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Factors of Maintenance of Rabies Transmission in Dogs in Kinshasa, Democratic Republic of the Congo

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Introduction

Rabies is a zoonotic disease responsible for an estimated 61 000 human deaths per year in the world, predominantly in Asia and Africa (WHO, 2013). A wide range of mammals are susceptible and can transmit rabies. The order of carnivora including domestic dogs (*Canis lupus*), raccoons (*Procyon lotor*), skunks (*Spilogale putorius*), foxes (*Vulpes vulpes*), jackals (*Canis aureus*) and the order of Chiroptera (bats) are considered as reservoirs

(Sedganti and al., 1990). The dog is responsible for 98% of rabies cases in Africa and Asia (Knobel et al., 2005).

The first rabies outbreak in Africa was reported in Algeria in 1858 (Steel, 1975). In the Democratic Republic of Congo (DRC), the first dog rabies outbreak was reported in 1923 (Repetto, 1932). Then, from 1938 to 2017, the published and unpublished laboratory data revealed that close to 1400 dog rabies cases were confirmed across the country by the three national veterinary laboratories of Kisangani, Lubumbashi and Kinshasa (Courtois et al., 1964; Makumbu, 1977; Bula and Mafwala, 1988; Twabela et al., 2016). In Kinshasa, the capital of the DRC, 152 dog-related human rabies cases were reported from 2009 to 2017. Most of these victims were children under 15 years old (Muyila et al., 2014; OVCR, unpublished data). It is likely that these official rabies data are under-reported. Indeed, active surveillance studies illustrated that official reports underestimate the abundance of rabies cases in low-income countries such as in Tanzania, Ethiopia and Bhutan (Cleaveland et al. 2002; Hampson et al., 2008; Deresa et al., 2010; Tenzin et al., 2011; Jemberu et al., 2013). In the DRC, field evaluations have evidenced the poor performances of veterinary services. These are explained by inefficient surveillance system and limited diagnostic capacity of national veterinary laboratories (Niang and Denormandie, 2008; Diop et al., 2012; Ministère de la Pêche et de l'Élevage, 2017).

Nearly one century after the first reported rabies outbreak (Repetto, 1932), dog rabies is still a public health threat in the DRC. Given that the World Health Organization (WHO), the World Organization for Animal Health (OIE) and the Food and Agriculture Organization (FAO) have set a global target of zero human deaths from dog-transmitted rabies by 2030 (Global Alliance for Rabies Control, 2015; Wallace et al., 2017; Fahrion et al., 2017), the challenge for DRC remains considerable. Despite mandatory rabies vaccination of dogs since 1938 in DRC (Royal Decree of 01 April 1938) disease control remains ineffective. It is

therefore important to investigate the reasons for the maintenance of rabies in dog populations and identify regions presenting the highest risk of rabies transmission. Risk factors such as dog density, poor dog management leading to free roaming, low vaccination coverage and wide biodiversity increasing the number of the rabies virus reservoirs have been identified in other countries such as in Zimbabwe, Tanzania (Foggin, 1988; Brooks, 1990; Cleaveland and Dye, 1995; Aréchiga et al.,2014), but no data are so far available for DRC.

Accordingly, the aims of this study were (i) to investigate the risk factors of rabies transmission between dogs in Kinshasa and (ii) to establish a risk map of rabies transmission by considering these risk factors. Risk factor assessment included the characterization of the dog population and its management as well as the evaluation of dogs' vaccination coverage against rabies in Kinshasa.

Materials and Methods

Study area

The study area was the capital of DRC, Kinshasa. This megalopolis is divided in 24 *communes*, further subdivided in *quartiers* including plots with one or more households (Decree N°08/016, 07 October 2008). The study was conducted from January 2017 to March 2018 in three *communes* where most dog rabies cases had been reported by the “Office de Vaccination et Contrôle de la Rage (OVCR)” in Kinshasa between 2003 and 2017 (unpublished data), ie Mont-Ngafula, Ngaliema and Lemba. In these *communes*, 22 *quartiers* were selected as primary sample units (study sites).

Characterization and management of the dog population

Dog density and population structure

A household questionnaire survey was conducted in the 22 study sites by selecting at least 20 dog-owning households per site. In each study site, the investigators walked in the streets, visited plots and contacted each household until 20 households with at least one dog were reached. All households with no dog were also recorded. This purposive sampling was done instead of random or systematic sampling because household lists and numbers for each study sites were not available. We assumed that households were sufficiently homogenous for important selection biases not occurring.

A questionnaire was used to collect data including: (i) the number of households on the plot, (ii) the number of dog-owning households, (iii) the number of households with no dogs, (iv) the number of people living in dog-owning households, (v) the number of people living in households with no dogs, (vi) the number of dogs owned per household and (vii) the individual description of owned dogs (gender, age and breed). The identified dogs were classified according to sex (male, female), age (puppies: less than 3 months, juveniles: from 3 to 12 months and adults: more than 12 months old) and breed (local breeds, crossed breeds, pure breeds). Data were expressed as relative frequencies.

The dog density was estimated from the ratio between the projected human density and the estimated Human to Dog Ratio (HDR). The projected human density data was obtained from the civil administration. The HDR is one of the best indicators of dog population abundance (WHO, 1987; Oboegbulem and Nwakonobi, 1989). It was calculated from the ratio between the total number of people recorded in visited households with or without dog and the total number of dogs recorded in visited dog owning-households. Ownerless dogs (see below) were excluded from the calculation of dog density.

Dog management

In order to estimate the proportion of restrained and free roaming owned dogs, the household questionnaire also addressed (i) the level of dog confinement (fully tied or caged,

intermittently tied or caged, free roaming), (ii) the type of plot (plot with or without fence/wall or any physical barrier that restrained dog's movement), and (iii) the dog feeding (provided by the owner or ensured by the dog itself during roaming). In addition, the reasons for dog abandonment were addressed in open questions.

An owned dog was considered as restrained if fencing, tying or caging completely prevented its roaming behavior. All intermittently or non-restrained dogs were considered as potentially free-roaming. Results regarding restrained and roaming dogs, as well as reasons for dog abandonment were also expressed as relative frequencies.

In order to evaluate dogs' roaming behavior, 16 free-roaming dogs (8 males and 8 females) owned by members of the academic staff of the University of Kinshasa (UNIKIN) and inhabiting the University campus were tracked during 24 hours using GPS collars. The majority of these dogs (15/16) were adults (≥ 12 months). The GPS I-GOTU GT-600 (I-gotU company) was programmed to take a GPS location each minute. The maximum distance covered by each dog was calculated based on GPS coordinates of the household and the most distant record using the formula available at <http://www.ipnas.org/garnir/donneesGPS>. In addition, the direct or indirect contact rate of tracked dogs with other free-roaming dogs was iteratively estimated in four steps by using the Quantum GIS software (<http://www.qgis.org>): (i) generation of a buffer zone which refers to the potential area covered by a tracked dog. The radius of the buffer zone corresponded to the maximum distance covered by each tracked dog, (ii) calculation of the area of the administrative (*quartier*) unit that was covered by the buffer zone and that we call "intersection area", (iii) estimation of the number of potentially free-roaming dogs per intersection area by considering the calculated dog density and the percentage of potentially free-roaming dogs in each respective *quartier*, and (iv) estimation of the contact rate with free-roaming dogs within the buffer zone by summarizing the number of dogs in intersection areas.

The percentage of ownerless or feral dogs was assessed in two study sites (Mitendi and Mongala) of the *communes* Monga-Ngafula and Ngaliema by the street count method, which is a modification of the sight-resight method (WHO, 1987). A total of 185 (Mitendi) and 110 (Mongala) owned dogs were identified with a yellow nylon rope used as collar. The following day, dog counters walked once in the morning (8 am) and once in the evening (6 pm) through the study sites and recorded identified and non-identified free-roaming dogs

Rabies vaccination

Vaccination coverage

The household survey also assessed the vaccination status of owned dogs by considering owner's report (history of vaccination and time point of last vaccination) or the vaccination certificate (if available). Reasons for not vaccinating dogs were addressed by semi-structured questions. The vaccination coverage was estimated for each study site from the ratio between the numbers of reported vaccinated dogs (independently of time since vaccination) and the number of identified dogs, including puppies of less than three months.

The vaccination status of the dogs (binary variable: vaccinated or not) was analysed using a cluster robust multivariable logistic regression in STATA software 11.0 (Stata Corp., college Station, Texas). Categorical explanatory variables were the sex of the animals (male, female), their age categories (puppies, juveniles, adults), their breed (local, crossed, pure breeds) and management (free, non-roaming). The robust model, which is more conservative, accounts for a possible design effects (DEFT) caused by the 22 study sites considered as clusters or primary sampling units. The relevance of the cluster robust model was evaluated by calculating and evaluating DEFT for each explanatory variable (Kreuter and Valliant, 2007).

The owner's reasons for not vaccinating dogs were aggregated and results were expressed as relative frequencies.

Serological evaluation of the immunization status of vaccinated dogs

Further to oral consent of the owners, 132 supposed vaccinated dogs aged between six months and fourteen years of Mont-Ngafula, Ngaliema and Lemba *communes* underwent venous blood collection. Serum was harvested after centrifugation and stored at -20°C. Anti-rabies antibody detection was performed by Sciensano National Reference Laboratory of Rabies in Belgium by use of Rapid Fluorescent Focus Inhibition Test (RFFIT), one of the WHO and OIE reference methods (Meslin et al., 1973; OIE, 2014).

Antibody titers were expressed in International Units per milliliter (IU/ml) and 0.5 IU/ml of anti-rabies antibody was considered as the minimum protective titer (WHO recommendations, 1992). Results were analyzed as regards of protective antirabies antibody titer ($<$ and ≥ 0.5 IU/ml) and the time span since last vaccination (≤ 1 year, 1-2 years, 2-3 years, > 3 years). Using the STATA software, a logistic regression model was used to explore if the percentage of vaccinated dogs with protective titer differed by the time span since last vaccination.

Risk map establishment

The risk of rabies transmission among dog populations was assessed by combining results of vaccination coverage, roaming behaviour and dog density in order to establish a risk of level 1 (low), 2 (medium) or 3 (high) for each study site. A weighting score was given to the different levels of the risk factors, namely vaccination coverage, roaming behavior and dog density. Thresholds were used in order to establish categories of vaccination coverage: $\geq 60\%$, 40-60% and $< 40\%$ (Coleman and Dye, 1996; Hampson et al., 2009); percentage of free roaming dogs : $\leq 25\%$, $> 25-50\%$, $> 50-75\%$, $> 75-100\%$ and dog density: < 5 and > 5 dogs/km² (see Table 1).

Research Agreement

The research project received the agreement N⁰ /012.20 /0171/IPAPEL/2016 of the Provincial Division of Agriculture, Fisheries and Livestock of Kinshasa. Oral informed consent was obtained from each dog owner prior to data collection or dog's blood sampling. Dogs participating in the serological study or the street count were gratuitously vaccinated against rabies. Dog owners were free to leave the study at any time.

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Results

Characterization and management of the dog population

Dog density and population structure

The household survey included 6122 households located in 2914 plots. In total, 504 dog-owning households with 922 dogs were recorded, corresponding to 9% (95% CI: 8- 10%) of all households. In most visited dog-owning households, the dog owner accepted to participate in the study. The average number of dogs per dog-owning household was estimated to 1.8 dogs (95% CI: 1.7-1.9). The Human to Dog Ratio equaled 53 (95% CI: 49-57) and the dog density was estimated to 49 dogs/km² (95% CI: 40-58), with a range of 22-90 dogs/km² in study sites.

Fifty eight percent of recorded dogs (535/922) were males. Furthermore, close to 60% of dogs were adults (≥ 12 months of age), whereas puppies (≤ 3 months of age) represented 15% of the population. The mean age of dogs was 2.5 years (95% CI: 2.2-2.8) and the majority (60%) of dogs belonged to local breeds (table 3).

Dog management

Between 5 and 100% (mean 56%) of plots were insufficiently fenced and did not prevent dogs' roaming. Regarding intermittently or continuously free roaming owned dogs, their percentage ranged from 2 to 100% across study sites (mean 60%, Fig 2a). The study also showed that 0% to 94% (mean 46%) of dogs were either partially fed or not fed by their owners across study sites.

The analysis of GPS data showed that the 16 tracked dogs covered maximum distances ranging from 0.046 to 2.34 km (mean 0.72 km). Maximum distances (> 2 km) were covered by males and highest roaming activities were recorded in the morning (before 8 am) and in the evening (after 6 pm). The estimated contact rate with other dogs equaled 30 (95% CI:23- 37) (data not shown).

Among 201 free-roaming dogs recorded by street count in two study sites, three were deemed to be ownerless (1/131 and 2/70). The average proportion of ownerless dogs was less than 2%. Regarding owners' attitude toward dog's abandonment, only 8% (40 among 504 owners) appeared to consider this option.

Rabies vaccination

Vaccination coverage

Fifty three percent (479/922) of dogs were reported to be vaccinated against rabies (the vaccination certificate was available for 89% of these dogs) and no differences with regard to sex and roaming behavior were found. Vaccination coverage increased with age and was higher in pure and cross breed dogs (Table 3). Vaccination coverage ranged from 24% to 81% among study sites and was below the critical threshold of 40% in 8 of the 22 study sites (Fig 2b). Associated costs and low age were reported as main reasons for not vaccinating dogs (Table 2).

Serological evaluation of the immunization status of vaccinated dogs

Seventy three percent of a subgroup of 132 reported vaccinated dogs displayed protective anti-rabies antibody titers (≥ 0.5 IU/ml). The percentage of protected dogs tended to decrease in function of time span since last vaccination from 81 to 63%, but this was not statistically significant ($p=0.4$, Fig 1). Regarding dogs' age at the time of the first or last vaccination, dogs were vaccinated at about 12 months of age (median), ranging from 3 to 115 months. Independently of dogs' age at vaccination, the median time span since last vaccination was 18 months. This period varied from 3 to 90 months (data not shown).

Establishment of a risk map

The combination of vaccination coverage, roaming behavior and dog density revealed that the risk level of rabies transmission among dog populations was 1 (low), 2 (medium) and 3 (high), respectively, in 27% (6/22), 32% (7/22) and 41% (9/22) of the study sites (Fig 2c).

Discussion

The present study aimed to investigate the risk factors of rabies transmission between dogs in Kinshasa and to establish a risk map by combining the dog density, dogs' roaming behaviour and dogs' vaccination coverage.

The method used to estimate the three factors was the household questionnaire survey for which the accuracy of estimates (vaccination coverage, dog density) was not proven to be significantly different of those from census method, which is considered as the gold standard method (Cleaveland et al., 2003; Minyoo et al., 2015). In particular for dog density calculation, our method aimed at increasing the accuracy by taking into account the number of people living in households with no dogs in the calculating of HDR given the poor accuracy of available human population data and the lack of dog population data. Indeed, the last population census in the DRC was conducted in 1984 and the rural-urban drift is increasing (Flouriot, 2013), thereby justifying an update. Although the registration of dogs at the veterinary services is mandatory since 1918 (Royal Decree of 22 January 1918) in the DRC, the law is not respected by owners.

Possible biases include response, classification and selection biases. The response bias was low since people were found in most households and very few refused to answer the questionnaire. Mis-classification could occur as people might fear to declare they owned dogs that were not vaccinated. Finally, a selection bias could have occurred because of the purposive sampling strategy.

Presently, the inclusion of the dog density among risk factors of rabies transmission in dog populations is debatable. On one hand, several field and modeling studies demonstrated a density-dependency of rabies transmission in Africa, where the disease persists in dog populations with a density > 5 dogs/ km² and only sporadically appears under this threshold

(Foggin, 1988; Brooks, 1990, Cleaveland and Dye; 1995; Kitala et al. 2002). On the other hand, the study of Morters et al. (2013) found no conclusive evidence that support the relationship between dog density and rabies transmission. In our study, dog density equaled 49 dogs/km², which is almost ten times more than the above threshold density (5 dogs/km²). Densities varied depending on *quartiers* (min 22 – max 90 dogs/km²).

The second risk factor was the poor dog management because more than 50% of owned dogs were free roaming in 60% of the study sites (Fig 2a). The main reasons for roaming were the absence of a physical barrier that permanently prevented dogs from roaming and the owners who voluntarily allow dogs to roam in search for food in public dumps and open markets.

The total roaming restriction of all dogs should be the first measure of rabies control at the community level as applied in parts of Europe before implementation of vaccination programs (Wallace et al., 2017). In Kinshasa, the total restriction of dogs is not feasible due to above mentioned reasons of abundance of free-roaming dogs. However, it can be considered that most free-roaming dogs might be easily captured and punctually caged or tied for vaccination as they have owners. Indeed, apparently ownerless dogs accounted for less than 2% of the free-roaming dog population in the two study sites. The term “apparently ownerless dog” was used instead of “ownerless dog” because the street-count method used to estimate the percentage of ownerless dogs could not exclude the presence of owned and ownerless dogs from neighbouring areas. The estimated percentage of ownerless dogs was low ($\leq 2\%$) and in line with estimates of 0–11% ownerless dogs in Zimbabwe, Tanzania and Chad (Butler and Bingham, 2000; Cleaveland, 2014). Considering the mean *quartier* size (6,1 km²), the mean roaming distance of dogs (0.72 km) and the roamed surface (1.6 km²), it can be hypothesized that roaming dogs, whether they are owned or not, mainly roam within one or two *quartiers*. Such information is important for vaccination campaigns because it suggests

that a high vaccination coverage could be achieved very locally. Given the reduced (n=16) number of dogs whose roaming behavior was assessed by GPS tracking, further investigations implying a larger number of dogs that are housed in different study sites would be useful.

Vaccination against rabies remains the key component of rabies control as shown by the strong correlation between high vaccination coverage and low rabies incidence demonstrated in several studies. Indeed, the empirical vaccination coverage of $\geq 60\%$ has led to a significant reduction of rabies outbreaks (Korns and Zeissig, 1948; Cleaveland et al., 2003; Hampson et al., 2009; Morters et al. 2013; Global Alliance for Rabies Control, 2015). In contrast, rabies outbreaks occur when the immunization coverage falls under the critical threshold of 40% (Coleman and Dye, 1996; Hampson et al., 2009). Several methods can be used to estimate the vaccination coverage (Minyoo et al., 2015). In the present study, the household questionnaire survey recorded vaccinated dogs regardless of the time span since last vaccination. Among 132 blood-sampled dogs, 73% showed a protective antirabies antibody titre (≥ 0.5 IU/ml) and the impact of time span since last vaccination was not demonstrated (Fig 1). This may be due to the small sample size. A decreased titer was observed as the time since vaccination increased and the recommendation for annual vaccination in dogs (Arrêté N°SC/151/BGV/MIN/AGRI & DR/SMI/2016) is still valid. These results further suggest that a proportion of vaccinated dog populations with a poor turnover would be protected against rabies for more than one year. Furthermore, it can be speculated that reported vaccinated dogs without protective antirabies antibody titres (27 % of dogs with < 0.5 IU/ml) had nevertheless been immunized against rabies and that they would display a rapid memory immune response upon exposure. On the other hand, a lack of quality (potency) of the vaccine due to an inadequate cold chain during vaccine storage or non-responding dogs could also account for absence of protection (Day et al., 2016).

By considering the vaccination history of all owned dogs through the household questionnaire, the overall vaccination coverage equaled 53% and was above the critical coverage level of 40% (Coleman and Dye, 1996; Hampson et al., 2009). However, the coverage significantly differed between study sites and ranged from 24 to 81%. In addition, coverage in 36% (8/22) of study sites was below the critical immunization of 40%, which is propitious for rabies outbreaks (Hampson et al., 2009). It is important to emphasize that the low coverage (<40%) was estimated particularly in areas with low proportions of restricted dogs (Fig 2b). The variability of coverage between study sites is likely to be linked to the differences of the socio-economic situation of their inhabitants. Despite mandatory vaccination of dogs against rabies in DRC (Royal Decree of 01 April 1938), vaccination is not fully applied in the field and must be afforded by the dog owners. Indeed, the current cost (20 USD) for rabies vaccination appears as the first reason of non-vaccination for 46% of the interviewed dog owners (Table 4). Given that in DRC 70% of people live under the poverty threshold (Moumami, 2010), it might be expected that low-income households own non-vaccinated dogs. Another consequence of poverty is a poor dog management: local and crossed breeds are less expensive (Kazadi et al., 2017) and are allowed to roam freely, whereas pure breeds predominantly live in fenced plots.

The dogs' age of was another factor limiting vaccination. Most of the dogs under one year of age, and mainly puppies (≤ 3 months of age), were often unvaccinated. The WHO recommends the inclusion of puppies of less than three months of age in the rabies vaccination programs (WHO, 2013). Indeed, puppies are susceptible sub-populations and published laboratory data show that 4 to 17% of confirmed rabies cases are puppies under three months (Perry, 1993; Widdowson et al., 2002; Reta et al., 2014; Morters et al., 2015). However, many owners, veterinarians and veterinary assistants consider that puppies are too

young for vaccination. As a consequence, 94% (121 of 129) puppies, presenting 14% of the dog population were not vaccinated (Table 3).

Finally, the combination of the three main risk factors in form of a risk map reflected the likelihood of rabies transmission. This risk was found to be high and medium respectively in 41% (9/22) and 32% (7/22) of study sites (Fig 2c). In addition, it is likely that *quartiers* that are close to high risk sites should be cautiously regarded as high risk sites. This key result correlates closely with the rabies epidemiological context (unpublished laboratory dog rabies data). The high risk level of rabies transmission was associated to poor dog-keeping practices and to low vaccination coverage. Both factors were tightly linked to the socioeconomic status of dog-owning households. Indeed, some dogs were not exclusively feed by the owners and were therefore allowed to roam freely. Furthermore, the cost of vaccination (ie 20 USD in DRC) is not affordable to most of owners in impoverished suburbs (Kazadi et., 2017). An association of increased risk for canine rabies and areas of low socioeconomic status has also been shown in Mexico and Bolivia based on positive rabies samples from different urban settings (Eng et al., 1993; Widdowson et al., 2002). In China, the low vaccination coverage and the growth of uncontrolled dog populations as a consequence of socio-economic changes were the main causes of rabies re-emergence in poor communities (Yin et al., 2013). Based on these evidences, the combination of the three main risk factors in form of a risk map provides a tool for the field assessment of rabies risk in urban settings. It should be added that in peri-urban and rural settings, the role of wild animals in the maintenance of rabies in dog needs further investigations.

In conclusion, our study shows that the risk of rabies transmission varies locally in urban settings in Kinshasa. Dog-keeping practices and vaccination coverage correlate with the socioeconomic status of households and thereby influence the risk level of dog rabies transmission. The establishment of a low scale risk map at the level of *quartiers* and by

considering vaccination coverage, roaming behavior and dog density provides a tool for local risk assessment and might be useful for targeting areas and/or action aiming at rabies control.

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References

- Aréchiga Ceballos, N., Karunaratna, D., Aguilar Setién, A., 2014. Control of canine rabies in developing countries: Key features and animal welfare implications. *OIE Rev. Sci. Tech.* 33, 311–321. <https://doi.org/10.20506/rst.33.1.2278>.
- Bula, M., Mafwala, L., 1988. Le diagnostic de la rage animale à Lubumbashi, Zaïre. *OIE Rev. Sci. Tech.* 7, 387–394. <https://www.oie.int/doc/ged/d8422.pdf>.
- Butler, J.R.A., Bingham, J., 2000. Demography and dog-human relationships of the dog population in Zimbabwean communal lands. *Vet. Rec.* 147, 442–446. <https://doi.org/10.1136/vr.147.16.442>.
- Brooks, R., 1990. Survey of the dog-population of Zimbabwe and its level of rabies vaccination. *Vet. Rec.* 127, 592–596. <https://www.ncbi.nlm.nih.gov/pubmed/2075689>
- Cleaveland, S., Dye, C., 1995. Maintenance of a microparasite infecting several host species rabies in the Serengeti. *Parasitology* 111, S33–S47. <https://doi.org/10.1017/S0031182000075806>.
- Cleaveland, S., Fèvre, E.M., Kaare, M., Coleman, P.G., 2002. Estimating human rabies mortality in the United Republic of Tanzania from dog bite injuries. *Bull. World Health Organ.* 80, 304–310. <https://doi.org/10.1590/S0042-96862002000400009>.
- 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. [https://doi.org/10.1016/S0264-410X\(02\)00809-5](https://doi.org/10.1016/S0264-410X(02)00809-5).
- Cleaveland, S., Beyer, H., Hampson, K., Haydon, D., Lankester, F., Lembo, T., Meslin, F.X., Morders, M., Mtema, Z., Sambo, M., Townsend, S., 2014. The changing landscape of rabies epidemiology and control. *Onderstepoort J. Vet. Res.* 81. <https://doi.org/10.4102/ojvr.v81i2.731>.
- Coleman, P.G., Dye, C., 1996. Immunization coverage required to prevent outbreaks of dog rabies. *Vaccine* 14, 185–186. [https://doi.org/10.1016/0264-410X\(95\)00197-9](https://doi.org/10.1016/0264-410X(95)00197-9).
- Courtois, G.H., Ninane, G., Thys, A., 1964. Sur les cas de rage diagnostiqué au Laboratoire de Stanleyville de 1938 à 1958. *Ann. Soc. Belge Med. Trop.* 44, 405–41.
- Deressa, A., Ali, A., Beyene, M., Selassie, B.N., Yimer, E., Hussien, K., 2010. The status of rabies in Ethiopia: A retrospective record review. *Ethiop. J. Heal. Dev.* 24, 127–132. <https://doi.org/10.4314/ejhd.v24i2.62961>.
- Day, M. J., Horzinek, M. C., Schultz, R. D., & Squires, R. A., 2016. WSAVA Guidelines for the vaccination of dogs and cats. *J Small Anim Pract.* 57, E1–E45. https://doi.org/10.1111/jsap.2_12431
- Diop, B., Ichou, S., Guidot, G., 2011. Analyse des écarts PVS. Rapport République Démocratique du Congo. OIE, Paris.
- Eng, T.R., Fishbein, D.B., Talamante, H.E., Hall, D.B., Chavez, G.F., Dobbins, J.G., Muro, F.J., Bustos, J.L., De los Angeles Ricardy, M., Munguia, A., Carraso, J., Robles, A.R.,

- Baer, G.M., 1993. Urban epizootic of rabies in Mexico: epidemiology and impact of animal bite injuries. *Bull World Health Organ.* 71, 615-624.
- Fahrion, A.S., Taylor, L.H., Torres, G., Müller, T., Dürr, S., Knopf, L., De Balogh, K., Nel, L.H., Gordoncillo, M.J., Abela-Ridder, B., 2017. The road to dog rabies control and elimination-What keeps us from moving faster? *Front. Public Heal.* 5, 1–8. <https://doi.org/10.3389/FPUBH.2017.00103>.
- Flouriot, J., 2013. Kinshasa 2005. Trente ans après la publication de l'Atlas de Kinshasa. *Les Cah. d'Outre-Mer* 66, 29–55. <https://doi.org/10.4000/com.6770>
- Foggin, C. M. 1988. Rabies and Rabies-related viruses in Zimbabwe. Historical, virological and ecological aspects. PhD thesis in Medicine, University of Zimbabwe.
- Global Alliance for Rabies Control, 2015. Report of the Rabies Global Conference. Geneva.
- Hampson, K., Dobson, A., Kaare, M., Dushoff, J., Magoto, M., Sindoya, E., Cleaveland, S., 2008. Rabies exposures, post-exposure prophylaxis and deaths in a region of endemic canine rabies. *PLoS Negl. Trop. Dis.* 2. <https://doi.org/10.1371/journal.pntd.0000339>
- Hampson, K., Dushoff, J., Cleaveland, S., Haydon, D.T., Kaare, M., Packer, C., Dobson, A., 2009. Transmission dynamics and prospects for the elimination of canine Rabies. *PLoS Biol.* 7, 0462–0471. <https://doi.org/10.1371/journal.pbio.1000053>
- Jemberu, W.T., Molla, W., Almaw, G., Alemu, S., 2013. Incidence of rabies in humans and domestic animals and people's awareness in North Gondar Zone, Ethiopia. *PLoS Negl. Trop. Dis.* 7. <https://doi.org/10.1371/journal.pntd.0002216>
- Kazadi, E.K., Tshilenge, G.M., Mbaio, V., Njournemi, Z., Masumu, J., 2017. Determinants of dog owner-charged rabies vaccination in Kinshasa, Democratic Republic of Congo. *PLoS One* 12, 1–9. <https://doi.org/10.1371/journal.pone.0186677>.
- Kitala, P.M., McDermott, J.J., Coleman, P.G., Dye, C., 2002. Comparison of vaccination strategies for the control of dog rabies in Machakos District, Kenya. *Epidemiol. Infect.* 129, 215–222. <https://doi.org/10.1017/S0950268802006957>.
- Knobel, D.L., Cleaveland, S., Coleman, P.G., Fèvre, E.M., Meltzer, M.I., Miranda, M.E.G., Shaw, A., Zinsstag, J., Meslin, F., 2005. Knobel DL et al. Re-evaluating the burden of rabies in Africa and Asia. *Bulletin of the World Health Organization*, 2005, 83(5):360–368. *Bull. World Health Organ.* 83.
- Kreuter, F., Valliant, R., 2007. A survey on survey statistics: What is done and can be done in Stata. *Stata J.* 7, 1–21. <https://doi.org/10.1177/1536867x0700700101>.
- Korns, R.F., Zeissig, A., 1948. Dog, fox, and cattle Rabies in New York State. Evaluation of vaccination in dogs. *Am.J. Public Health.* 38, 50-65. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1624236/>.
- Makumbu, D.S., 1977. Contribution à l'étude de la rage à Kinshasa (ZAIRE). Thèse pour obtenir le grade de Docteur Vétérinaire, Ecole inter-Etats des sciences et Médecine Vétérinaires de Dakar.
- Meslin, F.-X., Kaplan, M.M., Koprowski, H., 1973. Laboratory techniques in rabies - WHO 552, 56–94.

- Ministère de Pêche et Elevage, 2017. Evaluation du système de surveillance des maladies animales et des zoonoses en République Démocratique du Congo. Kinshasa.
- Minyoo, A.B., Steinmetz, M., Czupryna, A., Bigambo, M., Mzimiri, I., Powell, G., Gwakisa, P., Lankester, F., 2015. Incentives increase participation in mass dog rabies vaccination clinics and methods of coverage estimation are assessed to be accurate. *PLoS Negl. Trop. Dis.* 9, 1–17. <https://doi.org/10.1371/journal.pntd.0004221>
- Morters, M.K., Restif, O., Hampson, K., Cleaveland, S., Wood, J.L.N., Conlan, A.J.K., 2013. Evidence-based control of canine rabies: A critical review of population density reduction. *J. Anim. Ecol.* 82, 6–14. <https://doi.org/10.1111/j.1365-2656.2012.02033.x>
- 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. *Vet. Rec.* 177, 150. <https://doi.org/10.1136/vr.102975>
- Moumami, A., Abdul, B., John, C., 2010. Analyse de la pauvreté en République démocratique du Congo. Banq. Africaine Développement, Work. Pap. Ser. 25.
- 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. *Pathog. Glob. Health* 108, 317–322. <https://doi.org/10.1179/2047773214Y.0000000161>
- Niang, A.B., Denormandie N., 2008. Evaluation des services vétérinaires de la République Démocratique du Congo. Rapport PVS RDC. OIE, Paris.
- Oboegbulem, S.I., Nwakonobi, I.E., 1989. Population density and ecology of dogs in Nigeria : a pilot study. *OIE Rev. Sci. Tech.* 8, 733–745. <https://doi.org/10.20506/rst.8.3.426>
- OIE Terrestrial Manual, 2014. Manual of diagnostic tests and vaccines for terrestrial animals. Chapter 2.1.13. Rabies. OIE, Paris. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.13_RABIES.pdf (accessed 29 April 2016).
- Perry, B.D., 1993. Dog ecology in eastern and southern Africa: implications for rabies control. *Onderstepoort J. Vet. Res.* 60, 429–436.
- Repetto, R., 1932. A propos de l'existence de la rage au Congo belge. *Ann. Soc. Belge Méd. Trop.* 12, 147.
- Reta, T., Teshale, S., Deresa, A., Ali, A., Mengistu, F., Sifer, D., Freuling, C.M., 2014. Rabies in animals and humans in and around Addis Ababa, the capital city of Ethiopia: A retrospective and questionnaire based study. *J. Vet. Med. Anim. Heal.* 6, 178–186. <https://doi.org/10.5897/jvmah2013.0256>
- Sedganti, L., Superti, F., Bianchi, S., Orsi, N., Divizia, M., Panà, A., 1990. Susceptibility of mammalian, avian, fish, and mosquito cell lines to rabies virus infection. *Acta Viro.* 34, 155–163. <https://www.ncbi.nlm.nih.gov/pubmed/1975976>
- Steele, J.H., 1975. History of rabies. In: Baer, G.M. *The natural history of rabies*, 2nd Ed. Academic press, london, pp. 1-29. <https://doi.org/10.1201/9780203736371>

- Tenzin, Dhand, N.K., Gyeltshen, T., Firestone, S., Zangmo, C., Dema, C., Gyeltshen, R., Ward, M.P., 2011. Dog bites in humans and estimating human rabies mortality in rabies endemic areas of bhutan. *PLoS Negl. Trop. Dis.* 5, 30–32. <https://doi.org/10.1371/journal.pntd.0001391>
- Twabela, A.T., Mweene, A.S., Masumu, J.M., Muma, J.B., Lombe, B.P., Hankanga, C., 2016. Overview of animal rabies in Kinshasa province in the democratic republic of Congo. *PLoS One* 11, 1–9. <https://doi.org/10.1371/journal.pone.0150403>
- Wallace, R.M., Undurraga, E.A., Blanton, J.D., Cleaton, J., Franka, R., 2017. Elimination of dog-mediated human rabies deaths by 2030: Needs assessment and alternatives for progress based on dog vaccination. *Front. Vet. Sci.* 4. <https://doi.org/10.3389/fvets.2017.00009>
- Widdowson, M.A., Morales, G.J., Chaves, S., McGrane, J., 2002. Epidemiology of urban canine rabies, Santa Cruz, Bolivia, 1972-1997. *Emerg. Infect. Dis.* 8, 458–461. <https://doi.org/10.3201/eid0805.010302>
- World Health Organization, 1987. Guidelines for dog rabies control. Vph 92.
- World Health Organization, 1992. WHO Expert committee on rabies. Eighth report. World Health Organ. Tech. Rep. Ser. 824.
- World Health Organization, 2013. WHO Expert consultation on rabies. Second report. World Health Organ. Tech. Rep. Ser. 982.
- Yin, W., Dong, J., Tu, C., Edwards, E., Fusheng Guo, F., Zhou, H., Yu, H., Vong, S., for the Rabies Technical and Advisory Board, 2013. Challenges and needs for China to eliminate rabies. *Infect. Dis. Poverty* 2, 1-10. <https://doi.org/10.1186/2049-9957-2-23>

Figure captions

Figure 1. Distribution of serological status of reported vaccinated dogs in function of time since last vaccination: a: ≤ 1 year; b: 1-2 years; c: 2-3 years; d: >3 years. The proportion of dogs with protective titre (>0.5 IU/ml) does not differ between groups ($p=0.4$, logistic regression model).

Figure 2. Selected study sites (*quartiers*) in Mont-Ngafula, Ngaliema and Lemba *communes*.

(a) Estimated percentage of owned dogs which are potentially free to roam. (b) Estimated vaccination coverage. (c) Qualitative assessment of the risk of dog rabies transmission in study sites based on dog density, dog vaccination coverage and percentage of free-roaming dogs as the main risk factors.

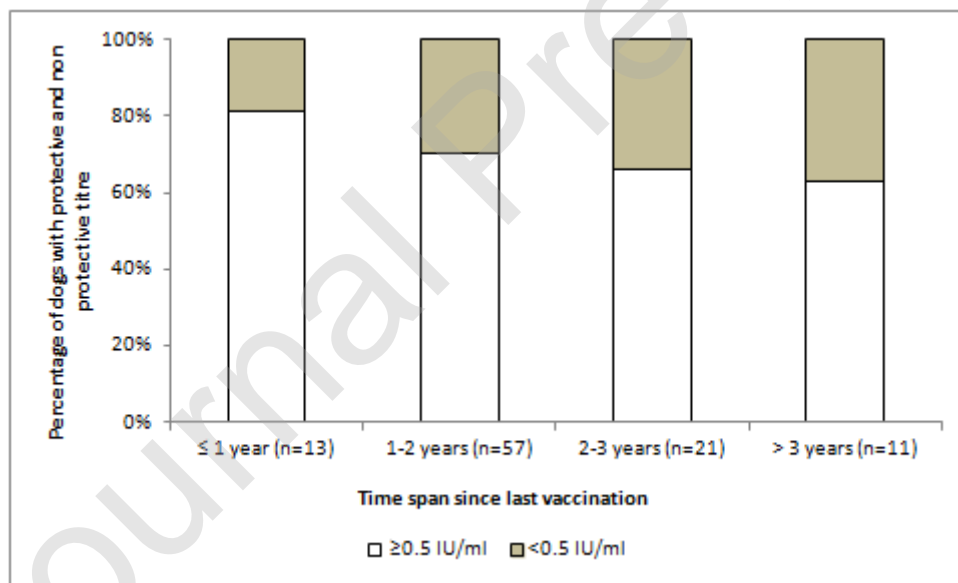


Fig1.

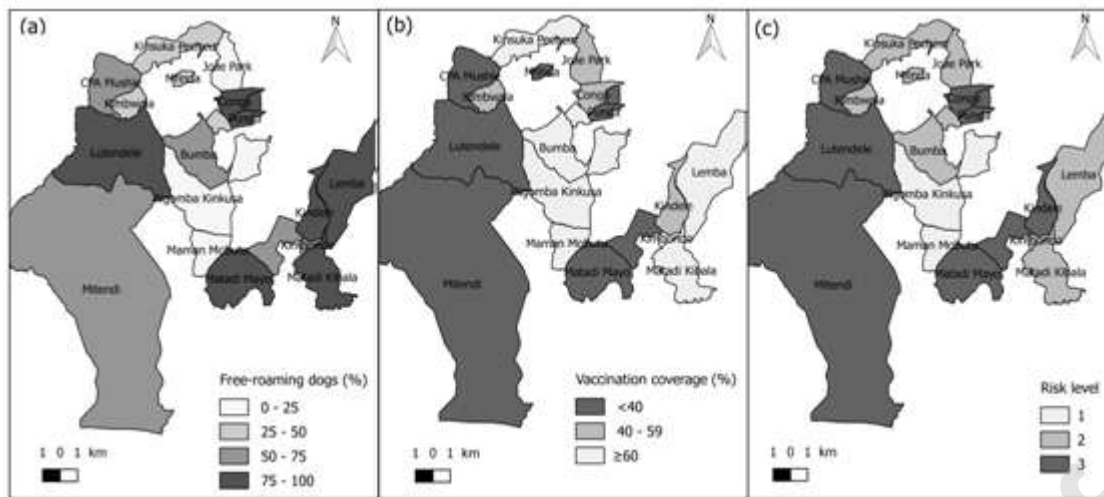


Fig2.

Table 1. Risk factor categories used for establishment of rabies transmission risks among dog populations

Risk factors	Weight (%) of each risk factor (w)	Threshold	Score of each threshold (s)	Weighted score of each threshold (w*s)
Vaccination coverage (%)	60	$\geq 60^*$	1	0.6
		40-60	2	1.2
		$< 40^{**}$	3	1.8
Percentage of free roaming dogs	30	≤ 25	1	0.3
		> 25-50	2	0.6
		> 50-75	3	0.9
		> 75-100	4	1.2
Dog density (dogs/km ²)	10	< 5	1	0.1
		$\geq 5^{***}$	2	0.2

* Empirical rabies control threshold (Cleveland et al., 2003; WHO, 2013).

** Under the critical threshold (Coleman and Dye, 1996; Hampson et al., 2009)

*** Dog density threshold for rabies maintenance in dogs in endemic regions of Africa (Foggin, 1988; Brooks, 1990, Cleaveland & Dye, 1995; Kitala et al. 2002, Lembo et al. 2008)

Notes: The risk per study site was the sum of three weighted scores by combination of three risk factors, which could be equal to 1 (low risk), 2 (medium risk) or 3 (high risk).

Table 2. Owner's stated reasons for not vaccinating dogs against rabies

Reasons of non vaccination	Number of answers	Percentage
The lack of money or the high cost of the rabies vaccination	194	46%
The dog is too young (≤ 3 months or < 1 year)	152	36%
The dog is not aggressive	68	16%
The lack of knowledge of the disease and the importance of vaccination	62	15%
The ignorance of the location of veterinary services	61	14%
The dog is completely restrained (no roaming)	59	14%
The negligence	32	8%
The vaccination side effects (loss of aggressivity, death), the vaccinator's credibility	26	6%
The rabies vaccination is the Government's responsibility	14	3%
The bitch was vaccinated	14	3%
No data	9	2%
Total of answers	424	

Table 3. Characteristics of reported rabies-vaccinated dogs among 922 owned dogs, using a cluster robust logistic regression and multivariable model

Factors	Number of dogs	Proportion (%)	Number of vaccinated dogs	OR(95%CI)	P value	Prediction of vaccination coverage (95% CI)
Sex						
Male*	525	57	305			58 (54-62)
Female	397	43	305	1.5 (0.9-2.3)	0.113	52 (47-57)
Age categories						
Adults (>12 mo)*	504	56	380			74 (70-78)
Juveniles (3-12 mo)	280	30	122	13 (2.3-76.7)	0.001	44 (38-50)
Puppies (\leq 3 mo)	129	14	8	61 (9-413)	0.001	6 (3-11)
Dog management						
Non-roaming dogs*	390	42	273			70 (65-74)
Free- roaming dogs	532	58	237	1.6 (0.8-2.9)	0.111	45 (40-49)
Dog breeds						
Pure breeds*	106	12	101			95 (89-98)
Crossed breeds	271	29	174	3 (2.2-5.2)	0.001	64 (58-70)
Local breeds	545	59	235	23 (8.6-62.7)	0.001	43 (39- 47)

* Reference variable represents the highest vaccinated category

Abbreviation: mo months