Studies on Bait Preference and Acceptance in Wolves
(\textit{Canis lupus lupus})

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ABSTRACT

Recently, wolves have returned to their historical range in several areas of Central and Western Europe. The renewed presence of this large carnivore in these areas raises many conflicts and challenges, including its potential as a host of rabies. Historically, dogs and wolves were considered the two main vector species for rabies in Europe, whereby wolf rabies appeared to dominate in rural areas. The potential risks that wolves could succumb to rabies upon re-emergence of this disease in Western and Central Europe cannot be ignored considering the reported cases in East-Europe and the Middle East where wolves and rabies coexist. It could also have a serious impact on the present general positive public attitude towards the renewed presence of this carnivore. Hence, tools to prevent this would have significant value for any wolf conservation management plan. Oral vaccination of different wildlife species against rabies has proven highly effective and this approach also may be applicable (locally) for wolves in the case of necessity. Besides a safe and efficacious vaccine, a well-accepted bait for the target species is another prerequisite for oral rabies vaccination. Therefore, bait preference and acceptance studies in wolves kept in enclosures were conducted. An experimental egg-flavored bait was tested, together with a positive control made from a natural product (intestine) and a negative control, a presently used oral vaccine bait matrix for foxes (vegetable fats and fish meal). The experimental bait was well accepted. No significant difference between the positive control and a significantly better acceptance rate than the negative control was found. Adding selected palatants to the bait did not improve bait acceptance. However, adding a rumen flour coating to the experimental bait increased its detectability significantly when distributed in the wolves’ enclosure.

Keywords: Bait; Preference; Acceptance; Wolf; Rabies; Oral Vaccination.

INTRODUCTION

Wolves (\textit{Canis lupus}) have re-occupied large areas of their historical range in Western and Central Europe since the latter part of the 20th century (1-2). The renewed presence of this large predator is unfortunately also associated with a growing number of conflicts, especially concerning predation of livestock (3). Another issue raised, is the deeply embedded fear of humans for an attack by such a large predator that can be traced back for centuries, although the possibility of encountering a wolf and subsequently being attacked is
extremely remote. Only few human fatalities because of wolf attacks have been documented in Europe during the last decades (4).

The most important factor predisposing wolves to attack people is rabies. Rabies is a fatal zoonotic disease that has been eliminated from their present principal reservoir species in Europe, the red fox (*Vulpes vulpes*), from most of the continent by distribution of vaccine-loaded baits (1). However, the disease is still endemic in parts of Eastern Europe and the Middle East. More than 500 cases of rabid wolves have been reported to the Rabies Bulletin Europe since 2000 (source: Rabies Bulletin Europe – www.who-rabies-bulletin.org) and also in the Middle East, rabies cases in wolves are frequently reported (5-7). Historically, dogs and wolves were considered the two main vector species for rabies in Europe, whereby wolf rabies seemed to dominate in rural areas (8). However, circulation of rabies virus in wolves independent of other reservoir species has not been documented unequivocally. The social nature of wolves usually leads to rapid extinction of packs before the virus is transmitted to neighboring packs (9-10). Rabid wolves are considered one of the most fearsome hosts of this disease due to their extreme ferocious attacks. For example, a rabid wolf entered the town Adalia in Anatolia, Turkey, in 1852 and wounded more than 100 people and killed 85 sheep (11). Also, from Western Europe historical data indicate that attacks of rabid wolves were frequent; Moriceau reported 2,602 human victims of attacks by rabid wolves during 1578-1887 in France (12).

In recent years, 2013-2015, seven rabid wolves were reported from Israel of which several attacked humans. Hence, rabies has been listed as one of the possible conflicts associated with the re-colonization of the wolf in Europe (8). Also, rabies spill-over infections from the reservoir species to wolves could lead to local extinction of this animal species as has been seen with other social canid species like the African hunting dog (*Lycaon pictus*) and Ethiopian wolf (*Canis simensis*) (13-14).

Oral vaccination of wolves against rabies could therefore be a potential solution to prevent rabies-induced mortality and possible human-wolf conflicts (5). The concept of oral vaccination of foxes against rabies has already been adapted to many other wildlife reservoir species; among others raccoon dog (*Nyctereutes procyonoides*), raccoon (*Procyon lotor*), striped skunk (*Mephitis mephitis*), coyote (*Canis latrans*), golden jackal (*Canis aureus*) and free-roaming domestic dogs (*Canis lupus familiaris*) (15-19).

A well accepted bait and suitable bait distribution strategy are pre-requisites for reaching the target population. In this study a potential bait candidate was tested in wolves kept in enclosures. Subsequently adding selected olfactory or taste enhancers to the bait matrix was examined to increase bait palatability and/or detectability.

## MATERIAL AND METHODS

### Baits

Wolves are considered opportunistic feeders that show great flexibility in food sources including plants and fruits. Even anthropogenic food sources like garbage are not avoided and are in some areas their primary food source. However, food neophobia in wolves has also been described and could influence bait acceptance (20). Hence, it was decided to include a positive and negative control besides the experimental bait. As a negative control, the presently used bait matrix for foxes was used, composed of vegetable fats and fish meal (abbreviation – Fish): This bait was shown to be poorly accepted by free-roaming dogs (21-23). As a positive control, boiled sections of pork intestine were used (abbreviation – Intest). Field studies showed that these baits were very attractive to free-roaming dogs (21, 24). The proprietary experimental bait (abbreviation – Egg) based on gelatin and egg-powder has been shown to be attractive to a variety of carnivores, among others the small Indian mongoose (*Herpestes auropunctatus*) and dogs (21, 25-26).

### Screening studies

For the screening studies, to confirm if the three selected baits (positive control, negative control and experimental bait) could be used for the intended purpose, 50 wolves in 5 different enclosures in Germany were tested [Family Table 1: List of the wolf enclosures used for the screening studies; n = number of animals kept together in the enclosure.

<table>
<thead>
<tr>
<th>Enclosure</th>
<th>Abbr.</th>
<th>n</th>
<th>Area (ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family Vogelsang</td>
<td>FV</td>
<td>3</td>
<td>0.20</td>
</tr>
<tr>
<td>Family Vogelsang</td>
<td>FV</td>
<td>2</td>
<td>0.12</td>
</tr>
<tr>
<td>Zoo Worms</td>
<td>TW</td>
<td>5</td>
<td>0.24</td>
</tr>
<tr>
<td>Game park Scharze Berge</td>
<td>SB</td>
<td>4</td>
<td>1.00</td>
</tr>
<tr>
<td>Game park Bad Mergentheim</td>
<td>BM</td>
<td>32</td>
<td>1.80</td>
</tr>
<tr>
<td>Game park Lüneburger Heide</td>
<td>LH</td>
<td>4</td>
<td>0.25</td>
</tr>
</tbody>
</table>
Fluctuating number of baits (2-8) of the 3 different types were positioned at 3-5 bait stations within the wolf enclosures. Bait uptake was recorded by direct observation and/or video cameras (RCX 1 & 2 Trial camera system, Firma Leupold & Stevens Inc.; 97006 Beaverton, Oregon, USA).

Optimization studies

The bait optimization studies were carried out in a wolf enclosure (0.6ha) at the Zoo in Osnabrück, Germany. The wolf pack consisted in 2014 and 2015 out of 13 and 10 animals, respectively. The wolves were predominantly fed cattle and goat meat four times a week.

Firstly, the three selected baits were tested against each other (two-food-preference) to confirm that the animals showed similar bait acceptance rates as the wolves in the other enclosures. Thereafter, different selected potential palatants to enhance bait acceptance were tested (Table 2). These substances were homogenously mixed with the components of the experimental egg-flavored bait. Cadaverine and putrescine (Sigma Aldrich Chemie GmbH, 82024 Taufkirchen, Germany) were selected as wolves also eat carrion (27–28). While blood contains iron, it was suggested that iron dextran (Serumwerk Bernburg AG, 06406 Bernburg, Germany) could enhance bait acceptance: three different concentrations were used (66.6, 133, 200mg/Kg). Salt (Carl Roth GmbH & Co KG, 76185 Karlsruhe, Germany) is often insufficiently available and therefore highly attractive for animals (29), hence a saline solution was added to the bait matrix. There are five different universal taste characteristics namely sweet, salty, sour, bitter and umami (30). The last one, umami, is often associated with meat and therefore a 0.01% mixture of guanosinmono-phosphate (GMP) and inosinmonophosphate (IMP) (Sigma Aldrich Chemie GmbH, 82024 Taufkirchen, Germany) was added to the bait (31).

When baits are distributed in the environment, first the animals need to locate (detect) the bait before it can be consumed. The bait matrix of the experimental bait does not have a strong smell and furthermore camouflages the potential olfactory enhancement of the selected palatants incorporated. Hence, it was decided to test a topical applied olfactory enhancer to increase detectability; rumen flour (Fleischeslust Tiernahrung, 94518 Spiegelau, Germany) as it is known that wolves consume rumen (28). Hence, additional baits were prepared and tested (Table 3). Bait preference was determined by placing two different

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**Table 2: The different bait preparations used together with the standard experimental bait for the bait acceptance optimization studies**

<table>
<thead>
<tr>
<th>Type</th>
<th>palatants</th>
<th>Abbr.</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>carrion</td>
<td>Cadaverine 5mg/kg</td>
<td>Cad</td>
<td>Sigma Aldrich Chemie GmbH, 82024 Taufkirchen, Germany</td>
</tr>
<tr>
<td>carrion</td>
<td>Putrescine 100mg/kg</td>
<td>Put</td>
<td>Sigma Aldrich Chemie GmbH, 82024 Taufkirchen, Germany</td>
</tr>
<tr>
<td>umami</td>
<td>0.01% 1:1 mixture of GMP and IMP*</td>
<td>Umami</td>
<td>Sigma Aldrich Chemie GmbH, 82024 Taufkirchen, Germany</td>
</tr>
<tr>
<td>iron</td>
<td>Iron dextran 66.6mg/kg</td>
<td>Fe66</td>
<td>Serumwerk Bernburg AG, 06406 Bernburg, Germany</td>
</tr>
<tr>
<td>iron</td>
<td>Iron dextran 133mg/kg</td>
<td>Fe133</td>
<td>Serumwerk Bernburg AG, 06406 Bernburg, Germany</td>
</tr>
<tr>
<td>iron</td>
<td>Iron dextran 200mg/kg</td>
<td>Fe200</td>
<td>Serumwerk Bernburg AG, 06406 Bernburg, Germany</td>
</tr>
<tr>
<td>salt</td>
<td>Saline solution (2% NaCl)</td>
<td>NaCl</td>
<td>Carl Roth GmbH &amp; Co KG, 76185 Karlsruhe, Germany</td>
</tr>
</tbody>
</table>

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**Table 3: The different bait preparations used together with the standard experimental bait for the detectability studies**

<table>
<thead>
<tr>
<th>Type</th>
<th>Preparation</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rumen</td>
<td>Fresh rumen was boiled and the generated brew was mixed with the gelatin (no egg-powder)</td>
<td>Rumen</td>
</tr>
<tr>
<td>Rumen flour</td>
<td>Instead of egg powder rumen flour was mixed with the gelatin</td>
<td>RF</td>
</tr>
<tr>
<td>Egg &amp; rumen flour</td>
<td>A 50% : 50% mixture of egg powder and rumen flour was mixed with the gelatin</td>
<td>Egg-RF</td>
</tr>
<tr>
<td>Rumen flour coated</td>
<td>The standard experimental bait was coated with rumen flour</td>
<td>RF-coated</td>
</tr>
</tbody>
</table>
baits at one of the three selected baiting sites within the enclosure. At every site, a camera was installed to record bait uptake; this was to complement direct observation from a platform overseeing the enclosure. Finally, to determine detectability of the rumen flour-coated bait and the standard experimental bait, 5 baits of each type were hidden randomly at 10 selected sites within the enclosure for every 9 runs, approximately 8-10 meters apart.

**Confirmation studies**

The final selected bait was tested against the positive and negative control for confirming purposes in two additional wolf enclosures; Zoo Worms and Game park Schorfheide with 4 and 5 wolves, respectively. Two different baits were placed at each of the three selected baiting sites within the enclosure and bait preference (first choice) was recorded by camera installed at the baiting sites. All three bait combinations were tested against each other.

**Statistical Methods**

Chi squared (screening and detectability studies) and the Wilcoxon test (optimization and confirmation studies) were used to determine differences between the groups. A probability of 5% or less (p<0.05) was considered statistically significant.

**RESULTS**

The bait percentage acceptance for the three bait types in the different wolf enclosures is shown in Figure 1. When the results of all 5 locations were pooled the positive control, negative control and experimental baits were accepted at rates of 100% (96/96), 42.3% (33/78) and 91.9% (91/99), respectively. The negative control bait was significantly less often taken than the other two baits; Chi²-test, \( \chi^2 = 104.3, \) df=4, p<0.001. These findings were confirmed in the Zoo Osnabrück; where, no significant difference was observed in bait preference between the experimental egg-flavored bait and the positive control. However, the negative control was significantly less accepted than the positive control and experimental bait (Table 4).

It was not possible to test all bait candidates containing the different additives against each other. After several days the wolves already showed less interest in baits and some-
times interruptions of several months had to be included. However, the combinations tested were interlinked in such a way that a final conclusion on bait preference was feasible (Figure 2).

Statistically significant differences regarding acceptance were only observed for three combinations tested (Figure 2); the bait containing iron dextran – 66mg/Kg (Fe66) was preferred over the bait with the highest amount of iron dextran – 200mg/Kg (Fe200); Wilcoxon-test, p=0.003. The standard experimental bait (egg) was better accepted than the umami bait (Wilcoxon-test, p=0.01) and the experimental bait containing not only egg powder but also rumen flour (Egg-RF) was consumed more often than the rumen flour bait (RF) (Wilcoxon-test, p<0.01). However, it can be concluded that none of the baits tested was significantly more often accepted than the standard experimental egg-flavored bait. During the detectability study, it was shown that the rumen flour-coated bait was significantly more often detected than the bait without the coating; Chi²-test, Chi²=5.324, df=1, p=0.02 (Table 5).

Hence, the rumen flour-coated egg-flavored bait was tested against the positive – and negative control in the two additional wolf enclosures. The results confirmed previous outcomes: the negative control was not taken at all, meanwhile all positive and rumen flour-coated experimental baits were taken, except for one positive control bait (Table 6). In direct comparison, there was no difference in preference (first choice) between the intestine bait and experimental egg-flavored bait.

**DISCUSSION**

Western and Central Europe are presently free of rabies, with the exception of certain bat lyssaviruses, therefore there is only a very small risk that the re-established wolf population will become infected (1). However, wolves are known to travel over large distances in a relatively short time (27-28) and thus the animals could re-introduce rabies from areas in

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**Table 4:** The results (number of observations) of the two-food-preference test in wolf enclosure Zoo Osnabrueck (XY: first bait X was consumed followed by bait Y, X-: only bait X was taken, Y-: only bait Y was taken, YX: first bait Y was consumed followed by bait X.

<table>
<thead>
<tr>
<th>Bait X</th>
<th>Bait Y</th>
<th>XY</th>
<th>X-</th>
<th>Y-</th>
<th>YY</th>
<th>Test.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>intestine</td>
<td>Fish meal</td>
<td>1</td>
<td>12</td>
<td>–</td>
<td>–</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>egg</td>
<td>Fish meal</td>
<td>–</td>
<td>10</td>
<td>–</td>
<td>–</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>egg</td>
<td>Intestine</td>
<td>6</td>
<td>–</td>
<td>–</td>
<td>16</td>
<td>p&gt;0.05</td>
</tr>
</tbody>
</table>

* – Wilcoxon test

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**Figure 2:** The results of the main bait acceptance optimization studies; see for the abbreviations of the baits main text and tables. The numbers shown near the arrows indicate the number of observations made and in case that there was a significant difference (Wilcoxon test), the arrow head indicates to the preferred bait.
Eastern Europe where rabies is still endemic. The last rabies outbreak in Finland at the end of the 1980’s was thought to be introduced by migrating wolves incubating rabies (34). Also, rabies can be re-introduced by, for example, illegally imported rabies-incubating dogs. Several cases of imported dog rabies in Europe have been documented in recent years (35).

In the event that rabies would re-emerge in Western and Central Europe, the population’s tolerance towards the wolf’s presence could alter drastically. This could be circumvented if the wolf population in the re-infected areas could be protected against this disease and thereby possible attacks by rabid wolves avoided. Oral vaccination of wildlife against rabies has been developed as a highly cost-efficient method to control and ultimately eliminate locally the disease from the targeted species. Distribution of baits targeted at wolves has so far only been used in campaigns to poison these animals and most baits were made of fresh meat products (36-38). As shown in this study, using natural products like intestine, are readily acceptable to wolves. Chicken heads have also been found acceptable by wolves (unpublished data).

Oral rabies vaccine baits made from natural products like intestine and chicken heads have shown to be highly efficacious in dogs and foxes (39-40). However, the preparation of such baits is very laborious and time consuming. Furthermore, there are certain regulatory requirements that would not permit using such bait matrices for the delivery of biologicals to wildlife in certain countries. For example, in several countries the bait matrix may not contain any product from (terrestrial) animal origin. The presently used vaccine baits for foxes and raccoon dogs are all composed of vegetable fats and/or fish meal and it seems that these baits are not notably palatable to the wolves. Chicken heads have also been found acceptable by wolves (unpublished data).

A bait must be attractive to a number of sensory capacities of the animal, including olfactory, taste, texture and sometimes also visual (41). The experimental egg-flavored bait that can be mass produced seemed to be also attractive for wolves. In this study, we found that the slightly lower bait uptake compared to the intestine bait was not significant in any of the enclosures tested. The egg-flavored bait had a relatively high moisture content compared to the fish meal bait and was therefore more palatable for animals. Using the same three baits during a two-food-preference test in beagles and free-roaming dogs in Thailand, it was shown that there was no difference in bait acceptance between the intestine – and experimental bait. However, a highly significant difference was observed between the intestine – and fish meal bait (21). The palatants selected in the present study did not improve bait acceptance by the wolves. Palatants in the pet food industry are normally added to induce the animal consuming food that may be inconsistent with their native diet. Rumen flour mixed homogenously with the bait matrix also did not increase bait acceptance but when applied topically it increased bait detectability and consequently overall bait uptake.

Further studies are necessary to confirmed that the selected bait is also accepted by free-roaming wolves. Furthermore, it needs to be investigated if the bait is suitable as a vaccine delivery vehicle. The vaccine must be released in the oral cavity to induce a protective immune response. If the wolves would swallow the bait in one mouthful, the vaccine will most likely not be released in the oral cavity and will rapidly loose its immunogenic potential in the gastrointestinal tract. Also, the size and shape of the bait must be optimized in such a way that spillage (e.g. leaking on the ground) is prevented.

The concept of oral vaccination consists of a well-accepted bait carrying suitable blister that can easily be perforated during consumption in order to release vaccine into the mouth, a safe and efficacious vaccine and finally a
baits by the target species (42). A safe and efficacious vaccine is most likely not an issue since rabies vaccines developed for domestic dogs are successfully used for wolves, although these concerns inactivated vaccines for parenteral use (43). It may be expected that efficacious oral rabies vaccine candidates for free-roaming dogs will also be able to protect wolves against a rabies infection.

Unfortunately, the bait distribution strategies used for foxes or other wildlife species do not seem to be suitable for wolves. Primarily, because wolf density is relatively low compared to the densities of smaller carnivores; therefore, these smaller carnivores and other bait competitors will most likely locate and consume the baits before the wolves. Hence, only targeted baiting at selected sites like the rendezvous sites seems feasible. At these rendezvous sites young wolves stay for several days to which adult wolves return regularly in late summer to early autumn (44-45). However, this requires further investigation.

CONCLUSIONS

Baits presently used for oral vaccination of wildlife against rabies in Europe have not been found acceptable by wolves. In the present study, we demonstrated that an experimental egg-flavored bait was orally well acceptable. Adding olfactory / taste enhancers to the bait matrix did not increase bait palatability however adding an olfactory attractant in the form of a coating did increased bait detectability. The optimal bait size and form in combination with a sachet containing the vaccine needs to be investigated in order to secure maximum release of the vaccine in the oral cavity.

ACKNOWLEDGEMENTS

We would like to express our gratitude to the staff of the wolf enclosures, especially of the Zoo in Osnabrück for their patience and support.

CONFLICT OF INTEREST

Two authors, SO and AV, are full-time employees of a company producing oral rabies vaccine baits. However, this had no effect or influence on the results and the interpretation thereof.

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