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Optimizing the baiting strategy for oral vaccine delivery to wild boar

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Abstract

Baits are a means of orally delivering toxicants, medicaments, or chemical markers to wild boar and feral pigs (*Sus scrofa*). We tested three bait types, the paraffin-feed-sugar-based IREC bait (green and colorant-free versions), a puffed leguminous bait with the same flavoring as the IREC bait (Flavor), and a plain puffed leguminous bait without flavoring (Plain). Baits were deployed in a fenced hunting estate with year-round feeding to assess bait type preference and study the effect of bait flavoring and coloring, pre-feeding, baiting device, and habituation on bait consumption and bait selectivity. Baits were deployed under heavy pavel stones, targeting adult wild boar, or in piglet-selective feeders. The main bait consumer was the wild boar (IREC 54%; Flavor 15%; Plain 16%) followed by azure-winged magpies (*Cyanopica cooki*; IREC 11%; Flavor 8%; Plain 17%). The most consumed bait was IREC (n = 164; 71%, mean 4.1 baits per site/day), followed by Flavor (102; 40%; 2.5) and Plain (70; 29%; 1.7). Pre-feeding increased bait consumption of all bait types (IREC 92%; Flavor 63%; Plain 40%). IREC baits were more consumed when deployed under stones (86%) than when deployed in piglet feeders (57%), while no difference between baiting devices was observed for Flavor and Plain baits. Birds preferred color-free baits (consumption ratio of 10% for color-free baits and 0% for green baits), while no other animal showed color preference. We suggest using green IREC-type baits, deployed after pre-baiting using species- and age-specific baiting devices.

Resumen Los cebos son un medio para administrar por vía oral tóxicos, medicamentos o marcadores a jabalíes y cerdos salvajes (*Sus scrofa*). Probamos tres tipos de cebo, el cebo IREC a base de parafina y azúcar (versiones verdes y sin colorantes), un cebo de leguminosas infladas con el mismo sabor que el cebo IREC (Flavor) y el mismo cebo de leguminosas sin sabor (Plain). Los cebos se colocaron en una finca de caza cercada con alimentación durante todo el año para evaluar la preferencia del tipo de cebo y estudiar el efecto del sabor y color del cebo, el precebado, el dispositivo de cebo y la habituación sobre el consumo y la selectividad. Los cebos se colocaron debajo de piedras, dirigidos a jabalíes adultos, o en comederos selectivos para rayones. El principal consumidor fue el jabalí (IREC 54%; Sabor 15%; Sencillo 16%) seguido del rabilargo (*Cyanopica cooki*; IREC 11%; Flavor 8%; Plain 17%). El cebo más consumido fue IREC (n = 164; 71%, media 4,1 cebos por sitio/día), seguido de Flavor (102; 40%; 2,5) y Plain (70; 29%; 1,7). El precebado aumentó el consumo de todos los tipos de cebo (IREC 92%; Flavor 63%; Plain 40%). Los cebos IREC se consumieron más cuando se colocaron bajo piedras (86%) que cuando se dispusieron en comederos para rayones (57%), mientras que no se observaron diferencias entre dispositivos para cebos verdes), mientras que ningún otro animal mostró preferencia en función del color. Sugerimos utilizar cebos verdes tipo IREC, distribuidos tras un precebado y utilizando dispositivos específicos para cada especie y edad.

Keywords African swine fever · Bait deployment devices · Feeding sites · Oral bait deployment · Sus scrofa

Introduction

Baits are a means of orally delivering toxicants, medicaments, or chemical markers to wild boar and feral pigs (*Sus scrofa*) (O'Brien et al. 1988a; Cowled et al. 2006; Rossi et al. 2015). Toxicants are used for wild boar and feral pig control in regions and situations where they cause significant environmental and agricultural damage (Gentle et al. 2014; Khan et al. 2017). The only disease currently targeted by wild boar/feral pig oral vaccination is classical swine fever (Rossi et al. 2015), although recent research is also aimed at targeting animal tuberculosis (Díez-Delgado et al. 2018) and African swine fever (ASF; Barasona et al. 2019) control. While ASF vaccines are not yet approved, research

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Extended author information available on the last page of the article

on strategies for vaccine deployment in wild boar is needed (Palencia et al. 2023).

A variety of means has been used for delivering products to wild boar or feral pigs. Meat chunks (Cowled et al. 2006), lupins (Twigg et al. 2007), and cereal grain (e.g., wheat, fermented wheat, barley, malted barley, or cracked or whole corn; O'Brien et al. 1988b; McIlroy et al. 1993; Twigg et al. 2005; 2007; Bengsen et al. 2011a) have been used to deliver toxicants for feral pig control. Baits for wild boar or feral pigs include a diversity of compressed bran/pollard pellet baits (O'Brien et al. 1988b), paste baits (Shapiro et al. 2016; Snow et al. 2017), wheat pasta filled with syrup or peanut butter (Baruzzi et al. 2017), or manufactured baits such as the ACP bait (Animal Control Products Ltd, Wellington, New Zealand), Riemser bait (IDT Biologika GmbH, Dessau-Roßlau, Germany) and their variants (Brauer et al. 2006), PIGOUT and HOGGONE (Animal Control Technologies, Somerton, Australia), and IREC baits (Ballesteros et al. 2009b). Some of the manufactured baits contain pig-specific attractants (flavoring) such as strawberry-flavored feed additive (Strawberry Aroma; QualiTech Incorporated, Chaska, MN, USA) or synthetic fermented egg flavor (FeralMone; Pestat, Bruce, Australia) (Campbell and Long 2009).

Bait delivery strategies include a broad range of options, from aerial deployment (Cowled et al. 2006) to shallow burial (Bengsen et al. 2011a). In Australia, but not in Texas (USA), shallow burial reduced bait consumption by nontarget species (Campbell and Long 2009; Bengsen et al. 2011a, b). However, making baits available only at night was less efficient regarding bait selectivity (Bengsen et al. 2011b). Using a plastic box to cover baits greatly reduced bait uptake by small nontarget omnivores (Bengsen et al. 2011a, b). Several species-specific baiting devices have been proposed, including the Boar Operated System (BOS; Massei et al. 2010), box- or feeder-like systems where pigs need to lift the top, such as the Hog-Hopper (Lapidge et al. 2012), or wooden (Shapiro et al. 2016), metal, or plastic bait stations (Lavelle et al. 2018), as well as simple heavy stones (Beltrán-Beck et al. 2014). To prevent birds from consuming baits, a problem that also occurs in the use of rodenticides for pest control, the possibility of using some sort of colorant as a deterrent has been studied, verifying the effectiveness of the use of blue and green colors to avoid primary consumption by birds (Cowan and Crowell 2017).

The IREC bait is made of pigfeed, wheat flour, paraffin, saccharose, and cinnamon-truffle flavoring. It is stable at high ambient temperatures and suitable for wild boar and feral pig adults and piglets (Ballesteros et al. 2009b, 2011b). This bait is also well accepted by several non-target species including cattle and red deer (*Cervus elaphus*), whereas badgers (*Meles meles*) showed varying bait acceptance (Ballesteros et al. 2011a). The IREC baits seem less attractive for birds (Beltrán-Beck et al. 2014). Bait delivery devices such as heavy pavel stones

or selective piglet feeders enable a more species-specific and age-specific bait deployment (Ballesteros et al. 2011a; Beltrán-Beck et al. 2014). Using these deployment devices, IREC bait uptake rates (success) of 24 to 74% by wild boar piglets have been achieved in natural sites. In managed sites (fenced and with year-round feeding at fixed stations), IREC bait uptake rates by wild boar piglets ranged from 16 to 92% (Ballesteros et al. 2011b; Díez-Delgado et al. 2018). The only field trial targeting adult wild boar found uptake rates between 15 and 26% in natural sites and 14% in one managed site in this age class (Ballesteros et al. 2011b). There is no previous information on bait uptake rates for puffed leguminous baits.

In the present study, we tested three bait types: the IREC bait, a puffed leguminous bait with the same flavoring as the IREC bait (Flavor), and a plain puffed leguminous bait without flavoring (Plain). Baits were deployed six times at ten sites in a wild boar fenced hunting state with year-round feeding. In addition, a final experiment was carried out where green and uncolored IREC baits were compared to assess the effect of color on bait selectivity. We investigated (1) bait type preference, (2) effect of flavor on bait consumption and bait selectivity (species), (3) effect of pre-baiting on bait consumption and bait selectivity (species), (4) effect of the baiting device on bait consumption and bait selectivity (species and age-class), (5) effect of habituation on bait consumption and bait selectivity (species), and (6) effect of color on bait consumption and bait selectivity (species). We hypothesized that wild boar would prefer flavored baits (IREC and Flavor); that pre-baiting with pig feed would increase bait consumption but decrease bait selectivity; that baiting device would improve bait selectivity and enable targeting specific age-classes; that habituation to baits would improve bait consumption through reduction of bait selection; and that the green color would increase bait selectivity by reducing their consumption by birds.

Material and methods

Animal use in research

This research was performed using non-invasive phototrapping, and no animals were captured, handled, or sampled. The protocol was designed by specifically trained and certified scientists according to the EC Directive 86/609/ EEC and approved by the Animal Experiment Committee of Castilla-La Mancha University and the Regional Ethic Committee (PR-2022–01-01).

Study site

The study was conducted in a private hunting estate located in Ciudad Real province (central Spain), with a total surface of 900 ha and surrounded by a wildlife-proof game fence. Within the hunting estate, 700 ha are exclusively dedicated to game rearing for hunting purposes, mostly red deer and wild boar, and the remaining 200 hectares are dedicated to rainfed crops, horse breeding, and agricultural facilities. Climate is continental Mediterranean, characterized by cold winters and dry and hot summers, as well as seasonal rainfalls. The habitat is dominated by scrubland and evergreen oak woodland interspersed with pastures and crops. Artificial feeding for the game species is regularly provided at specific feeding points. The overall wild boar population in the study site is estimated to be around 150 individuals.

Bait types

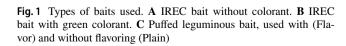
Three bait types were used in the study namely IREC (green and without colorant), Plain, and Flavored baits (Fig. 1). IREC baits were prepared according to the established protocol using piglet feed, wheat flour, saccharose, paraffin, and cinnamon truffle flavoring, with disc/flat cylinder shape $(3.4 \times 1.6 \text{ cm})$ and weighing around $11.74 \pm 1.44 \text{ g}$ (Ballesteros et al. 2009b). Cinnamon truffle powder (Novel S.A., Madrid, Spain) was used as flavoring agent, which has been found attractive to the wild pigs in previous field studies (Ballesteros et al. 2009b). Plain and Flavored baits were prepared from locally available spherical puffed leguminous plant product around 2.5 ± 0.5 cm in diameter and 5.0 ± 0.5 g in weight. For preparation of Plain baits, the leguminous plant product was impregnated with water whereas flavored water containing cinnamon truffle powder was used for preparation of the Flavored baits.

Bait deployment devices

A

We used age-selective bait deployment devices to target each age group (piglets and adults): five piglet feeders and ten pavel stones (two associated to each piglet feeder), respectively (Fig. 2). Piglet feeders consisted in $3 \times 2 \times 0.75$ m metal-grid cages installed on the ground. Its grid opening did not allow the access of wild boars older than 8 months

В



(Ballesteros et al. 2009a). These feeders were already in use prior to this study. Two concave pavel stones were placed outside the piglet feeder. Each stone weighed 7 kg and measured $35 \times 20 \times 9$ cm, with a 4-cm-deep hollow. Its shape allowed to place the baits under it, and its weight prevented other animals than adult wild boar from accessing the baits.

Field trial design

Bait uptake was monitored visually in the bait consumption, bait preference, and baiting strategy experiments, and using ten camera traps (Prometheus Group, AL, USA) with the following settings: trail or video mode, capture delay 1 s, fast multi-shot mode eight shots, and low image size. Pre-baiting consisted in providing corn regularly in and around three piglet feeders. The remaining two feeders only received corn in and around when the bait deployment started. During the trial, baits were placed in double-paired combinations at each site, following a rotation scheme (see Table 1). Bait deployment took place before sunset during three consecutive nights (Tue to Thu) for 2 weeks (6 days in total). Bait preference was assessed by comparing how many of the deployed baits were consumed, and how often (number of sites/day) one type of bait was preferred over the other one during choice experiments. The total number of sites/days was 60. The total number of deployed baits was 690: 230 IREC, 230 Flavor, and 230 Plain. In the last experiment to test the effect of color on bait selectivity, there were no rotations as only two types of bait (green IREC and uncolored IREC) were compared. Baits were placed both inside and outside the piglet feeder allowing a separation of 50 cm between types. Daily, five baits of each type and for each age group were placed (20 baits placed per site daily), except the first day, when quantities were double. The effect of bait color was tested at 36 sites/days. The total number of baits deployed was 210 green IREC baits and 210 uncolored IREC baits.

Statistics

All statistical analyses were conducted using R 4.2.2 computing software (R Core Team 2018). We followed the protocol for data exploration described by Zuur et al. (2010) to avoid type I or type II errors and potentially erroneous ecological conclusions. This included studying outliers, checking for homogeneity of variance, normal data distribution, and zero inflation. We also checked for collinearity among covariates, studied the relationships between variables, and considered possible interactions. Finally, we checked if the observations were independent of the response variable. We used generalized linear mixed effect model (GLMM) with a random effect of "site" with a binomial response (significance based on α =0.05) to compare differences in number of sites/ days visited by the animals between treatments (bait type,



Fig. 2 Bait deployment devices used in this study. A Wild boar piglet feeder (inset, IREC type baits deployed). B Pavel stones covering baits intended for adult wild boar. C Azure-winged magpie lifting one IREC bait. D Wild boar lifting a pavel stone to gain bait access

Site 2

IREC vs Flavored

Plain vs Flavored

IREC vs Flavored

Plain vs Flavored

IREC vs Flavored

IREC vs Plain

Site 3

Plain vs Flavored

IREC vs Flavored

Plain vs Flavored

IREC vs Plain

IREC vs Flavor

Plain vs Flavored

pre-baiting, delivery device, habituation, and species), and differences in bait consumption (expressed as baits consumed/ baits deployed) depending on the factors listed above. In those cases where we needed to analyze selectivity and preference of Plain and Flavored baits, and green and without colorant baits, we excluded the sites/days in which no baits were consumed to focus our analysis purely on bait uptake data.

Results

Bait consumers

Baits deployed under pavel stones were only accessible to other mammals and birds after wild boar lifted the stones.

Day 3

Day 4

Day 5

Site 1

Day 1 IREC vs Plain

Day 2 IREC vs Flavored

Plain vs Flavored

IREC vs Flavored

Plain vs Flavored

In two exceptional occasions, however, azure-winged magpies (*Cyanopica cooki*) were recorded accessing baits under the stone. Baits deployed in selective piglet feeders were unavailable for ruminants and for adult wild boar, but accessible for wild boar piglets and smaller juveniles, as well as for birds. Only wild boar piglets and azure-winged magpies were recorded inside the piglet feeders. Regarding the species registered consuming baits, the main consumer was wild boar, with an average of 35% bait consumption (IREC 54.07%; Flavored 15.20%; Plain 16.55%) accessing 20 times baits deployed under stones and six times baits deployed in piglet feeders; secondly, *C. cooki* consumed an average of 13.81% of baits (IREC 10.71%; Flavored 7.69%; Plain 16.67%), eight times accessing baits deployed under stones, mostly after wild boar had lifted them, and seven times in

Site 4

IREC vs Flavored

Plain vs Flavored

IREC vs Plain

IREC vs Flavor

Plain vs Flavored

IREC vs Flavored

Site 5

Plain vs Flavored

IREC vs Flavored

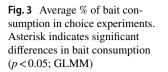
Plain vs Flavored

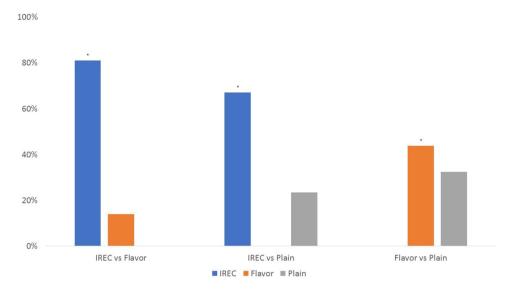
IREC vs Flavored

Plain vs Flavored

IREC vs Plain

Table 1Wild boar bait typerotation scheme during the fieldtrial. IREC bait as describedin Ballesteros et al. (2009b).Puffed leguminous baits wereeither Plain or Flavored withcinnamon truffle powder





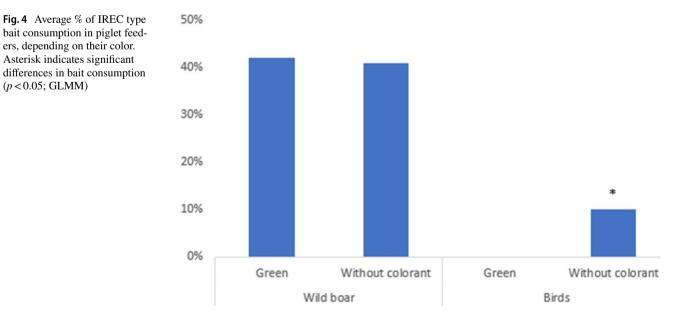
piglet feeders; and red deer consumed baits only once, eating four Plain baits from a stone which had previously been lifted by a wild boar.

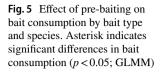
Bait preference

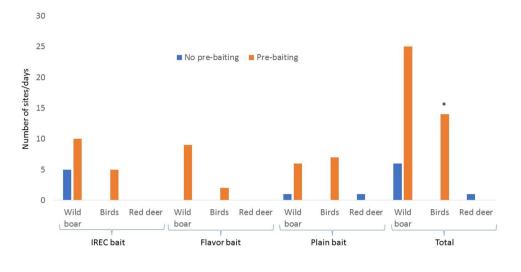
The most consumed one was the IREC bait (n=164; 71%, mean 4.1 baits per site/day), followed by the Flavor bait (102; 40%; 2.5) and the Plain bait (70; 29%; 1.7). When the IREC bait was compared with Plain baits, there was more consumption of IREC baits on 12 occasions, and equal consumption on 3 occasions. When the IREC bait was compared with the Flavor bait, there was more consumption of IREC baits on ten occasions, and equal consumption on seven occasions. Finally, when we compared Plain with Flavor, there was more consumption of Plain baits on three

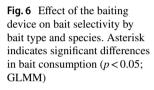
occasions, more consumption of Flavored baits on 5, and equal consumption on five occasions (GLMM p < 0.05 for sites/day differences in all three cases; Fig. 3).

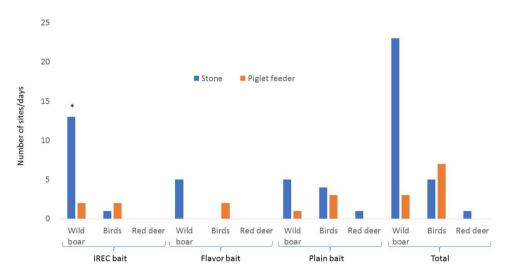
The uncolored IREC bait (n = 159; 76%; mean 4.4 baits per site/day) was consumed only slightly more than the green IREC bait (149; 71%; 4.1). Outside the piglet feeders (using the concave pavel stones), the only consumer was wild boar (100%). In the selective piglet feeders, where only wild boar up to 8 months of age and birds have access, the consumption ratio (baits consumed/baits deployed) of wild boar was 71% and the consumption ratio of birds was 3%. Regarding consumption by birds, a consumption ratio of 10% for uncolored IREC baits and 0% for green IREC baits was recorded (GLMM p < 0.05for differences in consumption ratio for the two types of bait) (Fig. 4).











Baiting strategy

Pre-baiting with corn increased bait consumption of all bait types (GLMM; IREC p = 0.003; Flavor p < 0.001; Plain p = 0.028). Regarding the species consuming baits (Fig. 5), pre-baiting slightly increased general bait consumption by wild boar (GLMM; IREC p = 0.199; Flavor p = 0.096; Plain p = 0.378; total p = 0.062; marginally significant) but also by birds (GLMM; IREC p = 0.969; Flavor p = 0.999; Plain p = 0.096; total p = 0.015). No effect of pre-baiting on nontarget mammals was found.

Regarding the baiting device, IREC baits were more consumed when deployed under stones than when deployed in piglet feeders (96 and 68 baits, respectively, GLMM p = 0.017). No difference between baiting devices was observed for Flavor and Plain baits (GLMM; Flavor p=0.600; Plain p=0.670). The effect of the baiting device on bait selectivity (Fig. 6) was significant for wild boar using the IREC bait (GLMM; IREC p=0.003; Flavor p=1.000; Plain p = 0.148; total p = 0.187) while no effects were found in birds (GLMM; IREC p = 0.611; Flavor p = 0.910; Plain p = 0.360; total p = 0.265). There were too few observations of non-target mammals.

There were no significant differences in bait consumption depending on the week, i.e., no habituation effect was observed (GLMM total p = 0.223).

Discussion

This experiment allowed gaining new insights into bait preference and the effects of bait flavoring, bait color, prebaiting, baiting device and habituation on bait consumption, and bait selectivity when targeting wild boar of different age classes. As initially hypothesized, we found that adult wild boar preferred flavored baits (IREC and Flavor); that pre-baiting with pig feed increased bait consumption (at the cost of decreasing bait selectivity); and that baiting device improved bait selectivity and enabled targeting specific age-classes. However, we found no habituation to the baits, meaning that previous exposure to baits did not improve bait consumption.

Bait preference towards the IREC bait, which was originally designed for wild boar (Ballesteros et al. 2009b), is no surprise. It is however important to note that, on the video footage taken at the pre-baited sites, wild boar always took the feed first and explored the baits only after most of the feed had been taken. This implies that there is still space for bait palatability improvement, even for the IREC bait.

The choice experiments demonstrated that adult wild boar (those able to lift the pavel stones) preferred flavored baits, since the rather flavor- and taste-neutral puffed leguminous baits were more consumed and more often chosen first (43.75%) when they contained the artificial flavoring. Again, other flavorings as well as the attractiveness of different bait components (e.g., sugar, wheat) deserve attention in further research.

Easily accessible and attractive food, such as cracked corn, is often used to lure wild boar or feral pigs to baiting sites (Bengsen et al. 2011a). In Texas (USA), approximately 18 days of pre-baiting allowed feral pigs to habituate to using pig-specific bait stations and consume a novel bait type (Snow et al. 2019). As expected, in our experiment, pre-feeding, or deploying the baits at already used wild boar feeding sites, increased bait consumption. This occurred consistently for all bait types, although at the cost of also losing more baits to birds. Comparing sites without prefeeding with sites with pre-feeding out of permanent feeding sites remains a pending objective. Field experience with tuberculosis vaccine deployment suggests that uptake rates are higher when permanent feeding sites are used (Díez-Delgado et al. 2018).

Selective piglet feeders (Ballesteros et al. 2009a, b) have the advantage of facilitating efficient bait deployment to piglets, which otherwise will get outcompeted by adults (Brauer et al. 2006; Díez-Delgado et al. 2018). Carnivores such as red fox (Vulpes vulpes) do only occasionally visit bait deployment sites in our study region (<2%; Beltrán-Beck et al. 2014). However, favoring piglets over adult wild boars comes at the cost of higher bait losses to birds. Thus, we tested green-colored baits to try to minimize these losses, proving that this is a useful improvement. In turn, heavy stones almost ensure bait delivery exclusively to larger juvenile and adult wild boar, with limited losses to other animals, but at the cost of not targeting piglets and potentially compromising the bait uptake rate in this epidemiologically relevant age class. Therefore, we postulate that the ideal approach for vaccine deployment is one taking advantage of both kinds of devices, although this needs to be confirmed with further experiments, probably including bait markers. For toxicants, however, neither option is truly suitable for operational use since non-target species accessed both baits frequently.

In this study, we found no habituation to the baits: exposure to baits in week 1 did not improve bait consumption in week 2. This was rather unexpected since habituation and social interactions are likely to overcome neophobia in pigs (Figueroa et al. 2013; Snow et al. 2017). This is relevant because, at least with the bait types used in this experiment, there was apparently no neophobia, so the baits can be used rapidly in new sites without previous habituation.

The baits used in this study did not contain markers. Hence, uptake rates (i.e., the proportion of wild boar consuming at least one bait) were not assessed. Increasing baiting intensity and bait station density increases bait uptake in feral pigs (Cowled et al. 2008), and good uptake rates have been reported in piglets (50-92%), which were the target age group of previous trials (Díez-Delgado et al. 2018). Since adult wild boar will be as much a target as piglets in the specific case of ASF, assessing uptake rates in adults in a range of density and management situations remains a pending issue. Furthermore, IREC baits and other larger baits targeting wild boar can contain a blister or a plastic tube carrying any pharmacological drug. This would not significantly change bait smell or taste. However, if the drug was mixed directly into the bait matrix, this could eventually affect uptake rates.

The ideal bait would be cheap and easy to produce, stable against water and extreme temperatures, hard enough to allow aerial deployment, species-selective, and suitable for all age classes. In addition to the pending issues already mentioned, seasonality (McIlroy et al. 1993; Bengsen et al. 2011a; Ferretti et al. 2014) and bait suitability for aerial deployment remain to be studied regarding the IREC bait.

Author contribution R. Pachauri: investigation, methodology, fieldwork, writing, review, and editing. J. Martínez-Guijosa: data processing, software, methodology, fieldwork, writing, review, and editing. E. Ferreras-Colino: methodology, fieldwork, review, and editing. J. Ferreres: methodology, fieldwork, review, and editing. D. Relimpio: data processing, investigation, methodology, fieldwork, writing, review, and editing.

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Availability of data and materials Data on the preference of the different species for the different baits used in this paper can be downloaded from the Zenodo repository (https://doi.org/10.5281/zenodo.8116453).

Declarations

Competing interests The authors declare no competing interests.

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