bird.
Closest side of the aviary matches	The side of the aviary recorded by the observer matches the side of the aviary of the antenna code of the RFID registration closest in time for the respective bird.

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Results

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From the video files, 304 locations of the three birds were detected of which the observer was certain (75.6% of all sightings of birds on antennae). Of these, in 91 % of the cases, the correct tier, side, and pen of the aviary was detected within 1 min. by the RFID system (Tab. 3a). In all but 7 cases, this was also the closest RFID detection in time. In 1 case, the correct tier, but at the opposite side of the aviary was indicated

132 by the RFID system. The correct tier regardless of the side of the aviary and the pen was detected in 93%
133 of the cases. Sensitivity fell precipitously to 39% when the focus was detecting the correct antenna within
134 one minute. In 3% of the cases a wrong pen was indicated and in 2% the wrong side of the aviary.

135 When the registrations of the litter were excluded, detection was much better (Table 3b). All
136 sensitivities on tier-level were between 98 and 99% whereas the sensitivities regarding the correct antenna
137 within tier remained below 50%.

138 The registration of the RFID system was on average 1.6 s (Stderror = 1.9 s) earlier than the video time
139 stamp if the tier identified by the RFID and observer matched and 3.6 s. (2.5 s.) earlier if the antennas by
140 the RFID and observer matched. Neither time differences were significantly different from zero (same tier:
141 Student's t = 0.82, P = 0.41, N = 293, same antenna: Student's t = 1.42, P = 0.16, N = 135).

142 Of the three hens, each accounted for 43.4% (132), 34.9 (106), and 21.7% (66) of all registrations. The
143 hens differed in the sensitivity of the registrations_ relative to tiers including the litter but not antennae
144 when all tiers included the litter were analyzed. However, with the exclusion of the antennae on the litter,
145 hens only differend when the same antenna within 1 min. was considered. The difference was due to the
146 two birds with the fewer registrations. Of those, one hen had about 5 times more correct than incorrect
147 registrations of the antenna within ~~one-1~~ min. and the other bird had twice as many incorrect than correct
148 registrations of the antenna within ~~one-1~~ min.

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151 **Table 3:** Sensitivities of the detection of locations of birds as seen on the video file by the RFID system. In
 152 some cases, the same antenna or tier was registered on RFID as the observer indicated within 1 min. but
 153 there was an earlier RFID registration event of another antenna/tier (named closest antenna etc. in time).
 154 a) all observations, N = 304. b) registrations on the litter excluded, N = 158.

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a)

Registered by RFID	N of event = true	Sensitivity	Difference between hens
Closest tier and side and pen in time	271	0.89	P = 0.002
Same tier and side and pen within 1 min.	278	0.91	P = 0.001
Closest tier and side in time	279	0.92	P = 0.02
Closest tier in time	284	0.93	P = 0.0005
Closest antenna in time	74	0.24	P = 0.12
Same antenna within 1 min.	120	0.39	P = 0.11
Closest pen in time	294	0.97	P = 0.02
Same (aviary) side	299	0.98	P = 0.53
Observer correct	234	0.77	P = 0.72

b)

Registered by RFID	N of event = true	Sensitivity	Difference between hens
Closest tier and side and pen	154	0.98	P = 0.58
Same tier and side and pen within 1 min.	156	0.99	P = 1.00
Closest tier and side in time	154	0.98	P = 0.58
Closest tier in time	157	0.99	P = 0.15
Closest antenna in time	48	0.29	P = 0.93
Same antenna within 1 min.	75	0.48	P = 0.0004
Closest pen in time	158	1.00	N/A
Same (aviary) side	155	0.98	P = 1.00
Observer correct	234 156	0.77 0.99	P = 0. 72 30

159 The detection rate of birds on the different tiers and in the litter of an aviary system was very high and
160 comparable to other efforts using different RFID systems in poultry with either equal or greater sensitivities
161 (In broilers: Li et al., 2019 (Ultra-high frequency); van der Sluis et al., 2020 (Ultra-Wide Band), laying hens:
162 Sales et al., 2015 (134.2 kHz); Wang et al., 2019; Sibanda et al., 2020 (UHF (915 MHz))). The findings were
163 also comparable to efforts using non-RFID systems (see review by Siegford et al., 2016) including those in
164 the same barn applying the same 'zone' approach (Rufener et al., 2018; Candelotto et al., 2022);but lower
165 than the reliability of 99% of the active low-frequency tracking system by Montalcini et al. (2022). Although
166 overall sensitivity was high, the correct antenna was detected in less than 50% of the cases. The poor
167 detection can be explained by the fact that the antennae were positioned adjacent to each other so that a
168 tag likely could be read intermittently by both antennae when the hen sat on both. The problem of birds in
169 between antennae has also been a problem for other efforts (van der Sluis et al., 2020). In addition to this
170 problem 'within' pens, the problem could also persist 'across' pens. As pens were adjacent, antennae of
171 one pen also touched antennae of the neighbouring pen leading to registrations in the 'wrong' pen. In case
172 that pens are connected and the movement of birds between pens is studied, this likely error would need
173 to be addressed. For instance, to resolve the problem of false pen registrations, the edges of antennae at
174 the extreme sides of the pen can be physically blocked (Ringgenberg, et al., 2015). In either case, our efforts
175 suggest the benefits of such a validation to help improve accuracy and determine potential solutions. More
176 critically, our results also indicate that the present set-up did not yield adequate precision to tell where
177 across the 225 cm wide tier the hen was located, i.e. we achieved only the registration of the tier and side
178 with acceptable levels. Given our validation results, tracking individuals at the side/tier level is possible,
179 but a higher resolution may be necessary depending on the research question.

180 In 20 instances, the RFID registration did not match the correct tier. In all but 1 of these cases, the bird
181 was seen on the litter but the antenna immediately above the litter on the first tier was recorded instead.
182 In one mismatch, the hen was seen on the antenna on the highest tier and it was recorded on that tier but
183 on the other side of the aviary. In each of these cases, the hen likely moved faster than the registration
184 window, e.g. up to the first tier / down to the litter or underneath the aviary to the opposite side. Speed
185 of registration has been shown to be a problem with fast moving laying hens with a similar RFID system
186 (Gebhardt-Henrich, et al., 2014). For the current validation, an improved system with faster registration
187 was used. However, it is possible that very fast moving hens may still be missed. The resolution of the
188 timestamp in the csv file generated by the RFID system was 0.1 s. Since it is impossible to synchronize the
189 video system with the RFID system with this accuracy, the time difference between the RFID registrations
190 and the video time stamps are not surprising.

191 Tier-specific, incorrect registrations also likely result from the system's physical set-up of the aviary and
192 the spatial configuration of the antennae. Interestingly, we found almost no mistakes in terms of tier
193 recordings except in the litter. The decreased sensitivity of the litter is likely because birds can more easily
194 enter the area without coming into contact with an antenna. In contrast, a bird transitioning between the
195 upper and nest box tiers would have to step onto an antennae at the edge of each zone. As a solution to
196 improve sensitivity in the litter, we have doubled the number of antennas there with a later setup.
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198 The three hens differed in the sensitivity of the registrations of the tier and the positions where they
199 were observed in the aviary. One hen was mostly seen on the litter while another on the uppermost tier.
200 The hen with the lowest sensitivity scores had fewer registrations but was seen both on the litter and the
201 uppermost tier. The sample size of three hens is too low to draw any conclusions whether certain
202 individuals would differ in the sensitivity of the registration of tiers. However, it is feasible that such a
203 difference exists due to variations in an individual's behavior (e.g., flying or jumping across antennas) or
204 preference of certain locations in the aviary which are less reliably registered on the antennas. In our
205 dataset the difference in the sensitivities likely resulted from differences in litter use because sensitivities
206 on tier-level no longer differed among hens when registrations of antennae on the litter were excluded.
207 Differences in the registration of the antennae within one min. were due to the 2 hens with fewer
208 registrations and the cause is not obvious unknown.
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210 This validation was done before the start of the ~~properfull~~ experiment so we do not have tracking data
211 from other hens for this period. However, density of hens and equipment of the pen was the same as in
212 the following studies except the addition of a second row of antennas on the litter in following experiments.

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214 It is important to note that a gold standard to determine the positions of the hens does not exist. The
215 observations from the videos were error prone and in almost one quarter of observations the combination
216 of antennas and side of the aviary were impossible and had to be corrected. Mistakes while coding videos
217 occur like in other easy tasks that do not require a high level of conscious attention esp. when the observer
218 is disrupted (Morrison, 2021). Furthermore, it ~~is-was~~ difficult to synchronize our video and RFID systems
219 with the resolution of less than 1 s. because both systems were not connected to the same network.

220 In conclusion, the employed RFID system reliably detected the position of hens on the different tiers
221 and the litter in an aviary with a sensitivity of 96% in a reliable way but tracking birds on the litter needs to
222 be improved.

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226 Data, scripts, code, and supplementary information availability

227 Scripts and code are available online on OSF: <https://doi.org/10.17605/OSF.IO/UHTSW>.

228 Conflict of interest disclosure

229 The authors declare that they comply with the PCI rule of having no financial conflicts of interest in
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