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Faculty of Veterinary Medicine and Animal Science

Canine rabies in Vientiane Prefecture, Lao PDR

Vaccination status and protection level

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SUMMARY

Rabies is a fatal viral disease that is primarily transmitted to humans by domesticated dogs. Canine rabies is estimated to be accountable for the death of 59,000 humans each year in the world and if post-exposure treatment is not initiated, death usually occurs within a month after first signs of symptoms. Rabies is however preventable through engaging the society and educating regarding rabies and the importance of post-exposure treatment. Another important measure is rabies vaccination of dogs. Research show that if 70% of the canine population is vaccinated against the rabies virus, it is enough to prevent human cases of dog-mediated rabies, which is why many endemic countries conduct mass dog vaccination campaigns.

The continents with most human cases of canine rabies are Africa and Asia. In this study, the vaccination status of dogs in Vientiane Prefecture in Lao PDR was investigated. Laos is a country located in Southeast Asia, with boarders to Cambodia, Thailand, Myanmar, China, and Vietnam. Every year, there are two to four human deaths due to rabies in Laos. To investigate the vaccination status, a survey was conducted in Vientiane Prefecture where dog owners were asked questions regarding the dog, the dog's living situation and vaccination status. Serum was then collected from the dogs and analysed for rabies virus neutralizing antibodies using the enzyme-linked immunosorbent assay (ELISA) test. The test distinguishes the samples that have detectable rabies virus neutralizing antibodies, which then are considered positive for antibodies. It also detects the samples that has an antibody level greater than 0.5 IU/ml. These dogs are considered as protected against rabies.

Our results showed that 28.38% of dogs included in the study had detectable antibodies against rabies, and 13.19% of the sampled dogs were protected against rabies. When asking the owners about any previous vaccinations, the answers were deviant from the results. The study also showed that more dogs in the districts closer to Vientiane capital city were vaccinated compared to dogs living in districts further away. The results in this study highlights the deficiency regarding rabies vaccination among dogs in Vientiane Prefecture and further mass dog rabies vaccination campaigns need to be conducted to make Lao PDR free of dog-mediated human deaths of rabies.

CONTENT

ABREVIATIONS

CDC	Centers for Disease Control and Prevention
CIA	Central Intelligence Agency
ELISA	Enzyme-linked Immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
FAVN	Fluorescent antibody neutralisation test
GARC	Global Alliance for Rabies Control
ID	Intradermal
IM	Intramuscular
IU	International unit
MNT	Mouse neutralization test
NAb	Neutralizing Antibodies
NAHL	National Animal Health Laboratory
OIE	World Organization for Animal Health
PCR	Polymerase Chain Reaction
PDR	People’s democratic republic
PEP	Post-Exposure Prophylaxis
PrEP	Pre-Exposure Prophylaxis
RABV	Rabies virus
RAPINA	Rapid neutralizing antibody detection test
RFFIT	Rapid fluorescent focus inhibition test
RIG	Rabies Immunoglobulin
SC	Subcutaneous
TRC	Thai Red Cross
UTRC	Updated Thai Red Cross
WHO	World Health Organization

INTRODUCTION

Rabies is a viral disease that can infect all mammals (Centers for disease control and prevention (CDC), 2019a) and once symptoms appear it is nearly always fatal (Wilde, 2007; World Health Organization (WHO), 2019a). Rabies is also a disease that is 100% preventable through vaccination (Taylor & Nel, 2015). Despite that, it is still endemic in many regions in the world, where most cases of human rabies are seen in Africa and Asia (Knobel *et al*., 2005; Hampson *et al.*, 2015).

Even though wildlife works as a reservoir for the virus, the majority of human rabies cases are caused by transmission from domesticated dogs (Weyer *et al*., 2011) where canine rabies is estimated to be accountable for the death of minimum of 59,000 humans in the world each year (Hampson *et al.*, 2015). A great part of these being children (Dodet *et al.*, 2008; Suraweera *et al.*, 2012), likely due to the fact that children enjoy interacting with dogs but lack the ability to read the dog's emotions and signals, therefore being at greater risk of getting bitten (Mbilo *et al.*, 2019).

Lao PDR, a country with estimated 7.2 million inhabitants, located in Southeast Asia with a land area of approximately 231,000 square kilometres which borders to Thailand, Myanmar, China, Vietnam and Cambodia (Central Intelligence Agency (CIA), 2019). Laos is endemic of rabies (Douangngeun *et al.*, 2017) and between the years of 2012-2017, there have been 33 reported cases of human rabies deaths in the country (WHO, 2019b). Like worldwide, dogs are the main transmitter of rabies virus (RABV) to humans in Lao PDR (Ahmed *et al*., 2015).

Some areas in the world, such as greater part of Europe, the United States of America, Japan, Australia, New Zeeland and many islands in Asia, Oceania and Caribbean are considered free of dog-mediated rabies (Public health England, 2019). A country is considered free of canine rabies when there have not been any confirmed cases of dog mediated rabies in humans or animals over the last two years (WHO, 2018a). For a country to be able to become free of canine rabies it is recommended that 70% of the dog population, both owned and stray, has an adequate immunization against rabies virus (Coleman & Dye, 1996; Cleaveland *et al.*, 2003). The World Health Organization recommends that the immune status among the canine population is evaluated regularly to be able to foresee the need of further vaccination campaigns and thereby uphold the immune status against rabies in dogs (WHO, 2018a).

In this report, the vaccination status and protection level against rabies among dogs in Vientiane Prefecture, Lao PDR, was investigated using the enzyme-linked immunosorbent assay (ELISA). The owners were also interviewed regarding the dogs' history and vaccination status.

LITTERATURE REVIEW

Rabies

Etiologi and epidemiology

Rabies virus

The rabies virus (RABV) is a single-stranded, non-segmented negative RNA (ribonucleic acid) virus of the *Lyssavirus* genus, family *Rhabdoviridae*. All viruses within the Rhabdoviridae family are distinctly bullet shaped and consists of two components: a helical ribonucleoprotein (RNP) with a surrounding envelope. The RABV genome encodes five proteins. The nucleoprotein (N), phosphoprotein (P) and polymerase (L), together with the negative RNA, forms the RNP complex, and the envelope consist of a lipid bilayer comprising the matrix protein (M) and glycoprotein (G) (Mebatsion, Weiland & Conzelmann, 1999).

Even though studies have shown that all lyssaviruses, including RABV, originates in bats (Badrane & Tordo, 2001), isolates of the classical rabies virus has been found in bats in the New World only (Rupprecht, Kuzmin & Meslin, 2017).

Susceptible species

All mammals are susceptible to rabies and, when infected, all mammals can transmit the disease. The primary reservoirs of the RABV are domestic dogs (Weyer *et al.*, 2011). Other species of the order Carnivora and species of bats, order Chiroptera, are also considered primary reservoirs, whereas in Southeast Asia the most common carnivorous wildlife reservoir is the red fox, *Vulpes vulpes* (WHO, 2018a). Foxes have also been proven to play a great role for the epidemiology of rabies in European countries (Freuling *et al.*, 2013; Polupan *et al.*, 2019) as well as in Russia (Shulpin *et al.*, 2018).

Even though all mammals are susceptible to rabies, dogs are the main transmitter of the virus to humans (Ahmed *et al*., 2015), being accountable for 99% of all human cases of rabies (WHO, 2019c) and resulting in at least 59,000 human deaths in rabies in the world each year (Hampson *et al*., 2015).

Transmission

Rabies virus is excreted in the saliva in rabid animals and humans and infection is generally through direct contact with saliva (Rupprecht, Kuzmin & Meslin, 2017). Research show that a patient with rabies can disseminate RABV in the saliva before onset of symptoms (Fekadu, Shaddock & Baer, 1982). RABV cannot penetrate intact skin, but it can be transmitted through a wound, most often caused by a bite from a rabid dog, or direct contact with saliva on skin lesions or a mucus membrane, such as in the mouth, eyes or nose (CDC, 2019b). Bites to the face, neck or hands are the most critical since these are highly innervated areas (Baltazard & Ghodssi, 1954; Wilde, 2007).

Less common transmission paths include contact with cerebrospinal fluid and nervous tissue (Wertheim *et al.*, 2009) which serves a greater risk in some professions, for example butchers, meat inspectors or veterinarians doing autopsy of suspected or confirmed animal cases.

Inhalation of aerosolized RABV is also a possible transmission path (Davis, Rudd & Bowen, 2007), but it is almost solely a risk for laboratory personal.

Rabies virus is not contagious once it becomes dry or is exposed to sunlight and petting or touching an animal or human with rabies does not serve a risk for infection. Neither does contact with non-infectious fluids, such as blood, urine or faeces (CDC, 2019b).

Human-to-human transmission of RABV is unusual, but there are reported cases where patients have gotten rabies from solid organ transplants (Zhang *et al.*, 2018) and one reported case where a man got rabies after sucking his son's bite wound caused by a rabid dog (Zhao *et al.*, 2019).

Pathogenesis

After transdermal entry, the virus can directly infect nerve cells or replicate in striated muscle cells and transfer to the central nervous system through retrograde axoplasmatic transport. Once in the central nervous system, the rabies virus starts to replicate rapidly, damaging nerve cells. After replication the virus spreads through peripheral nerves using anterograde axoplasmic transport, causing infection throughout the body and non-nervous tissues, such as the secretory tissues of the salivatory glands. The infectious cycle of rabies is considered completed when the virus is secreted in the saliva (WHO, n.d.; CDC, 2017).

Symptoms of rabies

There are two general clinical forms of rabies in humans and animals: furious and paralytic (Laothamatas *et al.*, 2008). The time between infection with RABV and the occurrence of clinical signs depends on the type of virus, if the individual has any immunity, the site of exposure and how far it is from the brain (CDC, 2019c), where exposure to the head and neck have a shorter incubation period than exposure to limbs (Qi *et al.*, 2018). Symptoms appears first when the virus is widely disseminated throughout the body, however first signs of rabies are usually seen within one month after exposure (Wilde, 2007) and death normally occurs within one month after first signs of symptoms (Wallace *et al.*, 2014; Dhayhi *et al.*, 2019).

Furious rabies

In furious rabies in humans, prodromal symptoms most often include those of general influenza with fever and pain or paresthesia at or around the site of exposure (Muyila *et al.*, 2014). Soon after, the symptoms become more acute and patients can present with abnormal vocalisation, hypersalivation, anxiety, agitation or restlessness and hallucinations and the patient can experience hydrophobia and even aerophobia (Wertheim *et al.*, 2009; Weyer *et al.*, 2011; Suraweera *et al.*, 2012). Death occurs due to cardio-respiratory arrest (WHO, 2019c).

Paralytic rabies

Paralytic rabies is not as usual as furious rabies and is seen in 20% of all reported human cases of rabies. However, this form is most likely underestimated since it is more often misdiagnosed because symptoms are not as acute or aggressive as the furious form, thus contributing to the under-reporting of rabies. Symptoms that can be seen are progressive muscle paralysis which often start at the site of exposure, slowly evolving into a coma and death (WHO, 2019c).

Rabies in animals

The symptoms of rabies in animals can be of great variety, which makes diagnostics upon clinical signs complicated. Symptoms that can be seen are similar to those in humans, with early onset of fever, lethargy, anorexia and vomiting. These often progress within days to signs of ataxia, hypersalivation, abnormal behaviour with unprovoked aggression and vocalisation, seizures, paralysis with difficulty breathing and eventually resulting in death (CDC, 2011; WHO, 2018a).

Diagnosis

Clinical diagnosis

Diagnosis in suspected cases of rabies is possible, however not recommended in animal cases because of the fact that a tests sensitivity varies depending on the animals' immune status, the progression of the disease and the fast that the virus is intermittently excreted. While a positive test can be useful, a negative laboratory test does not necessarily rule out rabies and the animal should continue to be treated as possible rabid (WHO, 2018a).

However, laboratory diagnostics are useful when it comes to suspected human cases of rabies and are, for instance, necessary for identification and characterization of pathogen and to rule out differential diagnoses, deciding on experimental therapeutic procedures and monitoring of viral loads and any decrease. It is also important for identification of other persons who may have been in contact with the possible source of infection as well as case closure and grief counselling to family members (Damodar, Mani & Prathyusha, 2019).

There are numerous tests for ante-mortem diagnosis of suspected rabies cases and, because a negative test does not rule out rabies, recommendations are to take serial samples of different body fluids and/or tissues to increase the sensitivity (Dacheux *et al.*, 2010; Damodar, Mani & Prathyusha, 2019).

Post-mortem diagnosis

Post-mortem diagnosis is suitable on suspected rabid animals to be able to begin appropriate actions regarding post exposure treatment for people who has been in contact with the animal.

Like ante mortem, there are numerous test available for post-mortem diagnosis, where the fluorescent antibody test (FAT) detecting rabies antigen is the most recommended (WHO, 2016a). Other tests that also detect antigen are the direct rapid immunohistochemical test (DRIT) on brain tissue (Nguyen *et al.*, 2015) and the immunohistochemistry test (IHC) on formalin-fixed samples from either brain or follicle-sinus complexes (Shiwa *et al.*, 2019).

Analysis can also be done for rabies virus RNA or rabies virus. A test that detects the rabies virus RNA is the polymerase chain reaction (PCR) test, which can analyse brain tissue, saliva, and cerebrospinal fluid (Damodar, Mani & Prathyusha, 2019). Tests that can detect rabies virus are the rabies tissue culture infection test (RTCIT) (Dacheux & Bourhy, 2015), the mouse inoculation test (MIT) and the virus isolation in cell culture test (VICC) (Corona *et al.*, 2018), all using brain tissue for analysing.

Prevention

According to the World Health Organization (WHO, 2019d) there are three proven effective ways to prevent rabies:

1. Awareness of rabies in communities to engage people and encourage them to seek medical attention when necessarily.
2. Thorough wound cleaning and easy access to post-exposure prophylaxis (PEP) and, when needed, rabies immunoglobulin (RIG) after suspected exposure to rabies.
3. Mass dog vaccination to prevent rabies outbreaks among the number one source for transmission to humans and thereby prevent human cases.

There are many successful cases where regions have been able to prevent and thereby eliminate rabies. For instance, Bohol, an island in the Philippines, managed to eliminate rabies through dog population control, mass dog vaccination campaigns, improved community involvement, establishment of veterinary quarantine, enhanced dog bite management including RIG and PEP and improved diagnostics and rabies surveillance (Lapiz *et al.*, 2012). Similar progress was made in Serengeti district in Africa, where four mass dog vaccination campaigns took place with approximately one year intervals, resulting in a significant decrease of human cases from exposure to suspected rabid dogs (Cleaveland *et al.*, 2003).

Vaccination

WHO recommends the use of inactivated vaccines only (WHO, 2018a). There are two types of vaccines available for both humans and animals; cell-culture-based or embryonated-egg-based (Malerczyk, Vakil & Bender, 2013). Depending on the vaccine and the injection route, the treatment procedure is different.

Human vaccination

Pre-exposure prophylaxis (PrEP) by vaccination creates a secondary immune response that generates a long lasting immunity which also responds well to booster vaccination, making RIG injections unnecessary in case of exposure to RABV (Suwansrinon *et al.*, 2006).

PrEP is recommended to people at risk of rabies exposure, such as veterinarians, laboratory personal, animal handlers and people travelling to endemic areas. It can be given either through intramuscular (IM) route where a full dose is given on day 0 and on day 7, or through intradermal (ID) route where 0.1 ml of rabies vaccine is given at two places on day 0 and on day 7 (Folkhälsomyndigheten, 2019). Studies have shown that there is no difference in immune response between the two different routes, and they can therefore be used interchangeably (Venkataswamy *et al.*, 2015).

Canine vaccination

To interrupt transmission of canine rabies, and thereby eliminating the risk of human cases, the recommendations are that 70% of the dog population is vaccinated (Cleaveland *et al.*, 2003; Conan *et al.*, 2015). Upholding this level of immunity is problematic and endemic countries often arrange rabies vaccination campaigns. Rabies vaccination to dogs can either be given

subcutaneously (SC) or orally. Oral vaccination does not provide an equally good immune response, however it is suitable for vaccination of free-ranging stray dogs that are hard to handle (Smith *et al*., 2017).

Nevertheless, the problem of not getting enough vaccination coverage remains, even with campaigns, due to lack of participation among dog owners. The number of participants can however be increased by improved information regarding upcoming vaccination campaigns, for example through sending out text messages to residents (Cleaton *et al*., 2018). Owners' intention to vaccinate can also be improved by increased knowledge regarding rabies, facilitating transports of dogs or by locating vaccination centers closer to target populations (Beyene *et al.*, 2018).

Adequate immunization and rabies virus titres

It is essential to evaluate the vaccination coverage among dogs to be able to have effective vaccination strategies. Knowledge about a dog's, or any other domestic animal's immunization status is also important to be able to prevent international spreading of the virus (Wasniewski *et al*., 2014). WHO recommends a minimum level of 0.5 IU/ml antibody titres in an individual to ascertain a sufficient immunization against rabies to fully consider the individual protected (WHO, 2013). This threshold is used for animals traveling between countries and for humans working with high-risk professions to prevent any outbreaks of rabies.

Validation of antibodies

A patient undergoing a PrEP or PEP routine does not generally need to be serologically tested for rabies virus neutralizing antibodies. There are however cases where evaluation of antibodies is indicated, for example where it counts as an occupational hazard such as for veterinarians, slaughter house workers and rabies virus laboratory personal. It is also indicated to evaluate the rabies antibody response in patients undergoing a PrEP or PEP and there has been aberrations in the protocol, the patient's immune status is reduced, or the initial vaccinations was made internationally with a product of unknown quality (CDC, 2016).

The first test for evaluating rabies virus neutralisation antibodies (RABV NAb) was the mouse neutralization test (MNT) which was developed in 1935 (Webster & Dawson, 1935). Since then there have been multiple methods for detecting NAb, and to date there are different tests available to evaluate the immunization status against rabies in animals and humans.

Rapid fluorescent focus inhibition test (RFFIT)

The RFFIT, first developed in 1973 (Smith, Yager & Baer, 1973), evaluates the level of RABV NAb in human or animal serum by mixing different levels of diluted test sera with rabies virus of constant amount. The mixture is incubated in a multi-chambered slide allowing any rabies virus neutralizing antibodies to neutralize the rabies virus. After a shorter incubation the mixture is transferred to cells and incubated approximately 20 hours allowing any active rabies virus to replicate. After second incubation the cells are fixed, stained and read with a fluorescent microscope to detect any rabies virus production. The plate is then validated against a control slide with reference dilutions and control serum (CDC, 2016).

RFFIT was proven to be compatible with the former most accepted and common test, MNT, (Smith, Yager & Baer, 1973) and is now classified as the gold standard test for evaluation of RABV NAb and to determine the potency of PEP vaccines and RIG by both the WHO (WHO, 2016b) and CDC's advisory committee on immunization practices (ACIP) (CDC, 2016).

Fluorescent antibody virus neutralisation test (FAVN)

The FAVN test, developed and described for detecting RABV NAb in 1997 (Cliquet, Aubert & Sagné, 1998), is a modified version of the RFFIT. Each test consists of 96-well microplates and positive and negative control sera. All serum samples, including the negative and positive control, are serially diluted four-fold with Eagle's minimum essential medium (EMEM), supplemented with 10% fetal bovine serum. Using the CVS 11 strain of RABV and growth medium and fixating the plates with 80% acetone solution before staining them with fluorescein isothiocyanate conjugated anti-rabies serum, the plates can by analysed by using an appropriate microscope and evaluated for RABV NAb (Cliquet, Aubert & Sagné, 1998; Ondrejková *et al.*, 2002).

The FAVN-test have been proven to be more accurate than the MNT and comparable to the RFFIT (Ondrejková *et al.*, 2002), nevertheless the test procedure is more complicated with longer incubations, in total reaching 51 hours, therefore taking longer time to perform compared to the RFFIT.

Enzyme-linked Immunosorbent assay (ELISA)

There have been numerous versions of the ELISA test since the 1970s (Atanasiu, Savy & Gilbert, 1978; Barton & Campbell, 1988; Muhamuda, Madhusudana & Ravi, 2007; Chabaud-Riou *et al.*, 2017). The test used in this study is a blocking ELISA that detects RABV NAb by comparing the samples against a negative control.

The test consists of microplates with 96 wells that are coated with rabies antigen. All samples, including controls, are diluted and incubated in wells overnight. After incubation biotinylated anti-rabies antibodies are added to the wells, and any existing RABV NAb in investigated samples will block binding of the coated antigens to the biotinylated anti-rabies antibodies, preventing formation of antigen-biotinylated antibody complexes. When streptavidin peroxidase conjugate is added to the wells, it will bind to any complexes formed, and any unbound conjugate is removed during washing. When adding TMB substrate, any antigen-biotinylated antibody complex is revealed and after a shorter incubation, stop solution is added and the optical density is read at 450 nm and the percentage of blocking (PB) antibodies is calculated. A PB of $\geq 40\%$ is considered as a sample positive for RABV NAb, and a PB $\geq 70\%$ is considered as a sample with NAb level ≥ 0.5 IU/ml (Wasniewski & Cliquet, 2012).

The test is user-friendly, however the total amount of incubations reaches up to 20-26 hours making it time consuming. Studies have proven the ELISA is comparable to the FAVN test, and being less dependent on serum quality it is well recommended for evaluation of immune status against rabies among carnivores post vaccination (Cliquet *et al.*, 2000).

Rapid neutralizing antibody detection test (RAPINA)

RAPINA was developed in 2009 (Shiota *et al*., 2009) and improved in 2012 (Nishizono *et al.*, 2012). It is a qualitative method, consisting of a test strip that indirectly measures RABV NAb by using a virus-neutralizing monoclonal antibody (mAb) against the CVS 11 strain of rabies virus, detecting any rabies virus glycoproteins (G) that are not bound to neutralizing antibodies.

Serum is diluted with the CVS 11 strain of inactivated rabies virus (iRABV) and transferred into the hole in the test strip. The RABV NAb in the sample will form complexes with the iRABV antigens. As the sample moves over the test strip it will travel over a pad where any unbound iRABV glycoproteins will form complexes with the virus-neutralizing mAb in the pad. These complexes will be trapped at the test line, creating a visible band. Any unbound virus-neutralizing mAb will instead be trapped at the control line, there creating a visible band. The total time for the test is 45 minutes. (Shiota *et al*., 2009; Nishizono *et al.*, 2012)

Interpretation of the RAPINA is based on the presence of visible bands at the test and control lines. If both test and control lines are visible, the results is interpreted as a rabies Virus NAb level of <0.5 IU/mL, if only the control line is visible the result is interpreted as a rabies Virus NAb level of >0.5 IU/mL and if no line is visible the test is considered invalid (Nishizono *et al.*, 2012).

The RAPINA has been proven to comparably detect antibodies in comparison to the RFFIT test, and is therefore considered a good, affordable and easy to use example for determining the antibody levels among dogs (Manalo *et al.*, 2017).

Post-exposure prophylaxis (PEP)

Anyone who suspects being exposed to rabies virus should thoroughly clean the exposed area with great amounts of water and soap for at least 15 minutes and, if available, apply a substance with viricidal activity, such as povidone iodine. If the site of exposure would be eyes or mucosal tissue, the area should be rinsed thoroughly with water. After initial wound care it is important that the patient seeks medical attention as soon as possible for assessment on whether further actions should be made (WHO, 2018a).

Additional post-exposure prophylaxis (PEP) measurements are critical for survival (Huang *et al.,* 2017) and consist of post-exposure rabies vaccinations and rabies immunoglobulin (RIG) injection. The World Health Organization (WHO, 2018b) has created general guidelines for countries or areas enzootic to rabies regarding PEP-treatment by dividing patients into three categories:

1. Touching, feeding or getting licked on intact skin by a suspected rabid animal
2. Minor scratches without bleeding or nibbling on uncovered skin,
3. Transdermal bites or scratches, licks on skin lesions, contamination of mucous membranes with saliva from licks or direct contact with bats

After risk assessment, the patients should be treated accordingly, where patients in category one has no indication of PEP, patients in category two should receive PEP immediately, and

category three should receive PEP as well as injection of rabies immunoglobulin (RIG) at the site of exposure immediately (WHO, 2018b). However the accessibility to affordable adequate post-exposure prophylaxis is a problem in many endemic areas due to the high demand, stockout periods at government facilities and high cost at private sectors resulting in lack of adequate PEP (Wilde, 2007; Wambura *et al.*, 2019).

Post-exposure vaccination

Post-exposure vaccination is given to humans after suspected exposure to rabies virus to enhance the immunity against rabies. Like PrEP, it can be given either intradermally or intramuscularly and the regimen and dosage depend on the route and any previous rabies vaccinations, PrEP or PEP.

There are different regimens of PEP vaccinations. Recommendations are to complete a regimen using the same brand of rabies vaccine once started. However, studies have shown that patients who has received different brands of vaccine or different regimens during a PEP-treatment still acquire a sufficient immunization of >0.5 IU/ml Rabies virus NAb (Ravish *et al*., 2014).

There are two intramuscular (IM) regimens that are currently accepted. The Essen regimen is the formerly recommended PEP-regimen where a full vial of rabies vaccine, either 0.5 or 1.0 ml, is given IM in one of the deltoid muscles on days 0, 3, 7, 14 and 28 (Global Alliance for rabies control (GARC), 2017). It is comparable to the Zagreb regimen where two full vials are given on day 0 (one in each deltoid muscle) and one full vial is given on days 7 and 21 (Ren *et al., 2015)*. The Zagreb regimen is proven to be more cost-effective than the Essen regimen since it reduces the amount of hospital visits from five to three as well as reduces the total dose of rabies vaccine given (Goswami *et al.*, 2005), however both regimens do require multiple visits to a caretaking facility and are expensive for people living in high risk areas.

Due to the high costs of IM PEP treatment, the WHO instead recommends intradermal (ID) administration of PEP (WHO, 2018c). With ID regimens, the dosage and number of hospital visits can be reduced, making it more accessible and affordable (Kundu *et al*., 2019). The formerly recommended ID-regimen was the Updated Thai Red Cross (UTRC) regimen which consists of two 0,1 ml ID doses of rabies vaccine given in the area around the deltoid muscles on days 0, 3, 7 and 28 (Khawplod *et al*., 2006). This regimen was proven to reduce the costs of PEP by almost 60% (Kundu *et al.*, 2019).

The UTRC regimen was later substituted and recommendations regarding post-exposure vaccinations are now a one week, two-site ID regimen known as the Institute Pasteur of Cambodia (IPC) regimen. Two vaccine doses of 0,1 ml are given intradermally in the area of each deltoid muscle on days 0, 3 and 7. This regimen further lowers the costs for PEP and is proven to be sufficient with no detectable added risks (WHO, 2018a; Tarantola *et al*., 2019).

For individuals that has received a full dose of PrEP before, the PEP-regimen is modified, and recommendations for ID-regimens are either one dose vaccine given on first and third day or four doses given at different sites on the first day. Recommendations for the IM-regimen is one full vial given on the first and third day (WHO, 2018a). A study by Soentjens *et al*. (2019) showed that, in patients who received PrEP during the previous 28 months, even a two dose,

single visit PEP ID regimen is sufficient to attain an adequate immune response up until seven days after PEP. However, more studies need to be done where immunisation levels are investigated longer than seven days after PEP to be able to fully state that single visit, two dose PEP is as sufficient as a single visit, four dose ID regimen.

Rabies immunoglobulin

Rabies immunoglobulin (RIG) can be used to provide passive immunization before the patients, after post-exposure vaccination, are able to produce their own antibodies at the site of infection. RIG should be given to all category three patients, if they have not yet completed a full PrEP or at least two previous doses of PEP. These patients should already have antibodies and thus the indication for RIG is lower (WHO, 2018a). There are two types of rabies immunoglobulin available: human RIG and equine RIG (Bharti, Madhusudana & Wilde, 2017). The maximum dose varies between the two, where human RIG has a maximum dose of 20 IU/kg body weight and equine RIG is 40 IU/kg bodyweight (Wilde *et al*., 2016). However, these recommendations are based on previously available RIG, and studies have proven that a reduced dosage of RIG still is sufficient to attain a good passive immunity (Madhusudana, Ashwin & Sudarshan, 2013; Bharti, Madhusudana & Wilde, 2017).

Treatment with RIG is given once, and if possible, at the same time as the initial dose of post-exposure vaccination or as soon as possible after. According to WHO, it is still indicated to be given up until seven days after first dose of PEP. After seven days the immune response from the post-exposure vaccination will be active and giving RIG would not give any extra immunization (WHO, 2018a). However, in a study by Ma *et al.* (2015), Chinese adults first showed seroconversion 14 days after vaccination. Giving RIG even after day seven after first dose of post-exposure vaccination might therefore still be indicated.

WHO recommendation of RIG protocol is that the full dose should be infiltrated at the site of exposure, or as close to it as possible, if anatomically possible without causing compartment syndrome. If the full dose cannot be given, giving the rest of the dose intramuscularly in close proximity to the site of exposure could be considered if it is suspected that there could be small unreported wounds (i.e. if the patient is a child who doesn't report all wounds), if exposure was to a bat or if exposure was through any other way than contact with saliva. Recommendations are the same if exposure only was through contact with mucosal tissue without any wounds, in which case intramuscular injection of RIG in combination with rinsing the area with RIG is recommended (WHO, 2018a). If none of these criteria are reached, studies have shown that intramuscular injection creates no significant rise in immune response compared to only infiltration in wound or site of exposure (Chomchay, Khawplod & Wilde, 2000), and muscular RIG injection is then not indicated. Instead of calculating the RIG dose based on bodyweight, the dose should then be calculated based on the number and size of wounds, (Bharti, Madhusudana & Wilde, 2017) making it more affordable and available for patients, especially in developing countries. On the contrary, intramuscular RIG injection without local injection in wound has been proven insufficient in many cases (Wilde *et al*., 1989).

Rabies in Lao PDR

Laos is part of the global strategic plan "Zero by 30", where the goal is that there should be zero dog-mediated human deaths of rabies by 2030. The plan is put together by United Against Rabies, a collaboration between the World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO), World Organization for Animal Health (OIE) and Global Alliance for Rabies Control (GARC) (WHO, 2018d). Dogs are the main transmitter of RABV to humans in the country (Ahmed *et al*., 2015), and between the years of 2012-2017, there have been 33 reported cases of human rabies deaths in Laos (WHO, 2019b). This number is however likely much larger, with a great amount of unreported cases.

In a study that took place during the years 2010-2016, 415 canine samples, consisting of either carcass or head, were submitted to the National Animal Health Laboratory (NAHL) in Vientiane capital. Out of the admitted samples, 284 samples were positive for rabies (68.4%). The proportion is however questioned, and most likely underestimated since the samples were submitted following a dog bite where diagnosis was critical for determining the need for PEP. The same study also showed a significant rise in positive samples during dry season in Laos, most likely due to canine behavioural pattern (Douangngeun *et al*., 2017).

MATERIAL AND METHODS

Study area and participants

The study took place in Vientiane Prefecture, Laos, primarily in the districts Naxaythong, Parknguem, Xaysetha and Xaytany. Participants were either from villages in the districts, a vaccination campaign held in Vientiane capital, or patients at an animal clinic. When conducting the study in the villages, an official worker from each district provided contact with the village chiefs and sampling was either done at a community building in the village, where participants got information regarding the study through the village speaker-system, or through door-to-door survey.

Dogs older than three months that showed no signs of aggression were included in the study. If there were more dogs in the household, only two dogs were used in the study. A total of 474 dogs participated, of which 437 were included in the study. 37 dogs were excluded due to lack of complete information about the dog or the dog escaped before any blood could be drawn.

Before sampling, all dog owners were informed of the testing procedure and gave a written consent. After sampling most owners received an information pamphlet regarding rabies that had been translated into lao. All dogs that took part in the study was given Zoetis - Defensor 3 rabies vaccine and ivermectin against endo- and ectoparasites, both injections given subcutaneously after sampling. The injections were also given to other dogs over three months in households with more than two dogs. If the household had other dogs that were younger than three months, these dogs solely received an injection of ivermectin.

Survey and sample collection of canine serum

The owners were asked questions about the dog, including age, the main purpose of the dog, the dog's living situation and history of previous vaccinations. After the owner had completed

the questions, the dog was prepared for sampling. All dogs were wearing muzzles and were handled by confident staff throughout the whole process.

Blood was collected from the cephalic vein from one of the front legs. In case of insufficient amount of blood both legs could come to be used. The materials used for collection of the blood was either butterfly cannula with a vacutainer luer adapter or cannula and syringe. The blood was collected in serum blood collection tubes and centrifuged with the Hettich EBA 20 centrifuge to receive serum. After centrifugation the samples were stored in a freezer at approximately -19 degrees Celsius for preservation until analysed.

Analysis for rabies virus neutralizing antibodies (RABV NAb)

After being thawed and brought to room temperature, the serum samples were analysed for rabies virus antibodies using the BioPro Rabies ELISA Ab kit. 283 of the 437 samples (64.76%) were run in duplicates to ensure the authenticity of the test. The mean value was then calculated for results. Out of the duplicates there were 14 samples (4.95%) with dubitable results, these samples were therefore analysed again.

Each sample, including positive and negative control and the three control serums, were diluted 1:2 with sample diluent. After dilution, 100 microliters of the diluted controls and samples were transferred into different wells and the microplates were incubated for 18-24 hours at 2-8 degrees Celsius. After incubation the wells were emptied, washed with wash solution 6 times and 100 microliters of diluted biotinylated anti-rabies antibody was dispensed in each well and the microplates were again incubated for 30 minutes at 37 degrees Celsius.

After the second incubation the wells were emptied, washed with washing solution 4 times and 100 microliters of diluted streptavidin peroxidase conjugate was dispensed in each well, following an incubation of 30 minutes at 37 degrees Celsius. The wells were then once again emptied and washed 4 times with washing solution, filled with 100 microliters of substrate solution (TMB) and incubated for 15-30 minutes in room temperature. Since none of the incubations were done with an orbital shaker this incubation lasted for more than 20 minutes, as recommended by the manufacturer. After the last incubation 50 microliters of stop solution was dispensed in each well and the optical density was read at 450 nm.

The results were then calculated into percentage blockage of antibodies according to instructions and samples with a percentage equal or higher than 40% are considered positive for RABV NAb. Samples equal or higher than 70% blockage are considered to have an antibody level reaching up to, or higher than 0.5 IU/ml based on the FAVN test. These samples are considered protected against rabies.

Data analyses

Data analyses were made using the Pearson's chi-squared test and logistic regression (logit) in the statistics software program STATA 14.2.

RESULTS

A total of 437 dogs were included in this study. The majority of dogs were sampled in villages (n = 415) and, as shown in figure 1, originated from the districts Naxaythong (n = 92), Parknguem (n = 107), Xaysetha (n = 108) and Xaytany (n = 108). The rest of the dogs (n = 22) were sampled at either a vaccination campaign in the capital Vientiane (n = 18) or at an animal clinic (n = 4). 3 of these dogs originated from Xaysetha and 1 dog originated from Xaytany and are classified thereafter. The remaining 18 dogs originated from the districts Sikhottabong (n=9), Chanthabouly (n=6) and Sisattanak (n=3) and are classified as "other" in this study.

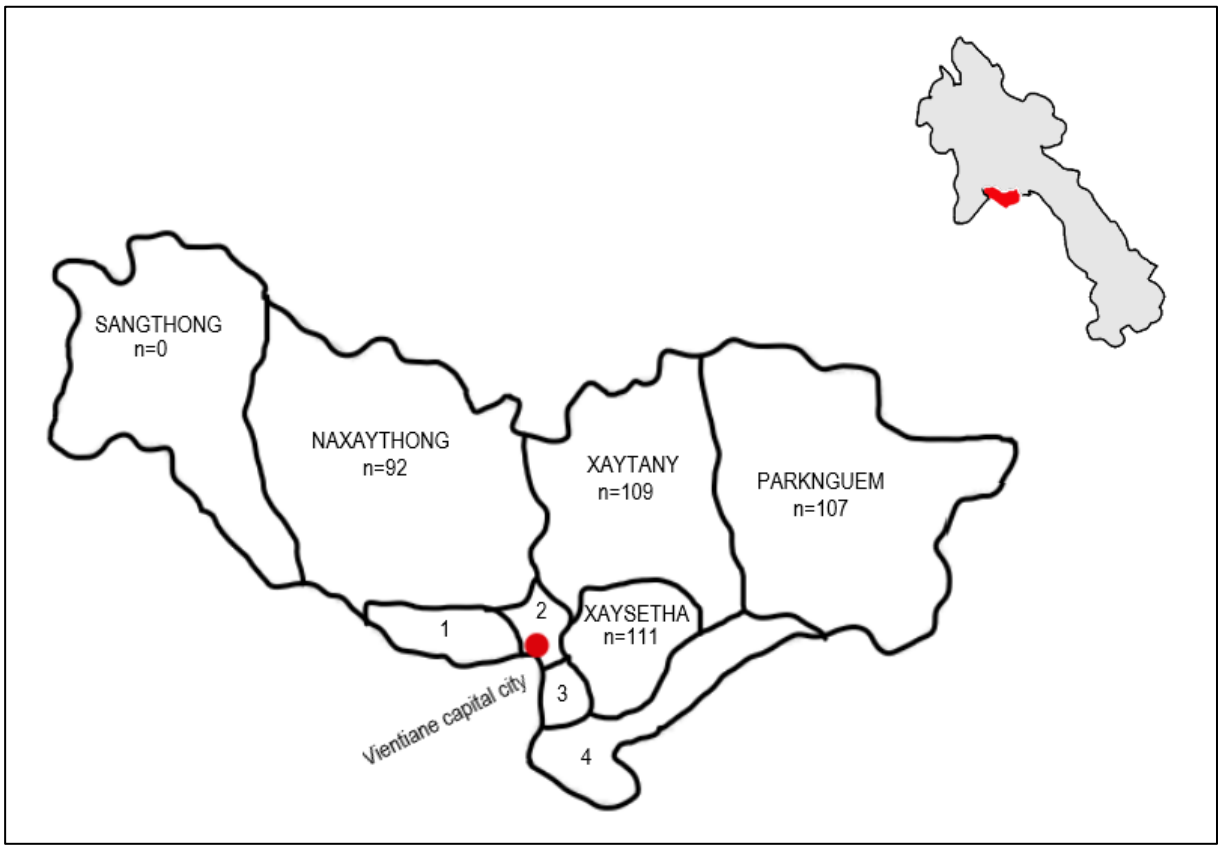

Figure 1. *Map of Vientiane prefecture in Lao PDR showing the number of samples in each district. 1: Sikhottabong (n=9), 2: Chanthabouly (n=6), 3: Sisattanak (n=3), 4: Hadxaifong (n=0).*

Out of the 437 dogs, 124 dogs (28.38%) were positive for RABV NAb. The average age among positive dogs was 3.6 years, significantly higher than dogs negative for RABV NAb, where the average age was 1.9 years ($p < 0.001$).

Five dogs out of the 124 dogs could not be determined whether they had a sufficient amount of RABV NAb due to borderline results. They are therefore not included in analyses that involves protection level, making 57 of 119 (47.90%) dogs with a RABV NAb level >0.5 IU/ml. Looking at the whole population, 13.19% (57/432) of the sampled dogs were protected against rabies and out of the dogs being protected against rabies, the average age was 3.5 years, significantly higher than the average age of dogs not being protected, which was 2.2 years ($p < 0.001$).

Owner's awareness of previous rabies vaccination

Presented in figure 2, out of the 56 dogs where the owner stated that the dog had been vaccinated against rabies, 34 dogs (60.71%) had detectable antibodies against rabies virus. Compared to the dogs where the owner stated that the dog had not been vaccinated against rabies (n = 375), 89 dogs (23.73%) had detectable RABV NAb, making it significantly lower than the dogs reported to be previously vaccinated (p = <0.001).

When instead investigating the protection level among dogs, where the owner stated that the dog had been vaccinated against rabies before (n = 56), only 27 dogs (48.21%) were protected against rabies, significantly higher (p <0.001) than where the owner stated that the dog had never been vaccinated against rabies (n = 370), out of which 30 dogs (8.11%) were protected against rabies (figure 3).

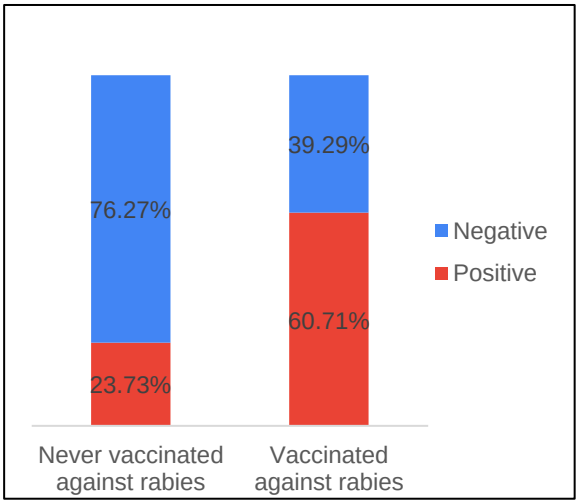

Figure 2. *Comparison of detectable RABV NAb between dogs reported being vaccinated against rabies and dogs reported never knowingly being vaccinated against rabies.*

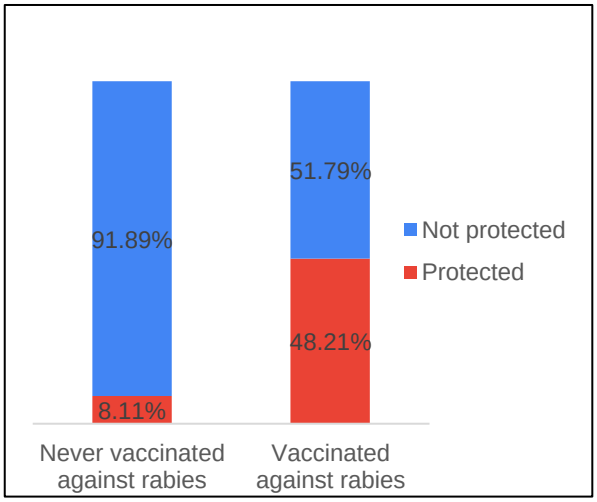

Figure 3. *Protection level comparison between dogs reported being vaccinated against rabies and dogs reported never knowingly being vaccinated against rabies.*

Vaccination status and protection level between the different districts

The distribution between the districts can be seen in figure 4. The districts with the highest percentage positive dogs were Xaysetha (34.58%) and Xaytany (25.93%), followed by Naxaythong and Parknguem who had 22.83% and 20.56% respectively. Among the dogs included in "other", 61.11% were positive giving the highest percentage overall.

When comparing protection level between the districts, the dogs sampled at the campaign was excluded and the significantly highest percentage of dogs with a RABV NAb level > 0.5 IU/ml was found in Xaysetha (18.27%) and Xaytany (14.02%), compared to Parknguem (7.48%) and Naxaythong (5.43%) (p = 0.015).

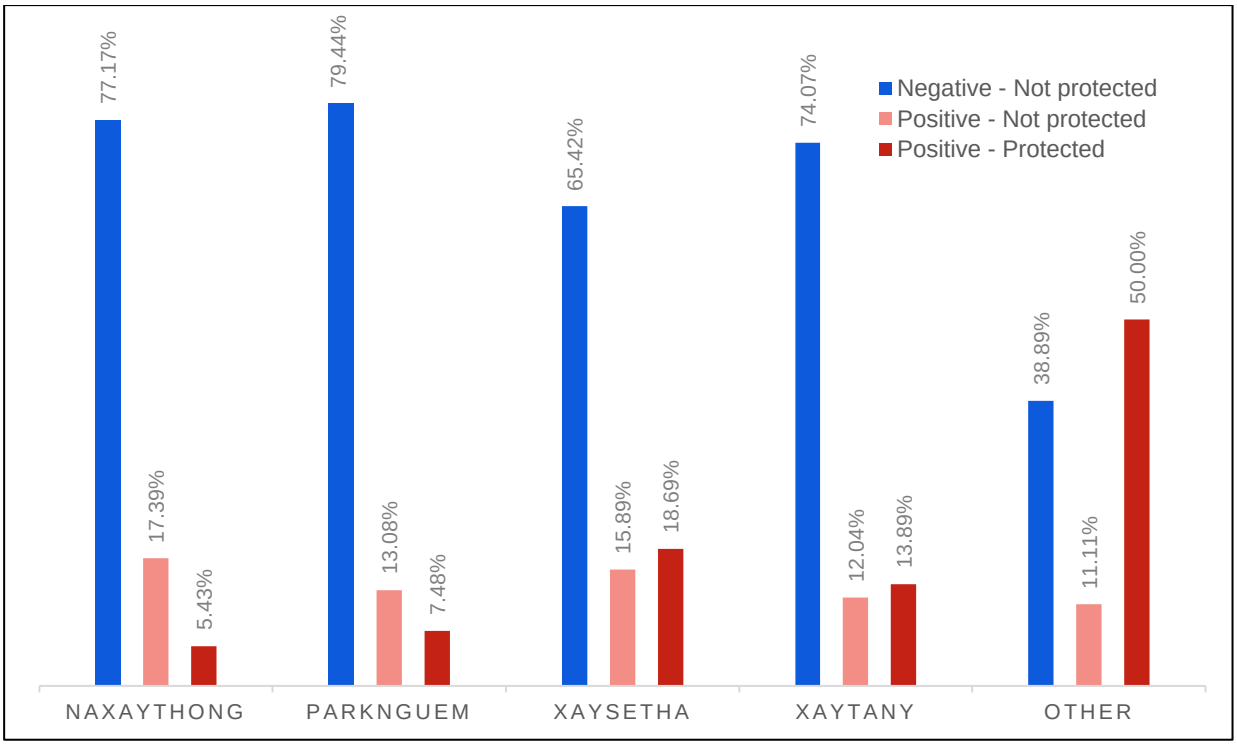

Figure 4. *Breakdown of the vaccination status among domesticated dogs in different districts in Vientiane Prefecture, Lao PDR.*

Impact on vaccination status by sampling location

Out of the dogs that were sampled at the vaccination campaign held in Vientiane capital, 8 of 18 dogs (44.44%) was reported to have been vaccinated against rabies before, significantly higher ($p<0.001$) than the dogs that were sampled in the villages, where 48 of 415 dogs (11.57%) had been previously vaccinated, and dogs sampled at the animal clinic (n =4), where no dogs were reported to be previously vaccinated against rabies (figure 5).

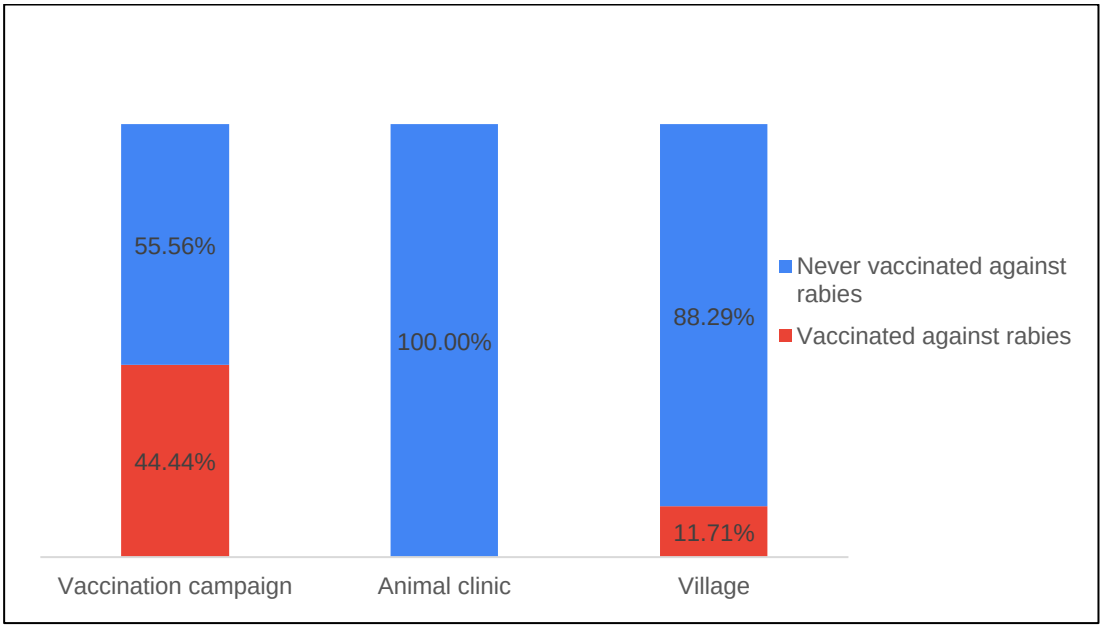

Figure 5. *Percentage of dogs stated previously vaccinated against rabies divided by sampling location.*

When looking at detectable RABV NAb among the dogs in the different sampling locations, the results were similar (figure 6). Out of the dogs who were sampled at the rabies vaccination campaign, 12 out of 18 (66.67%) were positive for RABV NAb, significantly higher than dogs sampled at the animal clinic (1/4) and dogs sampled in the villages (111/313) where percentages of positive dogs were 25% and 26.75% respectively (p=0.001).

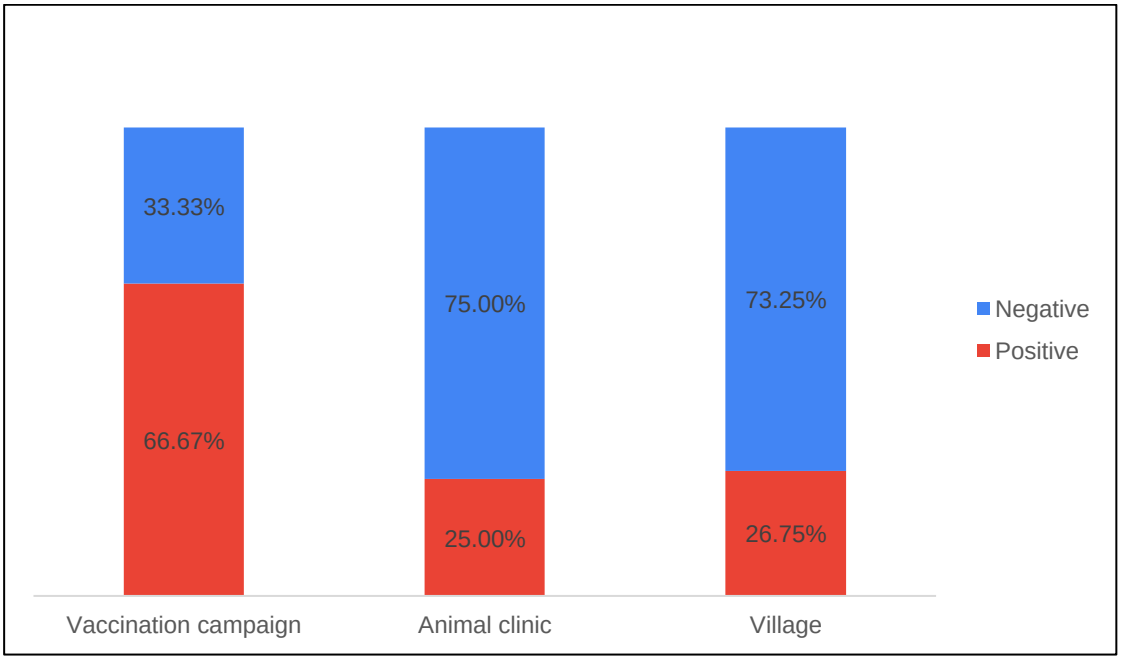

Figure 6. *Percentage of dogs with detectable levels of RABV NAb divided by sampling locations.*

Time impact on immune status

When investigating time impact on immunization, the owners were asked when the dog last was vaccinated against rabies. Out of the dogs where the owner stated it had been vaccinated against rabies (n = 56), 12 dogs had last been vaccinated more than one year before the survey (21.43%) and 44 dogs had been vaccinated within the last year (78.57%).

When comparing immunization between the two groups, 7 out of the 12 dogs (58.33%), who had last been vaccinated over one year before the survey, had detectable RABV NAb and 27 out of the 44 dogs (61.36%), who had been vaccinated within the last year, were positive for RABV NAb (figure 7, $p<0.001$). When instead looking at protection level (figure 8), 5 of the 12 dogs (41.67%) that had been vaccinated over one year before the survey were protected against rabies and 22 of the 44 dogs (50%) that had been vaccinated within one year of the survey were protected against rabies ($p<0.001$).

The odds of a dog having detectable RABV NAb is significantly higher for dogs who had been vaccinated against rabies over one year before sampling (OR 5.8, $p = 0.002$) and for dogs who has been vaccinated within one year prior to sampling (OR 4.6, $p <0.001$) compared to dogs who has never been vaccinated against rabies.

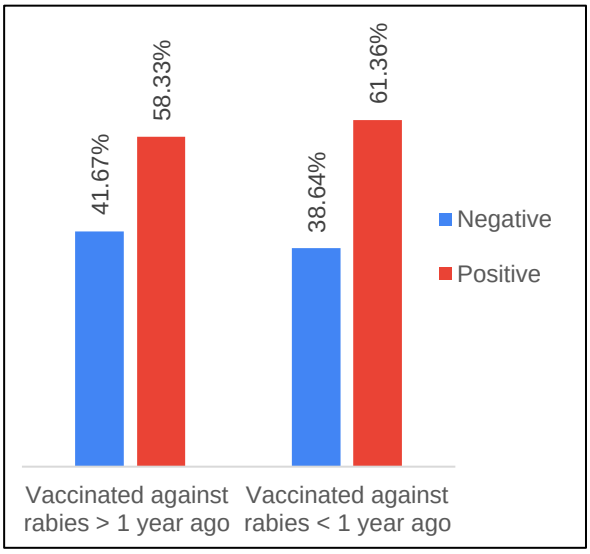

Figure 7. *Comparison of detectable RABV NAb between dogs reported being vaccinated against rabies over one year before the survey and dogs reported being vaccinated against rabies within one year of the survey.*

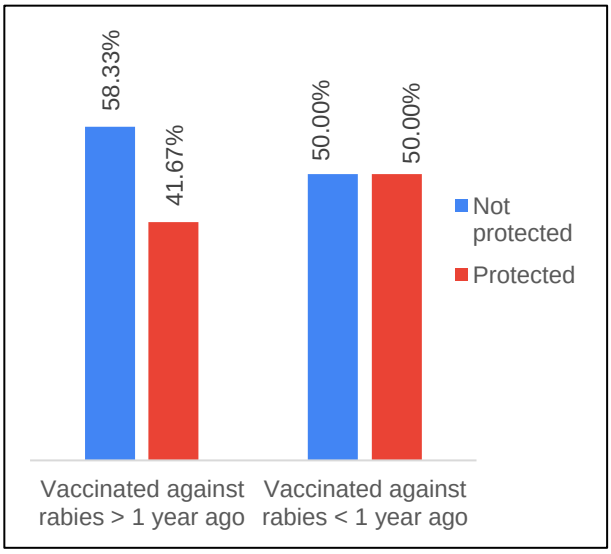

Figure 8. *Comparison of protection status between dogs reported being vaccinated against rabies over one year before the survey and dogs reported being vaccinated against rabies within one year of the survey.*

Impact on vaccination status by canine living situations

Dogs that were kept loose either indoors or outdoors were significantly less vaccinated, looking at all types of vaccines, compared to dogs that only lived indoors or outdoors in a leash or in an enclosed area (p=0.001). 11 out of 21 (52.38%) dogs only living indoors or outdoors in a leash and 36 out of 82 (43.9%) dogs living outdoors in an enclosed area were previously vaccinated compared to dogs held indoors and outdoors loose, or only outdoors loose, where 85 out of 326 (26.07%) were vaccinated.

When comparing duration since last vaccination, shown in figure 9, dogs that were kept only indoors or outdoors in a leash were more likely to have been vaccinated within the last year (42.86%), compared to dogs that were kept outside in an enclosed area (29.27%) or dogs that were kept loose either indoors or outdoors (15.64%) (p=0.001).

When instead looking at vaccination against rabies, there was no significant difference between dogs with different living situations regarding if they had been vaccinated against rabies or not (p=0.264). Neither was there a significant difference between the groups regarding time of last rabies vaccination (p=0.222), presence of detectable RABV NAb (P=0.106) or protection level against rabies (P=0.094).

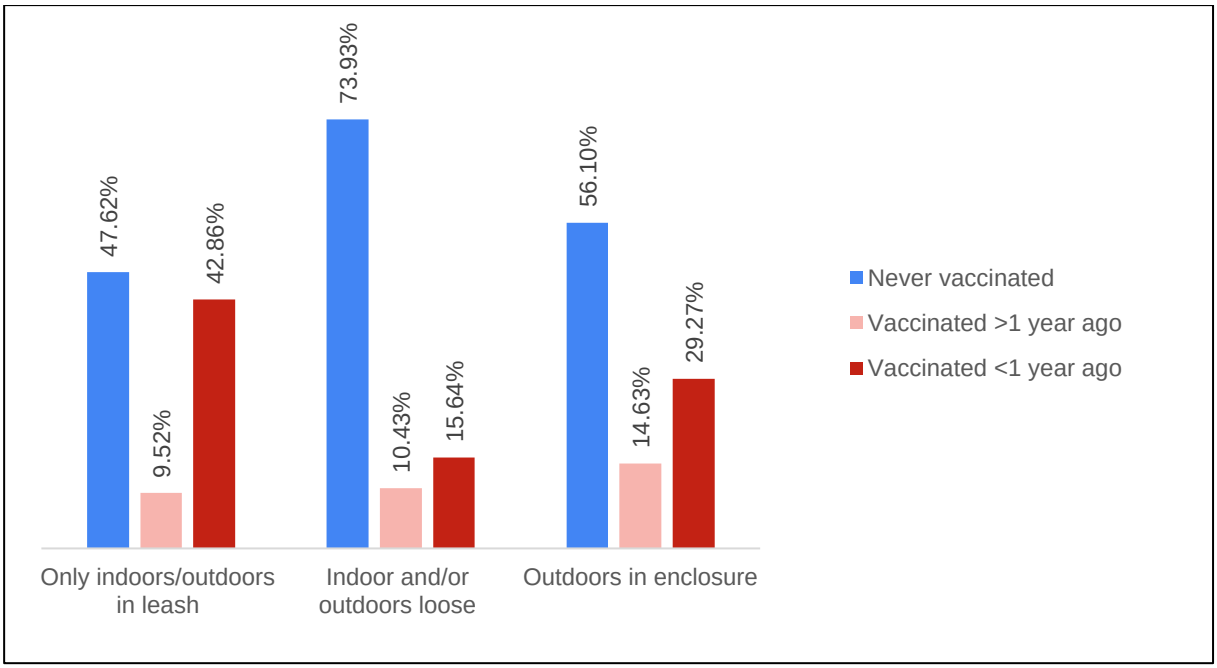

Figure 9. *Comparison of vaccinations against any diseases between dogs with different living situations.*

DISCUSSION

This study was conducted in Vientiane Prefecture, Lao PDR, in order to investigate the vaccination status and protection level against rabies among the canine population. Our results show that the proportion of positive dogs for rabies antibodies is not sufficient to prevent human cases of dog-mediated rabies. It also showed that there are discrepancies between dog owners' information regarding previous rabies vaccinations and the results of detectable antibodies of the dogs.

Vaccination and protection status

Vaccination is recommended to be given to dogs over 12 weeks, though studies have shown that there is no statistically significant difference in immune response between dogs being vaccinated under the age of 12 weeks and dogs vaccinated above the age of 12 weeks (Morters *et al.*, 2015; Wallace *et al.*, 2017). In this study the mean age of dogs not being protected against rabies was 2.2 years. Even though significantly lower than the dogs being protected against rabies (3.5 years), it is still much higher than the 12 weeks limit. Therefore, when designing rabies vaccination campaigns, it could be of interest to also vaccinate dogs under 12 weeks to be able to maintain a good vaccination level in the canine population. This can also be motivated by the fact that even small gaps of immunization against rabies within the canine population can profoundly delay elimination of rabies (Townsend *et al.*, 2013).

Taking into consideration the recommendations that 70% of the canine population should be vaccinated against rabies to be able to prevent human cases of canine rabies (Cleaveland *et al.*, 2003; Conan *et al.*, 2015), the vaccination status of dogs in Vientiane prefecture in Laos PDR is insufficient. Only 28.32% of sampled dogs having detectable RABV NAb and only 13.19% of sampled dogs reach a level of 0.5 IU/ml and are considered protected against rabies (WHO, 2013).

The studies that claims that a 70% vaccination coverage is sufficient, does not take into consideration the level of protection. As seen in this study, not all vaccinated dogs are protected against rabies. Vaccination coverage of 70% of the canine population in an area would therefore not necessarily result in the protection against rabies in the same number of dogs. This suggest that the number of protected dogs necessary to prevent human cases of dog mediated rabies may be less than 70%. However, to be able to fully conclude this, a study with greater participants is necessary. When investigating the proportion of dogs vaccinated in a population, it is more complicated to investigate the amount of dogs that has a detectable level of RABV NAb than controlling the number of dogs reported to be vaccinated. Since detection of RABV NAb requires a blood sample, monitoring the number of dogs being vaccinated is therefore a more suitable measurement to be able to ensure that the immunization of the canine population is sufficient.

Lack of immunization coverage after rabies vaccination can be caused by different aspects concerning the vaccine and the dog being vaccinated. Factors affecting vaccines includes extreme temperatures during storage (Stitz *et al*., 2017) and wrongful administration of vaccine (Vitale *et al*., 2016). Factors regarding the dog includes the age of dog, where older dogs may have a reduced immune response (Pereira *et al.*, 2011; Sridharan *et al*., 2011), and the health of the dog, where sick (Da Silva *et al*., 2018) or immunocompromised (Kopel *et al.*, 2012) dogs might not seroconvert as efficient as healthy dogs. Difference in immunization between dogs can also be explained by the usage of different brands of rabies vaccines (Mauti *et al.*, 2017).

Since Laos is a country with mainly tropical climate and average temperatures between 25-27 degrees Celsius (United Nations Development Programme, n.d.), the immunization after vaccination can very well be decreased by inadequate handling of vaccines due to deficient cold chain. This especially when conducting vaccination campaigns, since these often take place in areas where the cold chain is hard to maintain. However, a study done by Lankester *et al*. (2016) showed that the Nobivac rabies vaccine stored in higher temperatures than recommended did not reduce the efficiency of the vaccines. Nevertheless, this was not proved for the longer periods (6 months) in the higher temperatures (30 and 37 degrees), included in the study suggesting that with rising temperatures and time, the impact on quality of the vaccine increases. This further suggests that shortcomings in the handling of commercially available rabies vaccines or cold chains during transportations still can be an influencing factor on the immune status of vaccinated dogs in Lao PDR, depending on vaccine used and period of time and temperature in which it was stored.

Owner's awareness of previous rabies vaccination

When asking the owners regarding their dogs' previous vaccination status, results showed that among the dogs whose owners stated that their dog had never been vaccinated against rabies, 23.73% were positive for RABV NAb. Deviation could also be seen among the dogs where the owner stated that it had been vaccinated against rabies, where only 60.71% of the dogs were positive for RABV NAb. These deviations are alarming since they mean that there is a great number of dogs (39.29%) that does not have any detectable levels of RABV NAb, but where the owner thinks that their dog is vaccinated against rabies. Even though this can also be

explained by factors affecting the vaccine and the dog vaccinated, there are other factors to be considered.

One of these factors being time since last vaccination, where the immunization against RABV will decline over time (Pimburage *et al.*, 2017). However, in this study, time since last rabies vaccination did not seem to have any greater impact, seeing that among dogs stated being vaccinated within one year before sampling, 61.36% were positive for RABV NAb, comparable to the 58.33% of dogs that was last vaccinated over one year before the sampling. What needs to be considered is the human error factor, where remembrance of time for last vaccination might be faulty.

The human error factor might also have affected other analyses in the study. Among the dog owners that participated in this study, very few had a vaccination card for their dog, thus making it harder to remember what vaccines have been given and at what time. 23.73% of the dogs reported never being vaccinated against rabies before had detectable levels of RABV NAb, proving that not all owners were aware that their dog had been previously vaccinated against rabies. This could be caused by forgetfulness of previous vaccination in the owner, or the lack of realization that vaccination against rabies was made and could be an effect of miscommunication during any previous vaccination.

Communication difficulties during vaccination campaigns is a well addressed problem (Kaufman *et al.*, 2017), and it could very well be the case during any spay and neuter or vaccination campaign that has taken place in Lao PDR, where information regarding vaccines given not always reach the owner. Reasons for this could be language differences or lack of time due to many visitors at the campaign. Taking that into consideration in this study, it creates an error regarding duration since last vaccination, and could explain both the case where owner thought their dog was previously vaccinated and where they thought it had never been vaccinated, but respective test results were contradictory.

Impact on vaccination status by living situation

In this study there was no significant difference regarding rabies vaccination status between dogs that were kept in different ways. However, when looking at general vaccination, dogs that were living only indoors and/or outdoors in a leash, or only outdoors but in an enclosed area, were significantly more vaccinated than dogs living loose outdoors. The results were fundamentally the same when looking at time since last vaccination, where dogs that live outside loose or outside in an enclosed area are less likely to have been vaccinated within the last year. However, human error needs to be taking into consideration here as well, where owner's statements regarding latest vaccinations may differ from the actual time their dog was vaccinated.

The majority of dogs in this study were kept loose outside (326/429). Similar results have been seen in comparable areas (Hergert, Le Roux & Nel, 2018) where most dog-owning households had free roaming dogs. Since free-roaming dogs are at a greater risk of getting in contact with free roaming rabid animals, compared to dogs that are held only inside, in a leash or in an enclosed area, the importance for vaccination of dogs in areas with a large amount of free-

roaming dogs is even greater. In the same study by Hergert, Le Roux & Nel (2018), the majority of households with one or more dogs stated that at least one person in the family could handle the dog and present it for vaccination at a vaccination campaign. This being a great necessity in areas with a large proportion of free-roaming dogs due to the importance of them being vaccinated against rabies regularly to uphold a sufficient immunity against rabies (Conan *et al*., 2015). Nevertheless, the problem with capturing dogs, and the possibility of the dog escaping, in the event of a vaccination campaign will always remain with dogs held loose, making it more difficult to uphold a good immunity in areas with a great amount of free-roaming dogs.

Impact on vaccination status by district and sampling location

The four districts, from which the sampled dogs originated, are all located within the Vientiane prefecture, where Xaysetha is located closest to Vientiane capital city, followed by Xaytany and Naxaythong, and Parknguem being the district located furthest away. All dogs included in the category "other" were from districts located close to Vientiane capital city, where the vaccination campaign occurred. Comparing the different districts, the dogs in districts closer to the capital were significantly more vaccinated, as well as had a greater protection level, than dogs in districts further away. However not surprisingly since none of the government officials, who assisted in the study when sampling was done in the villages, had any records of previous vaccination campaigns in the area (Keosengthong, A., National University of Laos, Faculty of Agriculture, pers. comm., 2019) This, in combination with the test results in this study, suggests that previous vaccination campaigns in Vientiane Prefecture took place in Vientiane capital city, making it more accessible to dog owners in closer districts.

In a study done by Castillo-Neyra *et. al.* (2019), the owners' willingness to participate in vaccination campaigns decreased in proportion to the distance from the owners' house to the place of vaccination campaigns, proving the importance of conducting vaccination campaigns in close proximity to target population. Therefore, to further improve the vaccination status among dogs in Vientiane Prefecture, it would be necessary to conduct vaccination campaigns that are accessible even to people living in districts further away from Vientiane capital city.

When instead dividing dogs by where the participants were recruited, the results were that more of the dogs that were sampled at the vaccination campaign had detectable RABV NAb compared to dogs sampled at the animal clinic and in the villages. This also reflected the results of when owners were asked about the dogs' history of rabies vaccinations, where more dogs sampled at the vaccination campaign had been vaccinated against rabies before, compared to dogs sampled in villages or at the animal clinic. The influence by knowledge regarding rabies (Mucheru, Kikuvi & Amwayi, 2014) and education level (Awuni *et al.*, 2019) on owners' willingness to vaccinate have been previously recorded. This, connected to the results in this study, might suggest that dog owners who take part of vaccination campaigns have more knowledge regarding rabies and are therefore more likely to have previously vaccinated their dogs.

Noteworthy, however, is that since the vaccination campaign took place in Vientiane capital city, and more dogs closer to the city tend to be previously vaccinated, these results might have been dissimilar if the vaccination campaign took place in a rural area instead of a city. To fully

conclude that dogs taking part of vaccination campaigns are more likely to have been vaccinated before, more research needs to be made.

CONCLUSIONS

In conclusion, further work needs to be done in Lao PDR regarding rabies elimination strategies. This by engaging the society and educating about rabies and the importance of post exposure prophylaxis, as well as conducting mass dog rabies vaccination campaigns easily accessible to target populations. By doing this, the vaccination status and protection level among the canine population will be improved, the United against rabies' goal of zero human deaths of canine rabies by 2030 is achievable, and Lao PDR could become free of dog-mediated human deaths of rabies.

POPULAR SCIENCE SUMMARY

Rabies is a viral disease that can infect humans and warm-blooded vertebrae animals (CDC, 2019a). The disease is virtually always fatal once clinical signs appear (Wilde, 2007; WHO, 2019a). The main source of infection to humans are dogs (Ahmed *et al*., 2015), and the virus is most often transmitted through bites or direct contact with the dog's saliva on skin lesions (CDC, 2019b). Dogs are accountable for 99% of all human cases of rabies (WHO, 2019c) and the death of estimately 59,000 humans each year (Hampson *et al*., 2015). However, it is a disease that is 100% preventable through vaccination (Taylor & Nel, 2015).

There are two forms of rabies vaccinations; pre-exposure and post-exposure. Pre-exposure vaccination is given as a precautionary measure and can be given to humans and animals at risk of rabies infection. It creates a secondary immune response and, in the case of exposure to rabies virus, the immune system is able to partly fight infection and protect from disease (Suwansrinon *et al.,* 2006). Post-exposure vaccination is given to humans after contact, such as a bite or a scratch, with a suspected rabid animal. This further protects the person from infection and disease.

Knowledge regarding any existing immunizaiton among the dog population is necessary in order to efficiently plan and perform vaccination campaigns. Research show that if 70% of the dogs in an area are vaccinated against rabies, it is enough to prevent the spreading of the disease and thereby also human cases of rabies (Coleman & Dye, 1996; Cleaveland *et al.*, 2003). Despite this knowledge, rabies is still a big problem in many regions in the world, and most cases of human rabies are seen in Africa and Asia (Knobel *et al*., 2005).

Lao PDR is one of the countries in Southeast Asia where rabies is still a problem (Douangngeun *et al.*, 2017) and between the years 2012-2017 there have been 33 reported cases of human deaths due to rabies in the country (WHO, 2019b). The country is part of the global strategic plan "Zero by 30", where the goal is that there should be zero human deaths caused by rabid dogs by the year 2030 (WHO, 2018d). In this study, the vaccination status and protection level against rabies among 437 dogs in Vientiane Prefecture in Lao PDR was investigated.

Our results show that the proportion of positive dogs for rabies antibodies due to vaccination was 28.38%, which is not sufficient to prevent human cases of dog-mediated rabies. It also showed that there are discrepancies between dog owners' information regarding previous rabies vaccinations and the results of detectable antibodies in the dogs. Alarming, but it can however be explained by time's impact since last vaccination, where studies show that the amount of antibodies will decline over time (Pimburage *et al*., 2017). Even though time did not seem to have an impact on antibody level in this study, the human error factor needs to be taken into consideration, where the remembrance of time for last vaccination might have been wrong.

The study further showed that dogs living in districts closest to the Vientiane capital city were significantly more vaccinated and protected against rabies compared to districts further away. Research shows that the owners' willingness to participate in vaccination campaigns decreases in proportion to the distance (Castillo-Neyra *et. al.*, 2019). Since all previous vaccination campaigns in Lao PDR has been in Vientiane capital city, the results in the study is not

surprising. Furthermore, out of the dogs that were sampled at a vaccination campaign, a greater amount was previously vaccinated when compared to dogs sampled at an animal clinic or dogs sampled in villages in the districts. When instead comparing vaccination status between different living situations, no significant difference could be seen.

In conclusion, for Lao PDR to be able to reach the goal “Zero by 30”, further work needs to be done regarding rabies elimination strategies. Strategies that include educating and engaging the society regarding rabies, the symptoms and the importance of post-exposure treatment. Another important measure is conducting mass dog rabies vaccination campaigns easily accessible to target populations.

REFERENCES

Ahmed, K., Phommachanh, P., Vorachith, P., Matsumoto, T., Lamaningao, P., Mori, D., Takaki, M., Douangngeun, B., Khambounheuang, B. & Nishizono, A. (2015). Molecular epidemiology of rabies viruses circulating in two rabies endemic provinces of Laos, 2011–2012: Regional diversity in Southeast Asia. *PLoS Neglected Tropical Diseases*, 9:e0003645.

Atanasiu, P., Savy, V. & Gilbert, C. (1978). Rapid immunoenzymatic technique for titration of rabies antibodies IgG and IgM results. *Medical Microbiology and Immunology*, 166:201–208.

Awuni, B., Tarkang, E., Manu, E., Amu, H., Ayanore, M.A., Aku, F.Y., Ziema, S.A., Bosoka, S.A., Adjuik, M. & Kweku, M., (2019). Dog owners' knowledge about rabies and other factors that influence canine anti-rabies vaccination in the Upper East region of Ghana. *Tropical Medicine and Infectious Disease*, doi:10.3390/tropicalmed4030115. [2019-12-09]

Badrane, H. & Tordo, N. (2001). Host switching in lyssavirus history from the Chiroptera to the Carnivora orders. *Journal of Virology* 75:8096–8104.

Baltazard, M. & Ghodssi, M. (1954). Prevention of human rabies; Treatment of persons bitten by rabid wolves in Iran. *Bulletin of the World Health Organization*, 10:797–803.

Barton, L.D. & Campbell, J.B. (1988). Measurement of rabies-specific antibodies in carnivores by an enzyme-linked immunosorbent assay. *Journal of wildlife diseases*, 24:246–258.

Beyene, T.J., Mindaye, B., Leta, S., Cernicchiaro, N. & Revie, C.W. (2018). Understanding factors influencing dog owners' intention to vaccinate against rabies evaluated using health belief model constructs. *Frontiers in Veterinary Science*, doi: 10.3389/fvets.2018.00159. [2019-11-04]

Bharti, O.K., Madhusudana, S.N. & Wilde, H. (2017). Injecting rabies immunoglobulin (RIG) into wounds only: A significant saving of lives and costly RIG. *Human Vaccines & Immunotherapeutics*, 13:762–765.

Castillo-Neyra, R., Toledo, A.M., Arevalo-Nieto, C., MacDonald, H., De La Puente-León, M., Naquira-Velarde, C., Paz-Soldan, V.A., Buttenheim, A.M. & Levy, M.Z. (2019). Socio-spatial heterogeneity in participation in mass dog rabies vaccination campaigns, Arequipa, Peru. *PLoS Neglected Tropical Diseases*, 13:e0007600.

Centers for Disease Control and Prevention (2011-04-22). *Clinical Signs of Rabies in Animals.* https://www.cdc.gov/rabies/specific_groups/veterinarians/clinical_signs.html [2019-10-15]

Centers for Disease Control and Prevention (2016-04-15). *Rabies Serology.* https://www.cdc.gov/rabies/specific_groups/doctors/serology.html [2019-11-04]

Centers for Disease Control and Prevention (2017-10-27). *The path of the rabies virus.* https://www.cdc.gov/rabies/transmission/body.html [2019-10-26]

Centers for Disease Control and Prevention (2019a-06-11). *What kind of animal did you come in contact with?* https://www.cdc.gov/rabies/exposure/animals/index.html [2019-11-05]

Centers for Disease Control and Prevention (2019b-06-11). *How is Rabies Transmitted?* https://www.cdc.gov/rabies/transmission/index.html [2019-10-26]

Centers for Disease Control and Prevention (2019c-06-11). *What are the signs and symptoms of rabies?* https://www.cdc.gov/rabies/symptoms/index.html [2019-10-24]

Central Intelligence Agency (CIA) (2019-10-01). *The World Factbook – East Asia/Southeast Asia: Laos.* https://www.cia.gov/library/publications/the-world-factbook/geos/la.html [2019-11-07]

Chabaud-Riou, M., Moreno, N., Guinchard, F., Nicolai, M.C., Niogret-Siohan, E., Sève, N., Manin, C., Guinet-Morlot, F. & Riou, P. (2017). G-protein based ELISA as a potency test for rabies vaccines. *Biologicals*, 46:124–129.

Chomchay, P., Khawplod, P. & Wilde, H. (2000). Neutralizing antibodies to rabies following injection of rabies immune globulin into gluteal fat or deltoid muscle. *Journal of Travel Medicine*, 7:187–188.

Cleaton, J.M., Wallace, R.M., Crowdis, K., Gibson, A., Monroe, B., Ludder, F., Etheart, M.D., Vigilato, M.A.N. & King, A. (2018). Impact of community-delivered SMS alerts on dog-owner participation during a mass rabies vaccination campaign, Haiti 2017. *Vaccine*, 36:2321–2325.

Cleaveland, S., Kaare, M., Tiringa, P., Mlengeya, T. & Barrat, J. (2003). A dog rabies vaccination campaign in rural Africa: Impact on the incidence of dog rabies and human dog-bite injuries. *Vaccine*, 21:1974–1982.

Cliquet, F., Aubert, M. & Sagné, L. (1998). Development of a fluorescent antibody virus neutralisation test (FAVN test) for the quantitation of rabies-neutralising antibody. *Journal of Immunological Methods*, 212:79–87.

Cliquet, F., Sagné, L., Schereffer, J.L. & Aubert, M.F.A. (2000). ELISA test for rabies antibody titration in orally vaccinated foxes sampled in the fields. *Vaccine*, 18:3272–3279.

Coleman, P.G. & Dye, C. (1996). Immunization coverage required to prevent outbreaks of dog rabies. *Vaccine*, 14:185–186.

Conan, A., Akerele, O., Simpson, G., Reininghaus, B., van Rooyen, J. & Knobel, D. (2015). Population dynamics of owned, free-roaming dogs: Implications for rabies control. *PLoS Neglected Tropical Diseases*, 9:e0004177.

Corona, T.F., Böger, B., Da Rocha, T.C., Svoboda, W.K. & Gomes, E.C. (2018). Comparative analysis of mouse inoculation test and virus isolation in cell culture for rabies diagnosis in animals of Parana, Brazil. *Revista da Sociedade Brasileira de Medicina Tropical*, 51:39–43.

Dacheux, L., Wacharapluesadee, S., Hemachudha, T., Meslin, F.X., Buchy, P., Reynes, J.M. & Bourhy, H. (2010). More accurate insight into the incidence of human rabies in developing countries through validated laboratory techniques. *PLoS Neglected Tropical Diseases*, 4:e765.

Dacheux, L. & Bourhy, H. (2015). Virus isolation in cell culture: The rabies tissue culture infection test. *Current Laboratory Techniques in Rabies Diagnosis, Research and Prevention*, 2:25–31.

Damodar, T., Mani, R.S. & Prathyusha, P. V. (2019). Utility of rabies neutralizing antibody detection in cerebrospinal fluid and serum for ante-mortem diagnosis of human rabies. *PLoS Neglected Tropical Diseases*, 13:e0007128.

Da Silva, E.N., Baker, A., Alshekaili, J., Karpe, K. & Cook, M.C. (2018). A randomized trial of serological and cellular responses to hepatitis B vaccination in chronic kidney disease. *PLoS ONE*, 13: e0204477.

Davis, A.D., Rudd, R.J. & Bowen, R.A. (2007). Effects of aerosolized rabies virus exposure on bats and mice. *The Journal of Infectious Diseases*, 195:1144–1150.

Dhayhi, N.S., Arishi, H.M., Al Ibrahim, A.Y., Khalaf Allah, M.B., Hawas, A.M., Alqasmi, H., Sairam, I., Thubab, A., Buraik, M. & Alali, A. (2019). First confirmed case of local human rabies in Saudi Arabia. *International Journal of Infectious Diseases*, 87:117–118.

Dodet, B., Goswami, A., Gunasekera, A., de Guzman, F., Jamali, S., Montalban, C., Purba, W., Quiambao, B., Salahuddin, N., Sampath, G., Tang, Q., Tantawichien, T., Wimalaratne, O. & Ziauddin, A. (2008). Rabies awareness in eight Asian countries. *Vaccine*, 26:6344–6348.

Douangngeun, B., Theppangna, W., Phommachanh, P., Chomdara, K., Phiphakhavong, S., Khounsy, S., Mukaka, M., Dance, D.A.B. & Blacksell, S.D. (2017). Rabies surveillance in dogs in Lao PDR from 2010-2016. *PLoS Neglected Tropical Diseases*, 11:e0005609.

Fekadu, M., Shaddock, J.H. & Baer, G.M. (1982). Excretion of rabies virus in the saliva of dogs. *The Journal of Infectious Diseases*, 145:715–719.

Folkhälsomyndigheten (2019). *Rekommendationer om förebyggande åtgärder mot rabies.* Solna: Folkhälsomyndigheten. https://www.folkhalsomyndigheten.se/publicerat-material/publikationsarkiv/r/rekommendationer-om-forebyggande-atgarder-mot-rabies/?pub=61367

Freuling, C.M., Hampson, K., Selhorst, T., Schröder, R., Meslin, F.X., Mettenleiter, T.C. & Müller, T. (2013). The elimination of fox rabies from Europe: Determinants of success and lessons for the future. *Philosophical Transactions of the Royal society*, doi: 10.1098/rstb.2012.0142. [2019-12-11]

Global Alliance for Rabies Control (GARC) (2017-11-21) *WHO work group on rabies vaccines and rabies immunoglobulins forms new recommendations* https://rabiesalliance.org/resource/who-work-group-rabies-vaccines-and-rabies-immunoglobulins-forms-new-recommendations [2019-11-23]

Goswami, A., Plun-Favreau, J., Nicoloyannis, N., Sampath, G., Siddiqui, M.N. & Zinsou, J.A.,(2005). The real cost of rabies post-exposure treatments. *Vaccine*, 23:2970–2976.

Hampson, K., Coudeville, L., Lembo, T., Sambo, M., Kieffer, A., Attlan, M., Barrat, J., Blanton, J.D., Briggs, D.J., Cleaveland, S., Costa, P., Freuling, C.M., Hiby, E., Knopf, L., Leanes, F., Meslin, F.X., Metlin, A., Miranda, M.E., Müller, T., Nel, L.H., Recuenco, S., Rupprecht, C.E., Schumacher, C., Taylor, L., Vigilato, M.A.N., Zinsstag, J. & Dushoff, J. (2015). Estimating the global burden of endemic canine rabies. *PLoS Neglected Tropical Diseases*, 9:e0003709.

Hergert, M., Le Roux, K. & Nel, L.H. (2018). Characteristics of owned dogs in rabies endemic KwaZulu-Natal province, South Africa. *BMC Veterinary Research*, doi: 10.1186/s12917-018-1604-z. [2019-12-09]

Huang, X.-Y., Li, X.-L., Wu, S.-Y., Gu, Y.-L., Lv, X.-J., Klena, J.D. & Xu, B.-L. (2017). Bites from the same dog, different outcomes for two patients: A case report. *Infectious Diseases of Poverty*, doi:10.1186/s40249-017-0321-3. [2019-11-28]

Kaufman, J., Ames, H., Bosch-Capblanch, X., Cartier, Y., Cliff, J., Glenton, C., Lewin, S., Muloliwa, A.M., Oku, A., Oyo-Ita, A., Rada, G. & Hill, S. (2017). The comprehensive 'Communicate to Vaccinate' taxonomy of communication interventions for childhood vaccination in routine and campaign contexts. *BMC Public Health*, doi: 10.1186/s12889-017-4320-x. [2019-12-09]

Khawplod, P., Wilde, H., Sirikwin, S., Benjawongkulchai, M., Limusanno, S., Jaijaroensab, W., Chiraguna, N., Supich, C., Wangroongsarb, Y. & Sitprija, V. (2006). Revision of the Thai red cross intradermal rabies post-exposure regimen by eliminating the 90-day booster injection. *Vaccine*, 24:3084–3086.

Knobel, D.L., Cleaveland, S., Coleman, P.G., Fèvre, E.M., Meltzer, M.I., Miranda, M.E.G., Shaw, A., Zinsstag, J. & Meslin, F.X. (2005). Re-evaluating the burden of rabies in Africa and Asia. *Bulletin of the World Health Organization*, 83:360–368.

Kopel, E., Oren, G., Sidi, Y. & David, D. (2012). Inadequate antibody response to rabies vaccine in immunocompromised patient. *Emerging Infectious Diseases*, 18:1493–1495.

Kundu, B.K., Meshram, G.G., Bhargava, S. & Meena, O. (2019). Cost savings of using updated Thai red cross intradermal regimen in a high-throughput anti-rabies clinic in New Delhi, India. *Tropical Medicine and Infectious Disease*, doi:10.3390/tropicalmed4010050 [2019-11-22]

Lankester, F.J., Wouters, P.A.W.M., Czupryna, A., Palmer, G.H., Mzimbiri, I., Cleaveland, S., Francis, M.J., Sutton, D.J. & Sonnemans, D.G.P. (2016). Thermotolerance of an inactivated rabies vaccine for dogs. *Vaccine*, 34:5504-5511.

Laothamatas, J., Wacharapluesadee, S., Lumlertdacha, B., Ampawong, S., Tepsumethanon, V., Shuangshoti, S., Phumesin, P., Asavaphatiboon, S., Worapruekjaru, L., Avihingsanon, Y., Israsena, N., Lafon, M., Wilde, H. & Hemachudha, T. (2008). Furious and paralytic rabies of canine origin: Neuroimaging with virological and cytokine studies. *Journal of NeuroVirology*, 14:119–129.

Lapiz, S.M.D., Miranda, M.E.G., Garcia, R.G., Daguro, L.I., Paman, M.D., Madrinan, F.P., Rances, P.A. & Briggs, D.J. (2012). Implementation of an intersectoral program to eliminate human and canine rabies: The Bohol rabies prevention and elimination project. *PLoS Neglected Tropical Diseases*, 6:e1891.

Ma, J., Wang, H., Li, J., Chang, L., Xie, Y., Liu, Z., Zhao, Y., Claudius, M., Ma, J., Wang, H., Li, J., Chang, L., Xie, Y., Liu, Z., Zhao, Y. and Claudius, M. (2015). A randomized open-labeled study to demonstrate the non-inferiority of purified chick-embryo cell rabies vaccine administered in the Zagreb regimen (2-1-1) compared with the Essen regimen in Chinese adults. *Human Vaccines & Immunotherapeutics*, 10:2805-2812.

Madhusudana, S.N., Ashwin, B.Y. & Sudarshan, S. (2013). Feasibility of reducing rabies immunoglobulin dosage for passive immunization against rabies : Results of in vitro and in vivo studies. *Human Vaccines and Immunotherapeutics*, 9:1914–1917.

Malerczyk, C., Vakil, H.B. & Bender, W. (2013). Rabies pre-exposure vaccination of children with Purified Chick Embryo Cell Vaccine (PCECV). *Human Vaccines and Immunotherapeutics*, 9:1454–1459.

Manalo, D.L., Yamada, K., Watanabe, I., Miranda, M.E.G., Lapiz, S.M.D., Tapdasan, E., Petspophonsakul, W., Inoue, S., Khawplod, P. & Nishizono, A. (2017). A comparative study of the RAPINA and the virus-neutralizing test (RFFIT) for the estimation of antirabies-neutralizing antibody levels in dog samples. *Zoonoses and Public Health*, 64:355–362.

Mauti, S., Traoré, A., Hattendorf, J., Schelling, E., Wasniewski, M., Schereffer, J.L., Zinsstag, J. & Cliquet, F. (2017). Factors associated with dog rabies immunisation status in Bamako, Mali. *Acta Tropica*, 165:194–202.

Mbilo, C., Kabongo, J.-B., Pyana, P.P., Nlonda, L., Nzita, R.W., Luntadila, B., Badibanga, B., Hattendorf, J. & Zinsstag, J. (2019). Dog ecology, bite incidence, and disease awareness: A cross-sectional survey among a rabies-affected community in the democratic republic of the Congo. *Vaccines*, doi: 10.3390/vaccines7030098. [2019-09-29]

Mebatsion, T., Weiland, F. & Conzelmann, K.K. (1999). Matrix protein of rabies virus is responsible for the assembly and budding of bullet-shaped particles and interacts with the transmembrane spike glycoprotein G. *Journal of virology*, 73:242–250.

Morters, M.K., McNabb, S., Horton, D.L., Fooks, A.R., Schoeman, J.P., Whay, H.R., Wood, J.L.N. & Cleaveland, S. (2015). Effective vaccination against rabies in puppies in rabies endemic regions. *Veterinary Record*, doi: 10.1136/vr.102975. [2019-11-13]

Mucheru, G.M., Kikuvi, G.M. & Amwayi, S.A., (2014). Knowledge and practices towards rabies and determinants of dog rabies vaccination in households: A cross sectional study in an area with high dog bite incidents in Kakamega County, Kenya, 2013. *Pan African Medical Journal*, doi:10.11604/pamj.2014.19.255.4745. [2019-12-09]

Muhamuda, K., Madhusudana, S.N. & Ravi, V. (2007). Development and evaluation of a competitive ELISA for estimation of rabies neutralizing antibodies after post-exposure rabies vaccination in humans. *International Journal of Infectious Diseases*, 11:441–445.

Muyila, D.I., Aloni, M.N., Lose-Ekanga, M.J., Nzita, J.M., Kalala-Mbikay, A., Bongo, H.L., Esako, M.N., Malonga-Biapi, J.P., Mputu-Dibwe, B., Aloni, M.L. & Ekila, M.B. (2014). Human rabies: A descriptive observation of 21 children in Kinshasa, the democratic republic of Congo. *Pathogens and Global Health*, 108:317–322.

Nishizono, A., Yamada, K., Khawplod, P., Shiota, S., Perera, D., Matsumoto, T., Wimalaratne, O., Mitui, M.T. & Ahmed, K. (2012). Evaluation of an improved rapid neutralizing antibody detection test (RAPINA) for qualitative and semiquantitative detection of rabies neutralizing antibody in humans and dogs. *Vaccine*, 30:3891–3896.

Nguyen, K.A.T., Nguyen, T.T., Nguyen, D.V., Ngo, G.C., Nguyen, C.N., Yamada, K., Noguchi, K., Ahmed, K., Hoang, H.D. & Nishizono, A. (2015). Evaluation of rapid neutralizing antibody detection test against rabies virus in human sera. *Tropical Medicine and Health*, 43:111–116.

Ondrejková, A., Süli, J., Ondrejka, R., Beníšek, Z., Franka, R., Švrček, S., Madar, M. & Bugarský, A. (2002). Comparison of the detection and quantification of rabies antibodies in canine sera. *Veterinarni Medicina*, 47:218–221.

Pereira, L.F., de Souza, A.P. D., Borges, T.J. & Bonorino, C. (2011). Impaired in vivo CD4+ T cell expansion and differentiation in aged mice is not solely due to T cell defects: Decreased stimulation by aged dendritic cells. *Mechanisms of Ageing and Development*, 132:187–194.

Pimburage, R.M.S., Gunatilake, M., Wimalaratne, O., Balasuriya, A. & Perera, K.A.D.N. (2017). Sero-prevalence of virus neutralizing antibodies for rabies in different groups of dogs following vaccination. *BMC Veterinary Research*, doi: 10.1186/s12917-017-1038-z. [2019-12-09]

Polupan, I., Bezymennyi, M., Gibaliuk, Y., Drozhzhe, Z., Rudoi, O., Ukhovskyi, V., Nedosekov, V. & De Nardi, M. (2019). An analysis of rabies incidence and its geographic spread in the buffer area among orally vaccinated wildlife in Ukraine from 2012 to 2016. *Frontiers in Veterinary Science*, doi: 10.3389/fvets.2019.00290. [2019-09-29]

Public Health England (PHE) (2019-05-01). *Rabies risks in terrestrial animals by country.* https://www.gov.uk/government/publications/rabies-risks-by-country/rabies-risks-in-terrestrial-animals-by-country [2019-11-13]

Qi, L., Su, K., Shen, T., Tang, W., Xiao, B., Long, J., Zhao, H., Chen, X. & Xia, Y. (2018). Epidemiological characteristics and post-exposure prophylaxis of human rabies in Chonqing, China, 2007-2016. *BMC Infectious Diseases*, doi: 10.1186/s12879-017-2830-x [2019-11-14]

Ravish, H.S., Sudarshan, M.K., Madhusudana, S.N., Annadani, R.R., Ashwath Narayana, D.H., Belludi, A.Y., Anandaiah, G. &Vijayashankar, V. (2014). Assessing safety and immunogenicity of post-exposure prophylaxis following interchangeability of rabies vaccines in humans. *Human Vaccines and Immunotherapeutics*, 10:1354–1358.

Ren, J., Yao, L., Sun, J. & Gong, Z. (2015). Zagreb regimen, an abbreviated intramuscular schedule for rabies vaccination. *Clinical and Vaccine Immunology*, doi:10.1128/CVI.00531-14 [2019-11-22]

Rupprecht, C., Kuzmin, I. & Meslin, F. (2017). Lyssaviruses and rabies: Current conundrums, concerns, contradictions and controversies. *F1000Research*, doi:10.12688/f1000research.10416.1. [2019-10-13]

Shiota, S., Mannen, K., Matsumoto, T., Yamada, K., Yasui, T., Takayama, K., Kobayashi, Y., Khawplod, P., Gotoh, K., Ahmed, K., Iha, H. & Nishizono, A. (2009). Development and evaluation of a rapid neutralizing antibody test for rabies. *Journal of Virological Methods*, 161:58–62.

Shiwa, N., Yamashita, H., Tomioka, K., Kimitsuki, K., Manalo, D.L., Inoue, S. & Park, C.H. (2019). Statistical analysis of the usefulness of follicle-sinus complexes as a novel diagnostic material for canine rabies. *Journal of Veterinary Medical Science*, 81:182–185.

Shulpin, M.I., Nazarov, N.A., Chupin, S.A., Korennoy, F.I., Metlin, A.Y. & Mischenko, A. V. (2018). Rabies surveillance in the Russian Federation. *Revue scientifique et technique (International Office of Epizootics)*, 37:483–495.

Smith, J.S., Yager, P.A. & Baer, G.M. (1973). A rapid reproducible test for determining rabies neutralizing antibody. *Bulletin of the World Health Organization*, 48:535-541.

Smith, T.G., Millien, M., Vos, A., Fracciterne, F.A., Crowdis, K., Chirodea, C., Medley, A., Chipman, R., Qin, Y., Blanton, J. & Wallace, R. (2017). Evaluation of immune responses in dogs to oral rabies vaccine under field conditions. *Vaccine*, 37:4743–4749.

Soentjens, P., De Koninck, K., Tsoumanis, A., Herssens, N., Van Den Bossche, D., Terryn, S., Van Gucht, S., Van Damme, P., Van Herrewege, Y. & Bottieau, E. (2019). Comparative immunogenicity and safety trial of 2 different schedules of single-visit intradermal rabies postexposure vaccination. *Clinical Infectious Diseases*, 69:797-804

Sridharan, A., Esposo, M., Kaushal, K., Tay, J., Osann, K., Agrawal, S., Gupta, S. & Agrawal, A. (2011). Age-associated impaired plasmacytoid dendritic cell functions lead to decreased CD4 and CD8 T cell immunity. *GeroScience*, 33:363–376.

Stitz, L., Vogel, A., Schnee, M., Voss, D., Rauch, S., Mutzke, T., Ketterer, T., Kramps, T. & Petsch, B. (2017). A thermostable messenger RNA based vaccine against rabies. *PLoS Neglected Tropical Diseases*, 11: e0006108.

Suraweera, W., Morris, S.K., Kumar, R., Warrell, D.A., Warrell, M.J. & Jha, P. (2012). Deaths from symptomatically identifiable furious rabies in India: A nationally representative mortality survey. *PLoS Neglected Tropical Diseases*, 6:e1847.

Suwansrinon, K., Wilde, H., Benjavongkulchai, M., Banjongkasaena, U., Lertjarutorn, S., Boonchang, S., Suttisri, R., Khowplod, P., Daviratanasilpa, S. & Sitprija, V. (2006). Survival of neutralizing antibody in previously rabies vaccinated subjects: A prospective study showing long lasting immunity. *Vaccine*, 24:3878–3880.

Tarantola, A., Ly, S., Chan, M., In, S., Peng, Y., Hing, C., Taing, C.N., Phoen, C., Ly, S., Cauchemez, S., Buchy, P., Dussart, P., Bourhy, H. & Mary, J.Y., (2019). Intradermal rabies post-exposure prophylaxis can be abridged with no measurable impact on clinical outcome in Cambodia, 2003–2014. *Vaccine*, 37:A118–A127.

Taylor, L.H, & Nel, L.H. (2015). Global epidemiology of canine rabies: past, present, and future prospects. *Veterinary Medicine: Research and Reports*, 6:361-371.

Townsend, S.E., Sumantra, I.P., Pudjiatmoko, Bagus, G.N., Brum, E., Cleaveland, S., Crafter, S., Dewi, A.P.M., Dharma, D.M.N., Dushoff, J., Girardi, J., Gunata, I.K., Hiby, E.F., Kalalo, C., Knobel, D.L., Mardiana, I.W., Putra, A.A.G., Schoonman, L., Scott-Orr, H., Shand, M., Sukanadi, I.W., Suseno, P.P., Haydon, D.T. & Hampson, K. (2013). Designing programs for eliminating canine rabies from islands: Bali, Indonesia as a case study. *PLoS Neglected Tropical Diseases*, 7:e2372.

United Nations Development Programme (UNDP) (n.d.). *Lao People's Democratic Republic.* https://www.adaptation-undp.org/explore/south-eastern-asia/lao-peoples-democratic-republic [2019-12-06]

Venkataswamy, M.M., Madhusudana, S.N., Sanyal, S.S., Taj, S., Belludi, A.Y., Mani, R.S. & Hazra, N. (2015). Cellular immune response following pre-exposure and postexposure rabies vaccination by intradermal and intramuscular routes. *Clinical and Experimental Vaccine Research*, 4:68-74.

Vitale, N., Radaelli, M.C., Chiavacci, L., Paoletti, M., Teodori, L. & Savini, G. (2016). Factors affecting seroconversion rates in cattle vaccinated with two commercial inactivated BTV-8 vaccines under field conditions. *Transboundary and Emerging Diseases*, 63:175–183.

Wallace, R.M., Bhavnani, D., Russell, J., Zaki, S., Muehlenbachs, A., Hayden-Pinneri, K., Aplícano, R.M., Peruski, L., Vora, N.M., Elson, D., Lederman, E., Leeson, B., McLaughlin, T., Waterman, S., Fonseca-Ford, M., Blanton, J., Franka, R., Velasco-Villa, A., Niezgoda, M., Orciari, L., Recuenco, S., Damon, I., Hanlon, C., Jackson, F., Dyer, J., Wadhwa, A. & Robinson, L. (2014). Rabies death attributed to exposure in Central America with symptom onset in a U.S. detention facility - Texas, 2013. *Morbidity and Mortality Weekly Report*, 63:446–449.

Wallace, R.M., Pees, A., Blanton, J.B. & Moore, S.M. (2017). Risk factors for inadequate antibody response to primary rabies vaccination in dogs under one year of age. *PLoS Neglected Tropical Diseases*, 11:e0005761.

Wambura, G., Mwatondo, A., Muturi, M., Nasimiyu, C., Wentworth, D., Hampson, K., Bichanga, P., Tabu, C., Juma, S., Ngere, I. & Thumbi, S.M. (2019). Rabies vaccine and immunoglobulin supply and logistics: Challenges and opportunities for rabies elimination in Kenya. *Vaccine*, 37:A28–A34.

Wasniewski, M. & Cliquet, F. (2012). Evaluation of ELISA for detection of rabies antibodies in domestic carnivores. *Journal of Virological Methods*, 179:166–175.

Wasniewski, M., Labbe, A., Tribout, L., Rieder, J., Labadie, A., Schereffer, J.L. & Cliquet, F. (2014). Evaluation of a rabies ELISA as an alternative method to seroneutralisation tests in the context of international trade of domestic carnivores. *Journal of Virological Methods*, 195:211–220.

Webster, L. T., & Dawson, J.R. (1935). Early diagnosis of rabies by mouse inoculation. Measurement of humoral immunity to rabies by mouse protection test. *Proceedings of the Society for Experimental Biology and Medicine*, 32:570-573.

Wertheim, H.F.L., Nguyen, T.Q., Nguyen, K.A.T., De Jong, M.D., Taylor, W.R.J., Le, T. V., Nguyen, H.H., Nguyen, H.T.H., Farrar, J., Horby, P. & Nguyen, H.D. (2009). Furious rabies after an atypical exposure. *PLoS Medicine*, 6:e1000044

Weyer, J., Szmyd-Potapczuk, A.V., Blumberg, L.H., Leman, P.A., Markotter, W., Swanepoel, R., Paweska, J.T. & Nel, L.H. (2011). Epidemiology of human rabies in South Africa, 1983-2007. *Virus Research*, 155:283–290.

Wilde, H., Choomkasien, P., Hemachudha, T., Supich, C. & Chutivongse, S. (1989). Failure of rabies postexposure treatment in Thailand. *Vaccine*, 7:49–52.

Wilde, H. (2007). Failures of post-exposure rabies prophylaxis. *Vaccine*, 25:7605–7609.

Wilde, H., Lumlertdacha, B., Meslin, F.X., Ghai, S. & Hemachudha, T. (2016). Worldwide rabies deaths prevention - A focus on the current inadequacies in postexposure prophylaxis of animal bite victims. *Vaccine*, 34:187–189.

World Health Organization (WHO) (2013-11-07). *Symptoms & pre-exposure immunization.* https://www.who.int/rabies/human/sympt_pre_exp/en/ [2019-11-13]

World Health Organization (WHO) (2016a-08-18). *Rabies – Diagnosis.* https://www.who.int/rabies/about/home_diagnosis/en/ [2019-11-13]

World Health Organization (WHO) (2016b-08-18). *Tests for the determination of rabies antibodies.* https://www.who.int/rabies/human/test_antibody/en/ [2019-11-04]

World Health Organization (WHO) (2018a). *WHO Expert Consultation on rabies, third report.* Geneve: World Health Organization. https://apps.who.int/iris/bitstream/handle/10665/272364/9789241210218-eng.pdf?sequence=1&isAllowed=y

World Health Organization (WHO) (2018b-01-15). *WHO announces new rabies recommendations.* https://www.who.int/neglected_diseases/news/Rabies_WHO_has_published_new_recommendations_for_immunization/en/ [2019-11-13]

World Health Organization (WHO) (2018c). *Rabies vaccines and immunoglobulins: WHO position*. Geneve: World Health Organization. https://apps.who.int/iris/bitstream/handle/10665/259855/WHO-CDS-NTD-NZD-2018.04-eng.pdf?sequence=1

World Health Organization (WHO) (2018d-08-28). *Rabies.* https://www.who.int/rabies/United_against_Rabies/en/ [2019-11-07]

World Health Organization (WHO) (2019a-11-27). *Rabies – Overview.* https://www.who.int/health-topics/rabies#tab=tab_1 [2019-11-06]

World Health Organization (WHO) (2019b-10-07). *Reported number of human rabies deaths - Data by country*. http://apps.who.int/gho/data/node.main.NTDRABIESHUMANDEATHS?lang=en, [2019-10-16]

World Health Organization (WHO) (2019c-09-27). *Rabies - Key facts.* https://www.who.int/news-room/fact-sheets/detail/rabies [2019-10-23]

World Health Organization (WHO) (2019d-10-13). *Human rabies prevention and management.* https://www.who.int/activities/human-rabies-prevention-and-management [2019-10-13]

World Health Organization (WHO) (n.d.). *Transmission and pathogenesis.* https://www.who-rabies-bulletin.org/site-page/transmission-and-pathogenesis [2019-10-26]

Zhang, J., Lin, J., Tian, Y., Ma, L., Sun, W., Zhang, L., Zhu, Y., Qiu, W. & Zhang, L. (2018). Transmission of rabies through solid organ transplantation: A notable problem in China. *BMC Infectious Diseases*, doi:10.1186/s12879-018-3112-y. [2019-11-08]

Zhao, H., Zhang, J., Cheng, C. & Zhou, Y.H. (2019). Rabies acquired through mucosal exposure, China, 2013. *Emerging Infectious Diseases*, 25:1028–1029.