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A field study on canine rabies in Lao PDR

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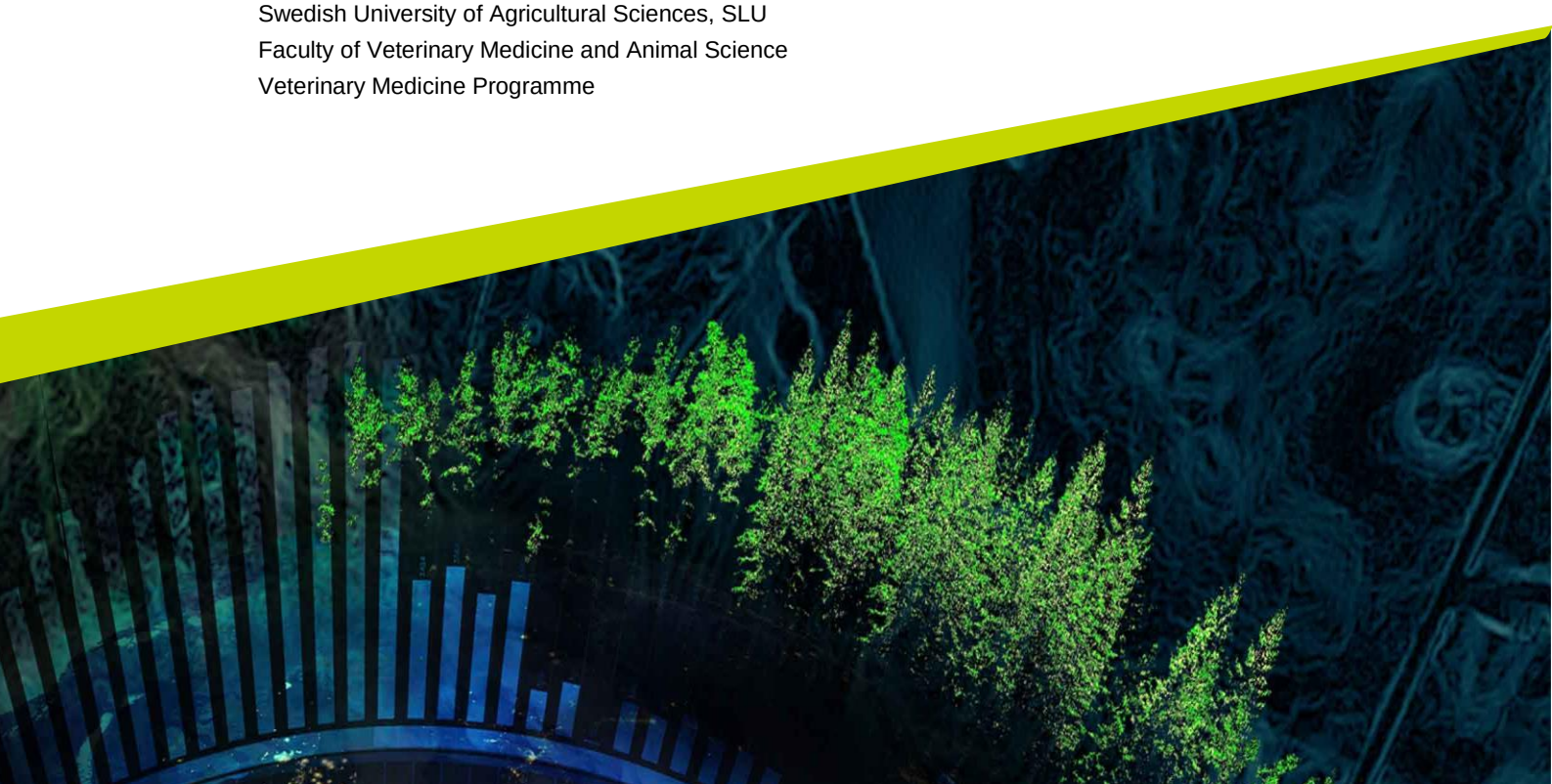

Dog owner knowledge and attitudes towards oral vaccination: A field study on canine rabies in Lao PDR

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Abstract

Rabies is a fatal viral disease causing around 59,000 deaths each year worldwide. Out of all deaths, 95% occur in Africa and Asia. In Lao People's Democratic Republic (Lao PDR) rabies is endemic with dogs being the main virus reservoir. All mammals can be infected by the rabies virus but up to 99% of human rabies cases are transmitted by dogs. The virus is mainly transmitted through saliva contact with wounds or mucosa and the incubation time is generally 20-60 days, but it can be several years. Symptoms include behavioral changes, anorexia, vomiting, excessive salivation, ataxia, paralysis and seizures and results in death through paralysis of the breathing musculature. Rabies is 100% preventable by vaccination. WHO (World Health Organisation), WOAH (World Organization for Animal Health) and FAO (Food and Agriculture Organization) have together formed the UAR (United Against Rabies) platform. The goal is to have zero dog-mediated human rabies deaths by 2030. WHO finds rabies vaccination of dogs to be an important measure to reach this goal, however it's considered difficult to reach adequate vaccination coverage with parenteral vaccination only. Therefore, there has been a promotion of oral rabies vaccination, which has helped eliminate rabies in wildlife in Europe. Oral rabies vaccines have been shown in studies to be safe, effective and stable in field conditions, however, there has been a few incidents of vaccine induced rabies cases in animals and vaccine induced skin infections in humans. Bait preferences have been shown to vary between countries. Oral vaccine baits can be distributed in different ways, including handing out the bait to dogs and distributing them in the environment. Oral rabies vaccination has been shown to be more cost effective and less time consuming than other methods. The goal of this study was to investigate knowledge and attitudes towards oral rabies vaccination campaigns among dog owners in Lao PDR. The study was carried out in three different provinces in Lao PDR during 2022. Participants included dog owners in these districts, who answered questionnaires and some also participated in group discussions, as well as village leaders who were interviewed. Dog owner knowledge was low regarding rabies hosts, transmission, vaccines, seriousness and symptoms. Most dog owners (87.6%) would prefer to vaccinate their dogs against rabies through an injection, while 12.4% would prefer the oral route. However, 60.2% thinks oral vaccination is a very good idea. Dog owners and village leaders considered oral vaccination to be easier, safer when vaccinating aggressive, and more ethical. It was considered an advantage that dog owners could perform the vaccination themselves. Concerns were the risk of the vaccine not being effective, being bitten while providing vaccine, potential side effects and the dogs not consuming the baits. Almost all (98.1%) dog owners claimed to be willing to pay to rabies vaccinate their dog. To reach the Zero by 30 goal, there need to be information campaigns on rabies to fill the knowledge gaps. This study shows an openness among dog owners to oral rabies vaccination, however further research is needed on this topic.

Keywords: canines, zoonoses, vaccine prevention, vaccine preferences

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1. Introduction

Rabies is a fatal viral disease with around 59,000 deaths each year worldwide (CDC 2020a). Out of all deaths, 95% occur in Africa and Asia (WHO n.d.-d). It is 100% preventable by vaccination (CDC 2020a). Rabies is considered a neglected tropical disease with around 80% of cases affecting people in poor rural areas where the daily income may be only US$ 1–2 per person (WHO 2021b). World Health Organisation (WHO), World Organization for Animal Health (WOAH) and Food and Agriculture Organization (FAO) have together formed the United Against Rabies (UAR) platform. The goal is to have zero dog-mediated human rabies deaths by 2030 (the Zero by 30 vision).

Lao PDR is located in southeast Asia and borders on Thailand, Vietnam, China, Cambodia and Myanmar (CIA 2022). The population is almost 7,750,000 people. Great progress has been made regarding poverty, as the poverty rate has decreased from 46% in the early 90s to 22% in 2012-2013. However, there are significant disparities between rural and urban areas, and income inequality is increasing.

In Lao PDR, dogs are the main rabies reservoir (Ahmed *et al*. 2015) and dog bites are the main source of transmission to humans (Douangngeun *et al*. 2017). Other typical host reservoirs for rabies in Asia are red fox (*Vulpes vulpes*), ferret badger (*Melogale mochata*) and golden jackals (*Canis aureus*) (WHO n.d.-b). From 2012 to 2017, there was 33 reported human rabies cases in Lao PDR (WHO 2018). A study from 2010-2016 analyzed brain material from suspected rabid dogs in the country and found 284 positive cases out of 415 submitted samples (Douangngeun *et al*. 2017). However, the study is based on the passive rabies surveillance that the country has, where testing is only done following a bite incident. Therefore, this study likely underestimates the true number of rabies cases in the country. The study also found an increase in positive cases during the dry season, likely due to changes in the dogs' behavioral patterns during this time.

1.1 Aim

The goal of this study was to investigate knowledge and attitudes towards oral rabies vaccination campaigns among dog owners in Lao PDR. The goal is to see whether there is an acceptance towards oral rabies vaccination campaigns, which would enable the implementation of such campaigns in the future. This could then help eliminate dog mediated rabies.

1.2 Hypothesis

The hypothesis is that people generally are positive towards oral rabies vaccination for dogs, but that knowledge about rabies is low.

2. Literature review

2.1 Rabies

2.1.1 Etiology and epidemiology

Rabies is a zoonotic and virtually 100% fatal disease caused by a lyssavirus (SVA 2021). The virus belongs to the Rhabdoviridae family and is an enveloped RNA-virus which can infect all mammals including humans (CDC 2020b). The disease is present mostly in Africa and Asia but also in North- and South America (SVA 2021). Most countries in Europe are free from rabies, however, the only continent entirely free is Antarctica (WHO 2021b).

Each year, 59,000 people are estimated to die from rabies, of which 95% of cases occur in Asia and Africa. Up to 99% of human rabies cases are transmitted by dogs, making dogs the by far most common animal to transmit the disease to people. 40% of dog bites caused by suspect rabid dogs are affecting children between 5-14 years of age, resulting in high numbers of rabies cases in children.

Typical host reservoirs are carnivores and includes for example red fox (*Vulpes vulpes*), raccoon (*Procyon lotor*), striped skunk (*Mephitis mephitis*), coyote (*Canis latrans*), golden jackals (*Canis aureus*) and small Indian mongoose (*Herpestes auropunctatus*) (WHO n.d.-b).

2.1.2 Pathogenesis

Following a bite from a rabid mammal, the rabies virus infects the subcutaneous tissues and muscles (Jackson 2016). The virus stays near the infection site for most of the incubation period. After infecting muscle fibers, the virus binds to nicotinic acetylcholine receptors found at the neuromuscular junction. The virus travels by retrograde transport up the motor and sensory axons. This enables infection of neurons and spread between neurons in the CNS. Once the virus reaches the brain, behavioral changes can be seen. The virus continues from the CNS to the parasympathetic nervous system and reaches salivary glands, skin, heart and other organs. Virus excretion into the saliva and behavioral changes facilitates transmission (Jackson 2016).

The disease is mostly characterized by inflammation and mild degeneration in the CNS and not neuronal cell death (Jackson 2016). Apoptosis is a host mechanism used to prevent the spread of the disease, rather than an important part of the rabies pathogenesis. Li and colleagues have found that infection with pathogenic rabies

virus in mice results in severe disruption of neuronal processes, while neuronal bodies show only small pathological changes (Li *et al*. 2005). This might be due to interruption of cytoskeletal integrity.

2.1.3 Transmission

Rabies is transmitted when saliva or CNS material from an infected individual comes in contact with non-intact skin or mucous membranes (CDC 2019d). The most common transmission path is through bites from a rabid animal, as the disease often results in aggressive behavior. Saliva contact with skin abrasions, scratches or open wounds are also a risk. Infection through corneal and organ transplants have been recorded but is rare. Inhalation of aerosols containing the virus and exposure of saliva from a rabies infected person are other theoretical transmission routes. However, there has not been any reported cases of a human transmitting the disease to another human.

Rabies virus can be excreted in the saliva up to 14 days before symptoms appear (SVA 2021). Incubation time is generally 20-90 days, but can in rare cases last longer than a year in humans (Jackson 2016).

2.1.4 Symptoms

In animals, symptoms vary among species, however changes in behavior is typical in the initial stages (SVA 2021). While wild animals lose their shyness and may approach humans, domestic animals can avoid humans and become aggressive. Incubation time is considered to be 4-8 weeks, but might vary from 10 days to 6 months (Epiwebb 2013a). Incubation time also varies between host species and depends on amount of virus, virus strain and location for infection. Death typically occurs 10 days after symptoms have arisen.

Generally, symptoms of rabies start with a prodromal stage, followed by either a furious and/or paralytic stage (Epiwebb 2013a). In dogs, the prodromal stage lasts 2-3 days, followed by either a furious or paralytic form of the disease (Epiwebb 2013c). The furious or paralytic stages lasts 3-7 days, followed by death. Both forms can be seen simultaneously, alternating between one another. The disease usually present with nonspecific symptoms initially, such as anorexia, lethargy, fever and vomiting (WHO n.d.-e). In the furious form, dogs typically become increasingly aggressive and make unprovoked attacks (Epiwebb 2013a; CDC 2019b). They may seem tireless, insensitive to pain, abnormally strong and might try to bite imaginary objects (Epiwebb 2013a). Aimless wandering, loss of corneal reflexes, mydriasis and changes in vocal sounds are other possible symptoms. In the paralytic form, dogs avoid social contact, become lethargic and do not show

aggression. The disease progresses with dysfunction in the cerebrum and cranial nerves, leading to ataxia, weakness, paralysis and seizures (WHO n.d.-e). Muscle paralysis result in difficulty swallowing, which makes ingestion of feed and water impossible and leads to excessive salivation (Epiwebb 2013a). The musculature gradually become increasingly paralyzed until it affects the breathing musculature which leads to death. Once paralysis is seen, death occurs within 5-6 days (WHO n.d.-e).

In people, the furious form is much more common than the paralytic form (Hankins & Rosekrans 2004). The prodromal stage occurs 2-10 days after exposure and is usually 1 day to 2 weeks long. During this period, anorexia, irritability, fever, headache, nausea and vomiting is common. Paresthesia, numbness and pain in the site for infection can also occur. After 2-7 days, this stage is followed by acute neurologic syndrome, characterized by anxiety, agitation, lethargy, manic behavior, dysphagia, salivation, polyneuritis, dysarthria, and nystagmus. The patient may also experience hallucinations, both visual and auditory, vertigo, diplopia, and hydrophobia due to spasms in the pharyngeal musculature which can be very painful. After 7-10 days, symptoms such as periods of apnea, generalized flaccid paralysis, seizures and coma ultimately leads to cardiorespiratory dysfunction and death. This usually takes 2-3 days without life support equipment.

2.1.5 Diagnostics

Clinical examination and observation can lead to a suspicion of rabies but not a diagnosis (WHO n.d.-a). To confirm or rule out the diagnosis, one of following diagnostic methods should be used: Detection of rabies antigen, detection of rabies virus replication (inoculation tests) or detection of rabies virus RNA. Serological assays are only used to assess immune response after vaccination in humans and animals and not as a diagnostic test. This is because rabies antibodies arise when clinical symptoms are already present (i.e when the disease will almost inevitably lead to death), or might not arise at all (Epiwebb 2013b). To detect antibodies, serological tests such as enzyme-linked immuno-sorbent assay (ELISA) or virus neutralization test can be used (WHO n.d.-a).

For detecting rabies antigen, the fluorescent antibody test (FAT) is most commonly used (Hanlon & Nadin-Davis 2013). This is considered the gold standard test by WHO and OIE since it is rapid and has high sensitivity and accuracy (WHO n.d.-a). There is also a direct rapid immunohistochemistry test (dRIT) that is based on the same principle as the FAT, but uses a different type of staining. The method is based on fluorescent antibodies binding to the rabies virus in impression smears from brain tissue, forming aggregates (Duong *et al*. 2016). The aggregates are identified using a fluorescence microscope.

Inoculation tests are mainly used to confirm unclear results from FAT or dRIT tests or to type the virus strain (WHO n.d.-a). This can be done by inoculating the virus in neuroblastoma cells or intracranially in live mice (Duong *et al*. 2016). However, the latter is problematic due to ethical reasons. The results must be confirmed by a FAT test since the rabies virus is not cytopathic.

Rabies virus RNA can be detected using polymerase chain reaction (PCR) which amplifies the RNA fragment in the genome of the virus (Hanlon & Nadin-Davis 2013). The results may be false positive or false negative, but the method has high specificity if performed carefully (Duong *et al*. 2016). It is useful for *intra vitam* diagnosis in humans.

In humans, several diagnostic methods need to be performed to diagnose rabies *intra vitam* (CDC 2019a). This can be done by using saliva for PCR or virus inoculation, skin biopsies for PCR or FAT, or serum/cerebrospinal fluid for FAT and virus neutralization test. For *post mortem* diagnosis, brain samples from the medulla, cerebellum and hippocampus are used to perform the FAT test (CDC 2021).

WHO strongly advices against *intra vitam* diagnosis in animals (WHO n.d.-a). Animals should be euthanized for diagnosis to be confirmed, since the diagnostic test requires material from two locations in the brain at minimum (CDC 2019a). Preferably, the cerebellum and brain stem are used. The brain material is used for the FAT test (Epiwebb 2013c). A positive result can be confirmed through inoculation in cell culture or mice. An animal that is infected but not yet excreting virus in the saliva, i.e not yet contagious, will likely be negative on the FAT test.

2.1.6 Treatment, prophylaxis and post exposure prophylaxis (PEP)

Rabies is a highly deadly disease with only 15 well documented survivals in humans ever, compaed to the 59 000 deaths it causes each year (WHO) 2018). The majority of surviving patients, however, experienced severe neurological deficits after recovering from the disease. In all these cases except one, the patients had received at least one dose of rabies vaccine before symptoms developed. In Milwaukee, USA, in 1969, one patient survived rabies due to an aggressive treatment protocol now known as the Milwaukee protocol (Willoughby *et al*. 2005). The protocol has been frequently used after that but without any further well documented survivals (WHO 2018). The WHO does not recommend aggressive protocols other than in cases where symptoms are very mild and where adequate resources and competent medical staff is available. This is due to the low survival rates despite

treatment and the high risk of permanent severe neurological deficits if the patient survives.

Ribavirin is one agent with known *in vitro* effect against rabies virus, however there are no studies showing efficacy when used to treat rabies inoculated mice or when used as treatment in rabies infected humans (Appolinario & Jackson 2015). Ribavirin is therefore not recommended as a treatment. Interferon-α (IFN-α) is another agent that is shown to inhibit the spread of the virus in muscle tissue, slow down spread to the CNS and delay mortality in inoculated mice. However, it does not inhibit progression of the disease after symptoms have developed in either monkeys or humans. Drugs like ketamine, minocycline and amantadine have also been included in research studies but without any strong evidence of effect against rabies virus. Research is currently focused on finding antiviral therapy inhibiting the replication of virus RNA. Viral enzymes such as polymerases and oligonucleotides like aptamers are two agents that might show promising results. Aptamers have been shown to inhibit replication of rabies virus in recent studies (Scott & Nel 2021).

Because there are no effective protocols to cure the disease today, treatment mostly consists of palliative measures (Scott & Nel 2021). Sedation with barbiturates and morphine to ease agitation, anxiety and pain is necessary, and benzodiazepines or midazolam can be used for muscle relaxation. Adequate hydration is also important. Life supporting measures such as intubation should be avoided. The patient is best treated in a hospital environment; however, cultural and religious needs should be respected. Emotional support and the possibility to be close to family is also essential since patients generally remain conscious and aware of the deadly nature of the disease.

A dog, cat or ferret that bites a person but does not show any signs of illness should be kept under observation for 10 days (CDC 2019c). If the animal develops any signs of illness during this period, a veterinarian should be consulted. If the veterinarian suspects the animal to be rabid, authorities should be contacted and the animal euthanized. The head of the animal is then sent for diagnostic tests to be performed.

Rabies is preventable by vaccination (pre-exposure prophylaxis) in both animals and humans (CDC 2019b). Vaccination is recommended for people who are likely to be exposed to the virus, such as people living in high risk areas or people travelling there, people working with potentially infected animals such as veterinarians, laboratory workers etc.

In case a person is bitten by a suspected rabid animal, post-exposure prophylaxis (PEP) is also very important. PEP includes thoroughly washing the wound with water and soap for at least 15 minutes immediately after exposure to decrease the amount of virus, receiving a dose of rabies vaccine approved by WHO and, if indicated, treatment with rabies immunoglobulin (RIG). About 80% of the rabies cases affects people in poor rural areas where the daily income may be only US$ 1–2 per person. PEP might not be afforded in these areas in since the cost is approximately US$ 108 (WHO 2021b)

2.2 Rabies vaccination in dogs

Vaccinating dogs is an effective way of preventing disease transmission to humans. Parenteral vaccination campaigns have been able to eliminate dog mediated rabies in high-income countries (Wallace *et al*. 2020). However, many countries in need of such campaigns do not have the necessary infrastructure, resources or enough accessible dogs to achieve 70% vaccination coverage, which is considered to give herd immunity (Wallace *et al*. 2017). Vaccination campaigns that do not require the same level of infrastructure and accessible dogs, such as capture-vaccinate-release (CVR) campaigns, are shown to give good vaccination coverage for free-roaming dogs but to be less cost- and time effective compared to door-to-door oral vaccination (Undurraga *et al*. 2020) and oral bait handout vaccination (Gibson *et al*. 2019). One study shows that even though door-to-door oral vaccination is an expensive method due to the relatively high cost of an oral bait, the cost per vaccinated dog is $1,97 compared to $2,20 for mobile static point (MSP) vaccination, and $2,28 for MSP and CVR combined (Undurraga *et al*. 2020). This is probably due to the higher accessibility of free roaming dogs with this method, making it possible to vaccinate a larger number of dogs in a shorter amount of time.

2.3 Oral rabies vaccination

2.3.1 The role of oral vaccination to eliminate dog mediated rabies

WHO finds both human rabies vaccination and rabies control in dogs to be important measures to eliminate dog mediated rabies in humans (WHO 2007). Since 1985, it has been considered a difficulty to reach adequate vaccination coverage of dogs only through the parenteral route. WHO has since then promoted research on oral vaccination of dogs, particularly on development of safe and effective vaccines and baits, and on dog population structure and immunization coverage in Asia, Africa, and Latin America. Research has also been focused on

safety for non-target species, especially humans, when exposed to oral dog vaccines, as well as different delivery systems for oral vaccines in the field. WHO also considers the economics of oral vaccination campaigns to be an important research area since adequate finances must be available for the campaigns to be carried out. For example, CVR requires significantly higher numbers of trained staff (Gibson *et al*. 2019), and a 2 week national campaign in India would require 1.1 million staff if CVR was used compared to 293 000 staff if oral bait handout was used, making CVR a difficult method to use in larger scale.

Europe has been able to control rabies in foxes and raccoons by using oral rabies vaccination (ORV) campaigns (EFSA 2015). Over the last 40 years, ORV has been performed in 30 European countries, and Western and Central Europe is now free from rabies (Müller & Freuling 2018). There are still cases of rabies in dogs being reported from Eastern European countries, probably due to spillover from wildlife, but dog rabies only persists in Turkey. Today, 12 European countries have declared themselves free from rabies according to the standards of the WOAH.

According to WHO, research regarding vaccine safety and bait development is needed to improve use of oral vaccines so they can be used to eliminate dog mediated rabies (WHO 2021a). They also recommend improvement of the vaccine licensure processes to make safe and effective vaccines more available. Education is also essential so that vaccinators and dog owners will understand how ORV works and understand why it is important.

2.3.2 Oral vaccine safety

Because of the proximity of dogs to humans and other animals, ORV campaigns would mean a high risk of vaccine exposure to humans and non-target animals. Therefore, oral rabies vaccines must be safe for not only dogs. There has only been a few instances where people have been affected after exposure to ORV, such as a pregnant woman with a skin condition who suffered a serious skin infection and cellulitis after being bitten by her dog who had previously chewed on an ORV-bait meant for raccoons (Rupprecht *et al*. 2001). After medical and surgical treatment, she survived, remained free of symptoms and gave birth to a healthy child. Another case was a woman on immunosuppressive medications who also had skin contact with the vaccine in an ORV-bait and suffered a skin infection, but recovered fully after treatment with human vaccinia immunoglobulin and antiviral agents (CDC 2009).

There are a few studies showing evidence of oral vaccine induced rabies in animals. Domestic animals such as a cow (Vuta *et al*. 2016) and cats (Esh *et al*. 1982) have been reported to develop rabies after intake of oral vaccine. Another study have

shown rabies in a fox caused by the SAD B19-vaccine strain (Hostnik *et al*. 2014). During a vaccination program for rabies in Canada from 1989 to 2004, 13 million baits were distributed in the environment containing attenuated ERA-strain rabies virus (Fehlner-Gardiner *et al*. 2008). During this time period, 4 red foxes *(Vulpes vulpes)*, two raccoons *(Procyon lotor)*, two striped skunks *(Mephitis mephitis)* and one bovine calf (*Bos taurus*) were found to have ERA-virus induced rabies. However, there were no evidence of establishment of the virus strain in the wildlife populations. Studies in the Baltic states have also shown vaccine induced cases in two non-target species (one badger and one marten), where the virus strains were closely related to the SAD B19-strain (Robardet *et al*. 2016). With other vaccine strains, such as the SAG2 vaccine, there has never been any vaccine induced cases or safety issues reported, even after distribution of 20 million baits in Europe (Mähl *et al*. 2014). This shows the importance of adequate safety regulations and testing for oral vaccines. Using the hand-out-model of baits instead of distribution in the environment would also help minimize unintended contacts between the baits and non-target species.

2.3.3 Oral vaccine efficacy

ORV have been shown to give protective levels of antibodies in dogs for at least 180 days after a single immunization (Zhugunissov *et al*. 2017). A study done by Leelahapongsathon and colleagues shows that parenteral vaccination achieved a slightly higher humoral immune response compared to the orally delivered vaccine, however there were no differences in seropositivity in the ORV group compared to the parenterally vaccinated group after one year (Leelahapongsathon *et al*. 2020). Another study showed protective antibody levels in 100% of dogs that were orally vaccinated and found no abnormal post-vaccination symptoms (Aly *et al*. 2022). Smith and colleagues found that oral vaccination gives detectable antibody levels in 77.8% of vaccinated dogs, compared to 92.7% in dogs that receive parenteral vaccination (Smith *et al*. 2019). This is similar to another field study in which 78% of orally vaccinated dogs seroconverted (Molini *et al*. 2021). For oral vaccination to be complete, the animal needs to be attracted to the bait, puncture the blister/ sachet containing the vaccine and have enough exposure time of the vaccine on the oral mucosa (Smith *et al*. 2019). Any failures in these steps may lead to inadequate immune response. Since these steps are harder to control in field conditions, it may explain why not all dogs achieve protective antibody titers when orally vaccinated in the field.

2.3.4 Bait development and evaluation of preferences

According to WHO, baits should be tested for acceptability both in owned dogs living in households and in free roaming/ownerless dogs in the area where the

vaccination campaign is to be carried out (WHO 2007). Baits should ideally be produced locally in large quantities and be as affordable as possible. To compare machine manufactured and hand-crafted baits, field trials should be carried out. Standardized protocols for these trials have been developed by the WHO and includes information about what dogs are included in the study (confined, household or free-ranging dogs), duration of bait presentation, composition, size and origin of the bait, the fate of baits and the vaccine container and the vaccine itself etc. The trials of new bait candidates should also involve a reference bait, for which chicken heads are recommended as they showed a high acceptance among dogs in Tunisia (Matter *et al*. 1995).

In general, bait candidates should be hygienic, easy to handle and fulfill the requirements for animal feed so that owners don't object to handling them (WHO 2007). The design of the bait should respect the dogs' food preferences so that it finds the bait appealing.

Many experiments have been done to determine the bait preference of dogs. In one study made in USA by Bergman *et al*. (2008), fish-meal-crumble coated sachets had the highest acceptance compared to vegetable based bait, dog food bait and fish meal bait. In a different study from Bangladesh made in 2020, intestine-based baits had the highest acceptance rate compared to fish and egg baits (Bonwitt *et al*. 2020). A study from Thailand showed the highest acceptance for intestine bait and slightly lower for egg bait, and significantly lower for the fishmeal bait (Kasemsuwan *et al*. 2018). In conclusion, the results seem to vary between different countries.

In the US, the two baits currently used for vaccine campaigns in wildlife (including raccoons, coyotes and gray fox) are produced by Merial, Inc (USDA 2022). The vaccine sachet is either covered by fishmeal coating or hidden inside a fishmeal polymer bait. The bait contains the RABORAL V-RG ® vaccine (USDA 2020). Another available oral rabies vaccine is ONRAB ® (Sobey *et al*. 2019). In the 80s, the Tübingen fox bait, made of fat and fish meal, was used in field trials in Austria, Luxembourg, Belgium and France and showed great results (Schneider *et al*. 1988). In Finland, ORV baits are currently being distributed on the border to Russia to prevent rabies from being reintroduced in the country (Vos *et al*. 2021). In these campaigns, a bait composed of fish products and vegetable fats are used.

2.3.5 Thermostability and resistance of vaccines and baits

Thermostability and resistance of oral vaccines and baits are important research areas to ensure their effects. Keeping a cold chain is easier for canine oral vaccination campaigns compared to wildlife campaigns, as the baits will be handed out directly to the dogs or left in certain areas and retrieved within 24 hours if not

consumed (see next section) (Cliquet *et al*. 2018). One study has shown the SAG2 vaccine to have a titer loss of log10 TCID50/ml every 3 days when placed under vegetation, but to have a marked titer loss if placed in direct sunshine (Bingham *et al*. 1999). In trials from 1999 to 2000, where all available oral vaccines in EU were tested (V-RG, SAG2, SAD B19 and SAD P5/P88), the titres of all vaccines were stable for 3 weeks when temperatures were under 30 degrees Celsius (European Commission 2002). However, titers became significantly lower when temperatures exceeded 30 degrees. The Rabigen SAG2 bait has been shown to stay intact after being dropped from airplanes/helicopters and to resist water and rainfall (Mähl *et al*. 2014). The bait can be stored at -20 or -40 degrees Celsius for 2 years or at 4 or 25 degrees for 7 days without negative impacts. The V-RG vaccine in fishmeal polymer block-baits has also been shown to be stable in field conditions, enduring temperatures from -20 to 20 degrees Celsius during 1 month (Maki *et al*. 2017). When the vaccine was placed in shade for 3 weeks, the titer loss was $10^{0.8}$ $TCID_{50}$/mL. When placed in direct sunlight, the titer loss was $10^{2.2}$ $TCID_{50}$/mL.

2.3.6 Bait delivery

The WHO presents four different methods for delivering oral vaccine baits to dogs (WHO 2007). First method is “door to door vaccination” of owned dogs, where vaccinators move between homes in a target area and give baits directly to the dog owners or the dogs in the home. Second is the “handout model”, which means offering baits directly to owned or unowned dogs in the street. Third method is “central point distribution”, where dog owners are provided with baits at specific sites, so they can offer the baits to their dogs at home. Lastly, we have the “wildlife model” (WIM), where baits are strategically placed in sites that free roaming dogs typically visit. The WHO has set up protocols to be used in studies of the most suitable delivery system. These include information about bait acceptance rates, socio-cultural acceptance, economics, dog population structure, feeding patterns etc. The goal is to find the most effective method, or combination of methods, and optimize the technique to reach the largest possible number of dogs. Oral vaccination can be seen as a good complement to parenteral vaccination. Risk assessment studies for human exposure should be included in future studies.

The central point distribution system has been effective in Tunisia, where 85-90% of the dogs in the study consumed the bait fully or partially (Youssef *et al*. 1998). This method targets primarily owned dogs, mostly those who are accessible for parenteral vaccination but also those who are not (Cliquet *et al*. 2018). This method might however require changes in some countries’ regulations regarding distribution of veterinary vaccines since it involves dog owners administrating the baits.

Door-to-door administration have also shown to be effective and gave 78% total

vaccination coverage in one study (Undurraga *et al*. 2020). Owned dogs that are hard to reach can more easily be targeted with this method, but stray dogs will likely not be reached. Also, the dog or dog owner might not be at home when the vaccinator arrives. It is also quite a time-consuming method. The behavior of the vaccinators can also influence the vaccination rates (Cliquet *et al*. 2018).

The WIM model is effective for targeting free roaming and stray dogs, which are more likely to contract and transmit rabies compared to contained dogs (WHO 2007). For example, 73% of baits disappeared over night in Morocco (Darkaoui *et al*. 2014) and in Tunisia, 40% of baits disappeared within 24 hours (Matter *et al*. 1998). A risk using this method is an increased number of unintentional exposure of vaccine to non-target animals and humans, especially children (Cliquet *et al*. 2018). The handout model limits the risk of unintentional exposure (Cliquet *et al*. 2018) and has been effective in for example the Navajo reserve in the US (Bender *et al*. 2017) and Morocco (Darkaoui *et al*. 2014).

3. Material and methods

3.1 Study design

The study was performed in the Bolikhamsai province, Vientiane province and Vientiane capital in Lao PDR. In each province, 2 districts with 2 villages in each were visited. In conclusion, the study involved a total of 12 villages. The data was collected from 21st of September to 8th of October 2022. Before data collection, translation of questionnaires was done from English to Lao, and afterwards the data collected was translated from Lao to English.

Before conducting the study, research staff from National University of Laos (NUoL) had contacted the village leaders and district veterinarians in each village and district, respectively, to inform about the study and ask for permission to carry out the project in that area. Upon arrival, the village leader and district veterinarian joined the team. The village leader arranged contact with the dog owners. The goal was to gather as many dog owners as possible at central public spaces, such as in government buildings, to facilitate collection of data. However, this was only possible in some villages or for a part of the day, since some people could not travel easily with their dogs or was working during that time. In many cases, the team instead had to go from house to house by foot or car. As a gratitude to the dog owners for taking their time to be part of the study, they were given deworming treatment for their dogs (ivermectin shots or tablets), snacks/candy, and books and pencils for their children.

Each dog owner that agreed to participate in the study had to sign a consent form and answer a questionnaire. After that, their dog/dogs were given an ivermectin shot/deworming tablet if appropriate. Some dog owners were also asked to join a group discussion. The village leader for each village was interviewed.

3.2 Ethical approval

The study was approved by the National University of Laos. In addition, ethical approval was given by International Livestock Research Institute (ILRI) Institutional Research Ethics Committee (approval number IREC2022-40) and Institutional Animal Committee on Use and Care (approval number ILRI-IACUC2022-32). Permission for the foreign (non-Lao) part of the research team to carry out field studies in the country was obtained through the Faculty of Agriculture at National University of Laos.

3.3 Participants

Dog owners living in the villages included in the study could participate. Each participant made a choice to take part in the study after receiving information and could discontinue their participation at any time. They were also informed that personal information would be confidential. Since not all dog owners were able to read and write, the consent forms and questionnaires could be read out loud by a research team member, and instead of a signature, the participant could make a fingerprint.

3.4 Collection of data

3.4.1 Consent forms and questionnaires

Each dog owner participating in the study had to fill in a consent form (appendix 1) and a questionnaire. The questionnaire contained questions about the dog owner and his/her habits, knowledge about zoonoses and rabies and attitudes towards vaccines and oral vaccination. There were also questions regarding the dog and its health and vaccination status. A total of 161 questionnaires were collected. The number varied between villages since the number of dog owners available varied. For the questionnaire, see appendix 2.

3.4.2 Village leader interviews

One research team member showed the village leader in each village a poster (for the poster text, see appendix 3) about what oral vaccination is and how it is performed, together with a brief explanation. The village leader was then interviewed by the same person, asking the questions in appendix 4, and the answers were written down and recorded. A total number of 12 interviews were collected.

3.4.3 Dog owner group discussions

One research team member gathered 3-6 dog owners in each village to join a group discussion and answer more in-depth questions about oral rabies vaccination (appendix 5). Before being asked these questions, the dog owners were also shown the poster about ORV and given a brief explanation. The answers were written down and recorded. A total number of 12 group discussions were conducted.

3.5 Data entry and analysis

Data was entered into Excel. Information from FGDs and KIIs was analyzed according to themes (Kiger & Varpio 2020). Data was analyzed descriptively.

4. Results

4.1 Questionnaire results

4.1.1 Information about study population

In total, 161 people answered the questionnaire, of which 61.5% were women and 38.5% were men. 1 person (0.6%) were less than 15 years old, 14.3% were 16-25, 14.9% were 26-35 and 70.2% were over 35 years old. 12.4% had no education, 15.5% had only gone to primary school, 38.5% had only higher secondary education and 33.5% had graduated or had even more education. See table 1.

Table 1. Information about the dog owners interviewed about their knowledge and attitudes towards rabies vaccination in Laos

	Number	Percentage
Women	99/161	61.5%
Men	62/161	38.5%
< 15 years old	1/161	0.6%
16-25 years old	23/161	14.3%
26-35 years old	24/161	14,9%
> 35 years old	113/161	70.2%
No education	20/161	12.4%
Primary education	25/161	15.5%
Higher secondary education	62/161	38.5%
Graduation and above	54/161	33.5%

4.1.2 Information about dogs

In total, 302 dogs were included in the study. 91.4% of participating dogs were used as guard dogs and 8.0% as company. Only one dog (0.3%) was used for both company and guarding. One dog owner in the study did not answer this question. 99.3% of dogs had a child in the family as the main caregiver and only two dogs (0.7%) was mainly taken care of by an adult in the family. The living conditions were quite mixed, however 69.8% of dogs were outside loose, all the time or partly. 4.6% of dogs were only kept inside. The rest were kept outside in fenced areas or always on a leash when outside. No participant stated that the dog lived with the family. 4.6% of dogs had been vaccinated against some disease sometime in their life according to the owner. See table 2.

Table 2. Information on dog usage, living conditions, main caregiver and vaccination status in Laos

	Number	Percentage
Dogs used as guards	276/302	91.4%
Dogs used for company	24/302	8%
Dogs used for both company and guarding	1/302	0.3%
Dogs with child as main caregiver	299/302	99.3%
Dogs with adult as main caregiver	2/302	0.7%
Dogs only outside loose	123/302	40.9%
Dogs only outside loose but in a fenced area	48/302	15.6%
Dogs only outside in a leash	13/302	4.3%
Dogs only indoor	14/302	4.7%
Dogs both indoor and outside, when outside loose	87/302	28.9%
Dogs both indoor and outside, when outside in a leash	17/302	5.7%
Dogs living with family	0	0%
Dogs that are vaccinated against some disease sometime in their life	14/302	4.6%

4.1.3 Rabies knowledge among dog owners

38.5% of the study population knew that animals can transmit diseases to humans, while the rest (61.5%) did not. 34.8% knew that animals can transmit rabies to humans, however 3 of these respondents said they did not know animals can transmit diseases to humans. 46.6% knew that dogs, specifically, can transmit diseases to humans, while 53.4% did not. See table 3.

Table 3. Information on participants' knowledge about disease transmission and rabies transmission

	Number	Percentage
Knows that animals can transmit diseases to humans	62/161	38.5%
Does not know that animals can transmit diseases to humans	99/161	61.5%
Knows that animals can transmit rabies to humans	56/161	34.8%
Knows that dogs can transmit diseases to humans	75/161	46.6%
Does not know dogs can transmit diseases to humans	86/161	53.4%

39.1% of participants knew there are vaccines for dogs, while 60.2% did not. One person did not answer this question. 36.6% knew there are rabies vaccines for dogs. The parvovirus vaccine and canine distemper vaccine was also mentioned by some participants. See table 4.

Table 4. Participants' knowledge on canine vaccines and rabies vaccines

	Number	Percentage
Knows there are vaccines for dogs	63/161	39.1%
Does not know there are vaccines for dogs	97/161	60.2%
No answer to question above	1/161	0.6%
Knows there are rabies vaccines for dogs	59/161	36.6%
Does not know there are rabies vaccines for dogs	102/161	63.4%

50.9% of participants said they know what rabies is. Out of these people, everyone said they know how rabies is transmitted except for one person, who did however know that bites are a transmission route. Out of all respondents, 49.1% knew that bites are a transmission route and 11.8% knew that contact with dog saliva is a transmission route.

Only 6.8% stated that all mammals can be infected by rabies, and 44.1% did not know what species could be infected at all. 41.6% of the answers included humans or all mammals, indicating that this is the percentage of people who know humans can contract rabies. 49.7% of people included dogs in their answer. 10 people (6.2%) did not answer this question. See table 5.

Table 5. Participants' knowledge on rabies existence, transmission routes and host species

	Number	Percentage
Knows what rabies is	82/161	50.9%
Does not know that rabies is	79/161	49.1%
Knows bites are a transmission route	79/161	49.1%
Knows saliva contact is a transmission route	19/161	11.8%
Knows that all mammals can be infected by rabies	11/161	6.8%
Does not know what species can be infected by rabies	71/161	44.1%
Knows that humans or all mammals can be infected by rabies	67/161	41.6%
Knows that dogs can be infected by rabies	80/161	49.7%
No answer to question about host species	10/161	6.2%

41.0% of people could state at least one symptom of rabies in humans, where salivation, aggressiveness and staggering were the three most common answers (in that order). 53.4% did not know any symptoms in humans at all. 9 people (5.6%) did not answer this question. 50.3% knew at least one correct symptom of rabies in dogs, where salivation, aggressiveness and staggering were the three most common answers (in that order). 44.1% did not know any symptoms in dogs at all. 9 people (5.6%) did not answer this question. See table 6.

Table 6. Participants' knowledge on rabies symptoms

	Number	Percentage
Knows at least one symptom of rabies in humans	66/161	41.1%
Does not know symptoms of rabies in humans	86/161	53.4%
No answer to question above	9/161	5.6%
Knows at least one symptom of rabies in dogs	81/161	50.3%
Does not know symptoms of rabies in dogs	71/161	44.1%
No answer to question above	9/161	5.6%

46.6% of participants answered that rabies infected dogs always die. However, 3.7% answered that most dogs survive but some die and one person (0.6%) answered that some dogs die but most survive. 79 people (49.1%) did not answer this question. Regarding the seriousness of rabies in humans, 44.7% stated that infected humans always die. 4.3% of people stated that most infected humans survive but some dies, and one person (0.6%) stated that most will die, but some survive. 81 people (50.3%) did not answer this question. See table 7.

Table 7. Participants knowledge on rabies seriousness

	Number	Percentage
Think rabies infected dogs always die	75/161	46.6%
Think most rabies infected dogs survive but some die	6/161	3.7%
Think most rabies infected dogs die but some survive	1/161	0.6%
No answer to question above	79/161	49.1%
Think rabies infected humans always die	72/161	44.7%
Think most rabies infected humans survive but some die	7/161	4.3%
Think most rabies infected humans die but some survive	1/161	0.6%
No answer to question above	81/161	50.3%

When suspecting rabies in a dog, participants stated they would take measures such as kill the dog, go to the hospital, sell the dog, take the dog to a veterinary clinic, follow up on symptoms and capture/cage the dog. The three most common answers were kill, follow up on symptoms, and go to an animal clinic (in that order). 8 people (4.97%) said they would sell the dog. 20.5% of people did not know what they would do. 37 people (23.0%) did not answer the question.

If suspecting rabies in a person, 55.3% of people would go to the hospital and 21.7% did not know what to do. 37 people (23.0%) did not answer this question. 7 people (4.3%) knew about a person in the area that had had rabies, while the rest (95.7%) did not.

On the question "Do you know if there is a vaccine against rabies?" 24.8% answered "yes, for dogs", 4.3% answered "yes, for humans", 11.2% answered "yes, for both dogs and humans", 47.8% answered "don't know" and 11.8% answered "no". See table 8.

Table 8. Participants' answers to the question "Do you know if there is a vaccine against rabies?"

"Do you know if there is a vaccine against rabies?"	Number	Percentage
Yes, for dogs	40/161	24.8%
Yes, for humans	7/161	4.3%
Yes, for both dogs and humans	18/161	11.2%
Don't know	77/161	47.8%
No	17/161	11.8%

4.1.4 Opinions on rabies vaccination among dog owners

98.1% of participants were willing to pay to rabies vaccinate their dog(s), and were willing to pay sums ranging from 10,000 kip (0.57 USD) to 200,000 kip (11.49 USD). The most common answer was 20,000 kip (48.4%), followed by 50,000 kip

(14.9%), 30,000 kip (14.3%) and the range 20,000-50,000 kip (8.7%). Only 2 people (1.2%) were not willing to pay, out of which one said it was because he/she "does not want to waste money", and the other person said it's because he/she "does not have money to pay for that". See table 9.

Table 9. Participants' willingness to pay for canine rabies vaccination

	Number	Percentage
Willing to pay to rabies vaccinate their dog	158/161	98.1%
Not willing to pay to rabies vaccinate their dog	2/161	1.2%
No answer	1/161	0.6%

87.6% of participants would prefer to vaccinate their dog against rabies through an injection, compared to 12.4% who would prefer to give the vaccine in food. At the same time, 60.2% think giving vaccine in food is a very good idea. 33.5% are not sure about giving vaccine in food and 6.2% think it's not a good idea at all. See table 10.

Table 10. Participants' preference on rabies vaccine administration and opinion on oral vaccination

	Number	Percentage
Prefers injection of vaccine	141/161	87.6%
Prefers giving vaccine in food	20/161	12.4%
Thinks oral vaccination is a very good idea	97/161	60.2%
Thinks oral vaccination is not a good idea at all	10/161	6.2%
Not sure about oral vaccination	54/161	33.5%

4.2 Group discussion results

Many dog owners could see potential positive aspects with oral rabies vaccination. It was considered more applicable by some because it is easier and more comfortable to administer compared to injections and requires less people. Answers included for example "it is easier than injections", "it is very good and comfortable giving vaccination like this" and "good because we don't use a lot of people like [for] injections" This was especially considered an advantage when vaccinating aggressive dogs. Another perceived advantage was that dog owners can administer the vaccine themselves. Some participants stated that it is "good because we can feed them by ourselves" and "very comfortable because we can feed our dogs".

The animal welfare perspective was also frequently mentioned. Oral vaccination was perceived as kinder since it does not need to be forced upon the dog. For example, participants commented that "it would be good if there was a vaccination

in oral way because it's not to force the animals" and "it's good for the dogs because we don't force them like injections". It was considered particularly good for dogs that are afraid of needles, and the fact that oral vaccination does not hurt was also raised. For instance, one participant stated that "is it good because our dogs are not hurting" and another one said that it's "very good because some dogs are afraid of needles".

Some disadvantages were also raised by the dog owners, such as a fear that the campaign or vaccination will not work. One participant was "afraid that it doesn't work". Possible side effects were also a concern. The oral vaccination was said to be "good if there is no influence on our animals".

4.3 Village leader interview results

Some village leaders considered oral vaccination to be easier compared to injections since dog owners can administer the vaccine themselves, which the dog owners also experienced as a positive aspect. Village leaders stated that "it's easy to practice by own" and that it's "good for those who have dog because they can use vaccination by themselves". Another participant commented that it would be "not necessary to have veterinarian come to help because it's easy to practice by our own". Another statement was that "it would be very good if there was an oral vaccine and it's easier to feed dogs".

On the other hand, many concerns were also raised among the village leaders. There were several statements regarding concerns that some dogs would not eat the baits, for example "it would be very good if there was a campaign using oral vaccination but I'm afraid some dogs would not eat it". One concern was that the dogs would not eat the vaccine and other animals would eat it instead. One leader said that "a disadvantage is I'm afraid that the dog is not eating it and other animals will take it". Other leaders also raised concerns regarding health risks when performing the vaccination. Some said "it would be very good if there was a campaign using oral vaccination, but it's difficult and I'm afraid the dog bites me" and "it would be good if there was an oral vaccine but I'm afraid there's a risk when feeding the dog". This concern was not mentioned among dog owners. Some village leaders were also concerned that the campaign might not work: "It's good but I'm afraid it [the dog] doesn't eat it and it [the campaign] does not work". This potential problem was also mentioned among dog owners.

5. Discussion

This study included an assessment of the knowledge about rabies among dog owners. It also included a first assessment about the perceptions of oral vaccination, both among dog owners and village leaders. The latter has never been studied before and therefore the results cannot be compared to other similar studies. It can, however, offer valuable insights when planning future research or when developing future oral vaccination campaigns.

5.1 Knowledge of rabies among dog owners

Only half of dog owner participants (50.2%) knew what rabies is. However, this answer might be biased since some participants may have interpreted the question in different ways than others. Some people might have said no because they don't know what kind of infectious organism rabies is or because they don't know details about the disease, while others might have said yes simply because they know it's a disease. This number is low compared to some other studies. For example, a study from Bangladesh showed that 84.5% of participants were aware of rabies (Mujibur Rahaman *et al*. 2020) and in Zimbabwe, 92% had heard of the disease (Spargo *et al*. 2021). Another study from India showed that 76% had heard of rabies (Sivagurunathan *et al*. 2021), which is similar to a study from Ethiopia where 77.9% were aware of the disease (Bihon *et al*. 2020). If we instead had asked "have you heard about rabies", the results may have been higher.

49,1% of respondents knew that dog bites are a transmission route, and only 11.8% knew that contact with dog saliva is a transmission route. This is lower compared to studies in Bhutan (Penjor *et al*. 2019), Cambodia (Ung *et al*. 2021) and Pakistan (Khan *et al*. 2019), where 99%, 98.7% and 62.9%, respectively, knew that rabies can be transmitted from dog bites. In a Nigerian study, 85.4% knew that dog bites are a transmission route and 10% knew that dog saliva in wounds can cause infection, the latter being similar to the results in this study (Al-Mustapha *et al*. 2021).

44.1% of dog owners did not know what species can be infected with rabies. This is significantly higher than studies from Zimbabwe, where the corresponding number is 8.4% (Spargo *et al*. 2021). In China, 5.91% did not know what animals can spread rabies (Li 2021). 53.4% did not recognize any symptoms of rabies in humans and 44.1% did not recognize it in dogs. The latter number is lower than in studies from Bangladesh (52%) (Alam *et al*. 2020) but higher than in Zimbabwe

(34.34%) (Spargo *et al*. 2021). Not knowing that humans or dogs can be infected and not recognizing the symptoms leaves people completely unaware of the risk of disease and what to look for to recognize the disease. This might lead to people not protecting themselves from potentially infected dogs and not seeking medical care in possible human rabies cases.

Regarding the seriousness of rabies, 46.6% stated that rabid dogs always die. However, almost half of participants (49.1%) did not answer this question. The reason to this is unclear. There were a few people (3.7%) who stated that most dogs survive but some die. This could potentially lead to people keeping rabid dogs hoping they survive instead of euthanizing them, especially if the dogs have emotional value or other value to the owner. This could both become an animal welfare issue as well as a safety issue regarding disease transmission. The same pattern was seen in the question regarding seriousness of rabies in humans, where half of participants (50.3%) did not answer this question and 44.7% said that infected humans always die. There were also some people (4.3%) who said that most infected humans survive. There are great varieties regarding knowledge of rabies seriousness in other studies. A study from China have shown that 40% of people does not know that rabies is virtually 100% fatal once clinical signs appear (Li 2021). In a study from Bangladesh, 8% of rabies bite victims think that rabies can be cured after symptoms occur (Alam *et al*. 2020), which is similar to an Indonesian study where 6.9% did not know rabies can cause death (Rehman *et al*. 2021). An Indian study showed that 13.6% did not know rabies was a fatal disease (Sivagurunathan *et al*. 2021). Not understanding the seriousness of rabies could lead to not prioritizing getting vaccinated and not taking adequate measures if exposed. According to WHO, awareness of rabies is important to engage communities in rabies prevention, and gives people the possibility of saving themselves in case of exposure (WHO n.d.-c). A study by Barbosa Costa *et al*. (2018) showed that people with increased wealth and knowledge about rabies are significantly more likely to seek medical care after potential exposure of rabies.

20.5% of participants did not know what to do when suspecting rabies in a dog. Among the people who did know, the most common answers were to kill the dog, follow up on symptoms or take the dog to an animal clinic, which are all appropriate actions. According to a study in India, most people would kill the animal, which is similar to the current study, followed by chasing away the animal and capturing the animal to send it to a laboratory (Herbert *et al*. 2012). In the current study, 4.97% stated that they would sell the dog. After asking the participants an open question about this, it became clear that they would sell the dogs to dog butchers or restaurants serving dog meat. This could lead to a rabies exposure risk for the buyer, as this person might not be aware that the dog has shown symptoms of disease and

will handle potential infectious material when butchering the animal. Also, there could potentially be a risk of transmission when consuming raw dog meat or other parts of the body. A research team in Ghana found 2.1% rabies virus RNA detection rate in apparently healthy dogs butchered for human consumption (Tasiame *et al*. 2022). This study also observed that raw meat and dog heads, which could possibly contain infectious material in the brain and salivary glands, were sold at markets. Human rabies cases resulting from consumption of rabid animals are extremely rare, but the risk from butchering and preparing the animal is likely higher (Wallace & Blanton 2020).

Similarly to when suspecting rabies in a dog, 21.7% did not know what to do when suspecting rabies in a human. 55% would go to the hospital. The 21.7% who did not know might not have easy access to a hospital or could maybe not afford medical care. However, this might also be due to people not being aware of rabies and its seriousness. 23% of people did not answer this question, and the reason to this is unclear. To the author's knowledge, no earlier studies assessing this has been done in the region.

Only 4.3% of participants knew about a person in the area that had had rabies. This can be compared to a study from Cambodia where 18.3% had heard of or seen a person with rabies (Ung *et al*. 2021). The relatively low numbers in the current study could be interpreted as a relatively low incidence of the disease in the studied areas. This is supported by reports from 2012-2017 showing 33 human cases of rabies in Lao PDR (WHO 2018). A low incidence might explain why some people do not know about the disease. It could also be the other way around, that the incidence is higher than the answers in this study suggest, but that people are simply not aware that people had rabies in particular, because there is a lack of knowledge.

91.4% of participating dogs were used only as guard dogs and 8% as company. No participant stated that the dog was living with the family. However, this might be a question of interpretation since dogs used for company typically involve close contact with the owner and/or the family of the owner. Guarding dogs might have less contact with the family since their purpose is not primarily to be social with people and might therefore pose a smaller risk of transmitting diseases to humans. However, a majority of dogs (69.8%) were outside loose all the time or partly, which enables frequent encounters with other dogs, and therefore a risk of contracting and transmitting diseases such as rabies. Almost all dogs were mainly taken care of by children in the family (99.3%). This could explain why 40% of dog bites by suspected rabid dogs are affecting children, which results in high numbers of rabies cases in the young population (WHO 2021b). Children are less likely to be aware of rabies and its transmission routes and might not tell an adult if they

become exposed to animal saliva or bites. In a study from Bhutan regarding rabies knowledge in school children, 15.8% of the students had experienced dog bites during the last 2 years, where 52.3% were caused by pet dogs (Penjor *et al*. 2019). 11.7% of these children did not visit a hospital after the bite and 3.2% did not complete the vaccine schedule.

When asked if there is a vaccine against rabies, 11.8% of respondents answered "no" and 47.8% answered "don't know". This can be compared to a Cambodian study where 6.4% did not think there was a rabies vaccine for humans and 31.1% did not know/did not reply (Sor *et al*. 2018). In the same study, 28.8% said that dogs cannot be vaccinated against rabies and 47.5% did not know/did not reply. These numbers represent gap in knowledge of rabies vaccines among dog owners.

Very few dogs (4.6%) had been vaccinated against some disease sometime in their life. However, this might be because non rabies vaccinated dogs mainly were chosen for the study because it was done simultaneously with another project where this was preferred. Some rabies vaccinated dogs were however still included in the study. For comparison, 21% of dogs in Nasarawa state in Nigeria was vaccinated (Kwaghe *et al*. 2019). The low vaccination rate could suggest a general reluctance to vaccinate dogs, which could be because of lack of knowledge, economic reasons, religious or cultural beliefs etc. These reasons should be identified and considered when planning future rabies vaccination campaigns to ensure a high vaccination coverage.

38.5% of participating dog owners claimed they know that animals can transmit diseases to humans, while 61.5% did not, which is quite concerning. This can be compared to a study from Zimbabwe where 77% of respondents were aware of pet zoonoses (Pfukenyi *et al*. 2010). However, there might have been some issues regarding the interpretation of the question in the current study. 3% of the people who claimed they did not know animals can transmit diseases to humans still said they knew rabies can be transmitted from animals to humans. This might also be due to participants achieving this information (i.e. that people can contract rabies from animals) after they've already answered the question whether animals can transmit diseases to humans. Also, 53.4% of dog owners did not know dogs, specifically, can transmit diseases to humans, which is also problematic since dogs live quite closely to people in these communities. Without people having the knowledge of disease transmission, it is of course difficult to prevent it.

Raising rabies awareness is a crucial part of the journey to eradicate rabies, according to researchers (Balaram *et al*. 2016). A study from India show that mass media could play a part in spreading information about rabies to the public (Herbert

et al. 2012) and another study from Rwanda shows that television and radio are two sources where people receive most of their information on rabies (Ntampaka *et al*. 2019). In conclusion, there needs to be information campaigns in the studied villages to raise awareness regarding zoonotic diseases, particularly rabies. These campaigns should include information about what rabies is, the possibility of humans and animals being infected by the disease, the possibility of animals (including dogs and other domestic animals) transmitting rabies to humans, how rabies is transmitted and the seriousness of rabies in both humans and animals. There should also be information about how to prevent rabies exposure, what measures to take when exposed to for example dog saliva or dog bites, and what to do when suspecting rabies in animals and humans. Information about rabies vaccination for both humans and animals, especially dogs since dogs cause a clear majority of rabies cases in humans, should also be included.

5.2 Opinions on oral rabies vaccination

A clear majority of participating dog owners (87.6%) would prefer to give their dog an injection instead of giving vaccine in food (12.4%) when rabies vaccinating their dog. However, 60.2% thought that giving vaccine in food was a good idea, while only 6.2% thought it's not a good idea at all. The rest was unsure. The opinions on oral vaccination have not been studied before and therefore cannot be compared to other studies.

Almost all participants were willing to pay to rabies vaccinate their dogs, and the sums mostly ranged from 20,000-50,000 kip (1.2-2.9 USD). This is similar to Cambodia, where 84% of respondents were willing to pay 2 USD to rabies vaccinate their dog (Ung *et al*. 2021). In a study from Ethiopia, 68.2% of people thought that rabies vaccine is important and 69.8% were willing to rabies vaccinate their pets (Bihon *et al*. 2020) In another study from Bangladesh, 73.37% had a positive attitude towards rabies vaccine (Mujibur Rahaman *et al*. 2020). This shows a general positivity for rabies vaccination campaigns. Many people in the studied areas have economic difficulties and were still willing to pay. This facilitates the implementation of future vaccination campaigns since the need for financial support decreases.

Dog owners could see many benefits with oral vaccination. For example, it was considered to be easier and to require less people than parenteral vaccination. This is in agreement with studies showing ORV to require less labor and education for the vaccinators compared to CVR (Gibson *et al*. 2019; Wallace *et al*. 2019). Some dog owners appreciated the fact that they can administer the vaccine themselves, without the need of a veterinarian. This was seen as a positive aspect among village

leaders as well. Another advantage mentioned among dog owners was the ethical aspect that oral vaccination does not need to be forced upon the dog and does not hurt the dog.

Disadvantages that were mentioned among dog owners was the risk of the vaccine or campaign not being effective, and possible side effects. However, there are several new studies showing the efficacy of the vaccine (Zhugunissov *et al*. 2017; Smith *et al*. 2019; Leelahapongsathon *et al*. 2020; Molini *et al*. 2021) and no abnormal postvaccination symptoms (Linhart *et al*. 1997). A few cases of vaccine-induced rabies have been reported in the past (Esh *et al*. 1982; Fehlner-Gardiner *et al*. 2008; Hostnik *et al*. 2014; Robardet *et al*. 2016; Vuta *et al*. 2016), but there are also vaccine strains such as the SAG2 strain where no such cases have been reported, even after distributing 20 million baits (Mähl *et al*. 2014). This highlights the importance of choosing well studied and safe vaccine strains when implementing campaigns.

Village leaders were concerned that the dogs would not eat the baits or that other animals would eat them instead, and that the campaigns would therefore not work. Studies have shown that bait preferences among dogs vary between countries (Bergman *et al*. 2008; Kasemsuwan *et al*. 2018; Bonwitt *et al*. 2020) and it is therefore important to study the bait preferences in the target area so that the bait with the highest acceptability can be chosen. The WHO recommends that bait acceptability is tested on both dogs living in households and on free roaming dogs in the area where the campaign is to be carried out (WHO 2007). Bait acceptability rates have been shown to vary from about 30-90%, commonly being as high as 70-90% (Linhart *et al*. 1997; Bender *et al*. 2017; Freuling *et al*. 2022).

Another concern among village leaders was the risk of dogs biting the person performing the ORV. A research team in Haiti studied the bite incidence among vaccinators participating in a parenteral mass rabies vaccination campaign for dogs (Kirkhope *et al*. 2021). In this study, the bite incidence during vaccinations was only 0.03%. The study revealed a worry among vaccinators to be bitten during the campaign and to contract rabies. Bite accidents while performing ORV can be avoided by keeping a distance to the dog through using the handout model, where the bait can be thrown to the dog, or using the wildlife model, where baits are distributed strategically in areas that dogs often visit (WHO 2007). An injection requires direct contact with the animal and is an invasive procedure that can cause discomfort for the dog. This is not the case with ORV, which should decrease the risk of bite accidents.

The concerns mentioned above could be the reason why most dog owner participants would prefer an injection despite the positive aspects of oral vaccination. Injecting a vaccine might be seen as more reliable since it's known to many dog owners and has been used for a long time. Implementing ORV campaigns is easier if dog owners and village leaders have a positive attitude towards them. Educating people about ORV can help them form a well-informed opinion and feel more secure in their knowledge.

6. Conclusion

In this study, we have found significant knowledge gaps regarding rabies among dog owners in certain areas of Lao PDR. To be able to reach the Zero by 30 goal, these gaps need to be filled, which can possibly be done through information campaigns raising rabies awareness and teaching people about transmission, seriousness, preventative measures including vaccines etc.

We have also found that while majority of dog owners (87.6%) would prefer to give their dog rabies vaccine as an injection, most also think oral vaccine is a good idea. Dog owners and village leaders could see many positive aspects of oral vaccination, but also had some concerns. Almost all dog owners (98.1%) were willing to pay to rabies vaccinate their dog. In conclusion, there is a general positive attitude towards rabies vaccination and majority of people seem open to the idea of oral rabies vaccination. Further research is needed on this topic to draw further conclusions.

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Popular science summary

Rabies is a fatal viral disease causing around 59,000 deaths each year worldwide. Out of all deaths, 95% occur in Africa and Asia. In Lao People's Democratic Republic (Lao PDR), rabies persists among dogs. All mammals can be infected by the disease, but up to 99% of human rabies cases are transmitted by dogs. The virus is mainly transmitted by saliva contact with wounds or mucosa and the incubation time is generally 20-60 days, but can be several years. Symptoms include behavioral changes, decreased food intake, vomiting, excessive salivation, staggering, paralysis and seizures and ultimately results in death. Rabies is 100% preventable by vaccination.

WHO (World Health Organisation), OIE (World Organization for Animal Health) and FAO (Food and Agriculture Organization) have together formed the UAR (United Against Rabies) platform. The goal is to have zero dog-mediated human rabies deaths by 2030. WHO finds rabies vaccination of dogs to be an important measure to reach this goal, however it's considered difficult to reach adequate vaccination coverage through giving injections only. Therefore, there has been a promotion of rabies vaccination through the mouth, also known as oral rabies vaccination, where the vaccine is hidden in baits for animals to eat. This method has helped eliminate rabies in wildlife in Europe. Oral rabies vaccines have been shown in studies to be safe, effective and stable in the environment, however, there has been a few incidents of the vaccine causing rabies infections in animals and skin infections in humans. Oral vaccine baits can be distributed in different ways, including handing out the bait to dogs and distributing them in the environment. Oral rabies vaccination has been shown to be more cost effective and less time consuming than vaccinating through injections.

The goal of this study was to investigate knowledge and attitudes towards oral rabies vaccination campaigns among dog owners in Lao PDR. The study was carried out in three different provinces in Lao PDR during 2022. Participants included dog owners in these districts, who answered questionnaires and some also participated in group discussions, as well as village leaders who were interviewed.

Dog owner knowledge was low regarding which animals can contract rabies, its transmission, vaccines, seriousness and symptoms. For example, only half of participants knew what rabies is and less than half knew what species can be infected by the disease. 61,5% of participants did not know that animals can transmit diseases to humans at all. Less than half knew that there is a vaccine against rabies. 4,3% thought that most rabies infected humans survive, while it is almost 100% fatal. This can lead to people not protecting themselves from potential exposures and not taking adequate measures if exposed to the disease.

Most dog owners (87,6%) would prefer to rabies vaccinate their dogs through an injection, while 12,4% would prefer the oral vaccine. However, 60,2% thinks oral vaccination is a very good idea. Dog owners and village leaders considered oral vaccination to be easier, safer when vaccinating aggressive dogs, and kinder towards the dog. It was considered an advantage that dog owners could perform the vaccination themselves. Concerns were the risk of the vaccine not being effective, being bitten while providing vaccine, potential side effects and dogs not eating the baits. Almost all (98,1%) dog owners claimed to be willing to pay to rabies vaccinate their dog, and most people were willing to pay around 20,000 kip (1,15 USD).

To reach the Zero by 30 goal, there need to be information campaigns on rabies to fill the knowledge gaps. This study shows an openness among dog owners to oral rabies vaccination, however further research is needed on this topic.

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Appendix 1

Consent Form - A field study on Rabies in dogs

Good morning/afternoon!

We are (name of field staffs in each country) and we study Veterinary Medicine in Sweden.

We are visiting you today to learn more about the knowledge of Rabies and the protection against Rabies among dogs. To do this we would kindly ask you to take part of an interview where you would answer some questions about Rabies. We would also like to take a blood sample from your dog if you are comfortable with that. Knowing this could help us design future projects. The blood sample from the dog will be used to analyze for antibodies against rabies, but we may also analyze the blood for other diseases later. If the dog is positive we may contact you and come back for a second sample, if you would be willing to do that.

Please be kindly informed that the participation in this study is entirely your choice. The discussion will not take much of your time and all you need to do is answer some of the questions mentioned in our questionnaire. You are free to decide if you want to share information with us today or not. Participating in this discussion will not cause you to lose or gain anything. We will keep all the research records private as required by law.

Before we begin, we would like to tell you a little more about how the session will go and ask for your consent to participate in the discussion.

We would kindly ask you to participate in an interview where you will answer questions from our questionnaire. After the interview we would kindly ask to take a blood sample from your dog. To do this we will use a muzzle on the dog for everyone’s safety. If your dog gets too uncomfortable during the process or if you at any point do not want to participate anymore just let us know and we will not continue.

The research team promise to respect your privacy and confidentiality. We will not tell anyone that you participated in this study and your identity will not be linked back to what you said or what the blood test showed. The information we talk about during the interview will be shared with the research team, however your name and other facts that might point to you specifically will not appear when discussed with others. Your participation in the discussion is completely voluntary, so if at any time you no longer want to participate, you are free to excuse yourself. Summarized information about this study will be published later, but it will not contain any information that could identify you.

You will not be paid for participating but to show our appreciation for your participation your dog will be given treatment against parasites.
Do you have any questions? If you have any further questions or have any concerns about the study, please feel free to contact the following people.

Study responsible:
Johanna Lindahl, DVM, PhD, Docent Department of Clinical Sciences, SLU Johanna.lindahl@slu.se+46730308822
Dr Vannaphone Phouthana, National University of Laos, Faculty of Agriculture, Nabong Campus. v.phouthana@nuol.edu.la (national partner contacts)

If you have any concerns regarding the way you are treated in this study, please contact (national partner contact)

At this point, we like to ask you if you are:

	Yes (Please sign or make finger print)	No
Willing to participate in the interview		
Willing to let us take a blood sample of your dog		

Appendix 2

Questionnaire – rabies for dog owners

Questionnaire code:
Name of participant:
Phone number of participant:

1. Location:
1.1 Which district and province do you live in:

2. Information about the dog owner:

2.1 Gender	Female Male
2.2 Age (years)	0-15 16-25 26-35 36 and above
2.3 Education level	No education Primary Class 5-10 Higher secondary Graduation and above
2.4 Are you the owner of the dog?	Yes No If no, whose dog is it?
2.5 Do you ever consume dog meat	Yes No
2.5a If yes, how often?	Every day Once a week Once a month Less often
2.5b If yes, where do you buy the meat?	
2.5c If no, why not?	
2.6 Are there live animals sold in the market in your village?	Yes No

2.6a If yes, which animals?	
2.7 Are there wild animals sold in the market in your village?	Yes No
2.7a If yes, which animals?	
2.8 Do you buy animals sold live at markets?	
2.8a If yes, which animals?	
2.9 Why do you buy live animals?	
2.10 Do you get the animals killed at market, or they are killed at home?	At market At home Other

3. General questions about zoonoses

3.1 Do you know if animals can transmit diseases to humans?	Yes No
3.1a If yes, which diseases do you know of that dogs could transmit to humans? (list all)	
3.2 Are you concerned about any health risks particularly in markets selling animals?	
3.2 Do you know if dogs can transmit diseases to humans?	Yes No
3.2a If yes, which diseases do you know of that dogs could transmit to humans?	
3.3 Do you know there are vaccines for dogs?	Yes No
3.3a If yes, which diseases do you know it is possible to vaccinate against?	

4. Rabies

4.1 Do you know what Rabies is?	Yes No
4.2 Do you know how rabies is transmitted?	Yes No
4.3 If yes, how is Rabies transmitted? Multiple options allowed:	Mosquitoes Faeces

	Bites Blood contact Contact with dog saliva Food Other, explain:
4.4 Who can get Rabies? **(More than one option can be selected)**	Humans Dogs Cats Cattle Birds All mammals Other, explain: Don't know
4.5 Symptoms of rabies in humans **(More than one option can be selected)**	Fever Vomiting / Diarrhoea Aggressiveness Salivation Abortion Staggering Difficulty breathing Weightloss Fatigue Skin lesions Don't know Other, explain:
4.5 Symptoms of rabies in dogs **(More than one option can be selected)**	Fever Vomiting / Diarrhoea Aggressiveness Salivation Abortion Staggering Difficulty breathing Weightloss Fatigue Skin lesions Don't know Other, explain:
4.6 How serious do you think rabies is for dogs?	Most survive, but some dies Most will die, but some survives They always die
4.7 How serious do you think rabies is for humans?	Most survive, but some dies Most will die, but some survives They always die
4.8 What do you do if you suspect rabies in a dog?	
4.9 What do you do if you suspect rabies in a human?	

4.10 Has any dogs in the area had rabies (that you know of)?	Yes No
4.11 Has any person in the area had rabies (that you know of)?	Yes No
4.12 Do you know if there is a vaccine against Rabies?	No Don't know Yes, for dogs Yes, for humans Yes, for both dogs and humans
4.13 Would you want to vaccinate your dog for rabies?	**No** **If, no why not?** **Yes** **If yes, would you be willing to pay for the vaccine?** **How much:**
4.14 If you were going to vaccinate you dog against rabies, what would you prefer	**Injection** **Give vaccine in food**
4.15 What would you think if vaccines were given to dogs in the whole village by given them vaccines through pieces of food?	**Not a good idea at all** **Not sure** **Very good idea**

5. Dog information one sheet per dog:

Sample code (questionnaire code plus number of the dog):

5.1 Age of the dog	
5.2 Breed	
5.5 The dogs' main use	Guard Company Meat Other, explain:
5.4 Main caregiver	Adult in family Child in family Other, explain:
5.5 Living situation	Only outside loose Only outside in a leash Only outside loose but in a fenced area Only indoor Both indoor and outside, when outside in a leash Both indoor and outside, when outside loose

	Lives with family
5.6 How did you come to own the dog?	Bought A gift A puppy from previous dog Other, explain:
5.7 Have your dog ever visited a veterinarian or veterinary technician?	No Yes If yes, what for:
5.8 Did your dog have any bite wounds the last six month?	Yes No
5.9 Has your dog ever shown signs of aggression?	Yes No If yes, towards humans or other animals or both?

6. Health status of the dog:

6.1 Has the dog ever gotten vaccinated?	Yes No (continue to 6.4) Don't know
6.2 How often does the dog get vaccination?	One time 1 time / year 1 time / 3 years Other: Don't know
6.3 Against which illnesses?	
6.4 History of illness of the dog (More than one option can be selected)	Bite wounds Vomiting/diarrhoea Parasites Lameness Other, explain:

Appendix 3

Poster explaining oral vaccination
The poster contained the following text:

"Oral rabies vaccination

Vaccine sachet: plastic bag, contains vaccine liquid

Vaccine is put inside bait

Bait: Fishmeal, eggs, meat etc. – tasty for the dog!

Rabies vaccine gives antibodies – minimizes risk of dog being infected and spreading rabies

Dog is vaccinated when it chews on the vaccine sachet and liquid comes in contact with inside of mouth. No injection needed.

Vaccine bait can be offered from a distance

NOTE: Rabies is a deadly disease that can be spread from dog bites to humans. If a dog bites you – wash wound with soap and water and seek medical help immediately."

Appendix 4

Interview questions for village leaders (key informant interviews):

Village name:

1. Do you know if there has ever been any vaccination campaigns for rabies in your villages? In that case, how did it happen, and when was it?
2. What do you think about rabies vaccine campaigns? Do you think they work?
3. How many dogs approximately are in your village? Is it common with people eating dogs?
4. Are there many dogs that no one owns?
5. Is there a problem with aggressive dogs or wild dogs?
6. What would be done with a dog if it aggressive and has bitten people?
7. If there was a campaign using oral vaccination, what would you think about that? What pros and cons do you see? Do you have any concerns?
8. Are there any markets selling live animals in your village?
9. Which animals are sold at these markets? Are there any wild animals sold?
10. Are you concerned about any disease risks at these markets?

Appendix 5

Group discussion questions for dog owners

These questions are for a group of 4-8 dog owners.
Show the slides about giving oral vaccines.
The discussion should be written down or recorded.

Village name:
Number of participants: Male: Female:

Questions to discuss:

1. What do you think about giving vaccines this way?
2. Would you feel ok if it was given to your dog? If not why?
3. Is there anything you would be worried about?
4. If there was a campaign in your village using oral vaccination, what would you think about that? Do you have any concerns?
5. Would you think it was ok if baits were left on the ground for wild dogs to eat? Would you worry about that?
6. What is important for you to want to vaccinate your dog?
7. Would people in your village be willing to pay for vaccination of the dogs?
8. How many dogs approximately are in your village?
9. Do any dogs ever come from other countries to your village?
10. Is it common with people eating dogs?
11. Are there many dogs that no one owns?
12. Is there a problem with aggressive dogs or wild dogs?
13. What would be done with a dog if it aggressive and has bitten people?

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