Studies for enhancement of strategies for the control of rabies in Zambia

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Summary (English)

The purpose of this study is to perform risk assessment of rabies infection in the Republic of Zambia (Zambia). Risk assessment of rabies virus infection will lead to cost-effective control measures for rabies, and the control measures will be implemented as risk management cooperating with the University of Zambia and the relevant authorities. As the first step of the research, in order to grasp the current situation on the occurrence of rabies and its control programmes in Zambia, a survey on the vaccination status and antibody retention ratio against rabies virus in domestic dog population, and a KAP survey against dog rabies and its control among dog owners were conducted. Furthermore, information on the rabies situation in Zambia was collected from the relevant authorities regarding the rabies control measures. The survey was conducted in two different areas which were Mazabuka and Monze districts in Southern Province, and Lusaka district in Lusaka Province. A total of 736 dogs were vaccinated and 265 canine sera were collected through mass vaccination in Mazabuka and Monze districts. In Lusaka district, a total of 278 canine sera were collected visiting households with dog(s) and 224 KAP survey questionnaires were collected at the same time. The results of the survey conducted by GLTP in Africa 2014 are now in the progress of analysis. The canine sera samples are going to be measured the antibody titre against rabies virus then the data of antibody titre will be collated with the vaccination record of each dog. Moreover, all of the data obtained in the survey may be analysed by multivariate analysis, then epidemiological factors correlating with vaccination status in dog population in Zambia may be identified. Finally risk analysis for the control of rabies will be conducted using all of the data which obtained in the survey and the data from the previous literatures to determine the factors associated with the risk of rabies occurrences in Zambia. It will contribute to risk management to carry out the cost-effective control measures for human rabies in the future. All of the outcomes of this research are going to be published as a doctoral dissertation in around 2017.

Summary (日本語)

本研究の目的はザンビア共和国(以下ザンビア)において狂犬病のリスクアナリシスを実施し、リスクアセスメントを通して費用対効果の高い狂犬病対策方法を検討し、大学および関連機関と協力してそれらの対策法をリスクマネジメントとして実施することである。その第一段階として、現在のザンビアにおける狂犬病発生の実態や狂犬病制御対策(主に飼い犬におけるワクチン接種)の実施状況について把握するために、Global Leadership Training Programme in Africa 2014 において、ザンビアの飼い犬におけるワクチン接種状況と狂犬病抗体保有についての調査、そして犬の飼い主を対象とした狂犬病についての Knowledge, Attitudes and Practices (KAP) 調査を実施した。また、ザンビアにおいて狂犬病制御対策を実施している関連機関においてザンビア国内の狂犬病の発生に関する情報を収集した。南部州に位置するマザブカ郡・モンゼ郡および首都であるルサカ郡が対象とされた。マザブカ・モンゼの両郡では、集団狂犬病ワクチン接種が実施され、合計 736 頭の飼い犬がワクチンを接種され同時に 265 頭の飼い犬が血液を採取された。ルサカ郡においては犬を飼育する家庭を訪問し、合計 278 頭の飼い犬が採血された。また、224 人の飼い主から KAP 調査の回答を得た。GLTP in Africa 2014 で得られた調査結果は現在解析中であり、採取された犬血清中の狂犬病ウイルスに対する中和抗体価とワクチン接種時期や使用されたワクチンの種類等の関連について結果を照合する予定である。そして、犬の個体情報や飼い主情報、KAP の回答を合わせた多変量解析を実施し、ザンビアにおける犬集団のワクチン接種状況に関連する疫学的要因を検討する。また、調査結果や関連機関、過去文献から得られたデータをもとに、ザンビアの狂犬病制御対策のためのリスクアナリシスを実施し、狂犬病発生のリスクと関連する要因を探し出し、今後の費用対効果の高い狂犬病制御対策法を実施するためのリスクマネジメントにつなげる。本研究は調査者の博士号論文として発表されるため、最終的な調査結果の発表は 2017 年ごろになる見込みである。

Background

Rabies has been known since antiquity as one of the most feared zoonotic diseases. Today, it still causes approximately 55,000 deaths per annum, predominantly among children in Asia and Africa (Knobel *et al*., 2005). Rabies is a viral disease caused by rabies virus belonging to a neurotropic species of *Lyssavirus*, a member of the family *Rhabdoviridae*, that has tropism for the central nervous system and the salivary glands (Stedman's Medical Dictionary, 2006). Transmitted by saliva from the bite of an infected animal, the symptoms are characteristic of a profound disturbance of the nervous system, e.g., excitement, aggressiveness, and madness, followed by paralysis and death, in the absence of timely post-exposure prophylaxis (PEP). In animals, clinical signs are variable, and sometimes salivation and tongue paralysis are the only signs. Although rabies may affect all species of warm-blooded animals, the majority of human rabies cases are intermediated by domestic dogs (Lembo *et al*., 2010).

Though rabies is fatal once clinical signs appear, it is fully preventable in the presence of appropriate vaccine programme and PEP. Nevertheless, rabies cases in human and animal have still been prevalent in most of the countries in the world. The main reason of this situation is considered that costly pre-exposure vaccine and post-exposure vaccine is enormous burden for both of the health sector of the government and individuals and its resultant incomplete distribution of pre or post-exposure vaccine against rabies (Knobel *et al*., 2005). Now dog vaccination is regarded as the most effective control strategy against animal as well as human rabies with the combination of dog population control, registration system for owned dogs, movement regulations and elimination of stray dogs, however, covering the entire dog population in the nation as the target for control is always accompanied by difficulties concerning about economic and administrative argument (Durr *et al*., 2009). Additionally some other reasons such as presence of wildlife or cross-border of rabid animals hamper the control of rabies in the world. Involving these various factors, execution of control strategy against rabies becomes complicated therefore, rabies is also considered one of the Neglected Tropical Diseases particularly in the developing countries.

In this study, rabies situation in Zambia was focused on. For collecting the fundamental information to consider the overall situation of rabies in Zambia, the basic data of dog population was researched and the features of the population were cleared. Besides, the vaccination status of the dog population was studied. The vaccination status in domestic dog population was commonly considered to be crucial for the control of rabies outbreak. Furthermore, the Knowledge, Attitudes and Practices (KAP) survey in human against rabies infection and its control was also conducted. The official information on rabies from the relevant authorities was also collected as much as possible.

Purpose of the study

The study was designed to understand the features of dog population and its vaccination status against rabies in target areas and to summarize KAP against rabies and its control in humans as the fundamental study to develop an advanced risk analysis of human rabies in Zambia in the future.

Strategies

For achieving the purposes, the following strategies were built up.

i) To understand the features of dog population and its vaccination status against rabies in target areas in Zambia

For achieving this, canine blood sampling and collection of information from dog owners were planned. It is to obtain the information of particulars of domestic dogs and vaccination status and to confirm that dogs have enough immunity to protect rabies virus infection conferred by vaccination.

ii) To summarize KAP against rabies and its control in humans

It is to evaluate KAP in humans against dog rabies. It will contribute to promote the control measures, particularly in education and sensitization against rabies.

iii) To collect the information about rabies from relevant authorities

It is important to grasp the situation in Zambia and to utilize for developing the risk analysis model to determine the high risk factors to be controlled and managed in the society.

Study areas

Two types of target areas were selected from local area and urban area in Zambia. The selected areas were Mazabuka and Monze districts in Southern province as the local area, and Lusaka district in Lusaka province as the urban area. In Mazabuka district, the area of the district is 6,242 km^2, human population is 261,268 (2010) and the dog population registered is 12,916 (2012). In Monze district, the area is 4,854 km^2, human population is 195,921 (2010) and 16,136 dogs are estimated to be kept (2014). These two areas are neighboured each other. Several times of outbreaks of rabies in humans and dogs have been reported in Mazabuka district recent years and the outbreaks also have been reported in Monze districts. However, little data was available about immunization coverage in dog population nevertheless the adequate level of immunization coverage in dog population highly contributes to prevention from outbreak in not only dog population but also human population. The area of Lusaka district is 360 km^2 and the population is 1,742,979 (2010), the dog population in Lusaka district is 26,000 (The National Livestock Epidemiology and Information Centre, 2009/Central Statistical Office, 2011). The high density area of humans like Lusaka is an important area for the control and containment of the disease not to spread the outbreak to the other areas in the nation.

Materials and Methods

1. Canine blood sampling in the target areas

The mass vaccine campaign was conducted to gather the domestic dogs in Mazabuka district (14th to 15th February, 2015) and Monze district (28th February 2015) under the cooperation of Mazabuka and Monze District Veterinary Office (DVO), respectively. Simultaneously with the mass vaccination against rabies, blood sample collections were carried out from cephalic vein on the foreleg of the dogs with the consensus of the owners. The information about particular of owners and dogs, vaccination history was collected for data analysis.

In Lusaka district, sampling was conducted from 23rd March 2015 to 17th April 2015 following WHO Immunization Coverage Cluster Survey (World Health Organization. Dept. of Immunization, Vaccines and Biologicals, 2005/World Health Organization. Expanded Programme on Immunization, 2008). The goal of this two - stage cluster sampling design is to estimate immunization coverage to within ± 10% points of the true population, with 95% confidence. In the first stage, 20 of clusters, which are wards of constituencies in Lusaka district, are selected with probability proportionate to the size (PPS) of the population in the cluster. Sampling with PPS allows the larger clusters to have a greater chance of being selected. In the second stage of sampling, 10 subjects are selected within each cluster. Although the sampling unit is the individual subject, the sampling is conducted on the household level. To identify the clusters, the number of households in Lusaka district is extrapolated for the population to be surveyed representing the dog population. In this study, 20 wards out of 33 wards in 7 constituencies in Lusaka district are selected to be surveyed as the clusters based on the PPS (details in appendix).

The minimum sample size is calculated by the following formula.

$$n = DE \times \frac{z_{1-\alpha/2}^2 \times p \times (1-p)}{d^2}$$

α: the desired confidence level

p: expected coverage

d: the desired width of the confidence interval

DE: the ratio between the variance from the cluster design to the variance that would be obtained from a simple random sample

$$n = 2 \times \frac{1.96^2 \times 0.5 \times (1-0.5)}{0.1^2} = 192.08$$

$$\frac{192.08}{20(clusters)} = 9.604$$

Ten subjects are sampled in each cluster, that is to say, 1 dog is sampled from each household which has dog(s) and 10 households are visited in one cluster in total. In the target households with dog(s), vaccination and simultaneous blood sampling were carried out and the information about particular of owners and dogs, vaccination history was collected for data analysis. It was also done with the consensus of the owners.

2. KAP survey in human

The KAP survey through questionnaires was implemented in Lusaka district at the same time the canine blood sampling in the same period. The dog owners in the district were interviewed about the knowledge on rabies and the control of rabies and attitudes and practices for the control of rabies using questionnaire sheet. The questions in the KAP survey were e.g. the numbers of dogs kept in the household, how to keep the dogs in the household, knowledge about the rabies clinical signs or transmission, etc. If the actual dog's owner

was absent when interviewers visited the household, a representative from the household was interviewed for questionnaire. In both cases, the interviewees were 16 years old and more based on the official age for suffrage in Zambia.

3. *Collection of the information regarding rabies cases in Zambia from relevant authorities*

The information regarding rabies in animals and humans in Zambia were collected from the relevant authorities. The information about rabies cases and its control in animals was obtained through the Director of Veterinary Public Health Sub-unit, Veterinary Services Unit, Department of Veterinary Services, Ministry of Agriculture and Livestock on 17th February 2015. The information about rabies cases and bite cases in human and the control of rabies from medical side was obtained from the Director of Disease Control and Surveillance, Ministry of Health on 7th April 2015. Furthermore, the any other information about rabies was collected through the activity period by contacting the relevant officers in the DVO or professors and lecturers in the university.

Results

1. *Canine blood sampling in the target areas*

A total of 736 dogs were vaccinated and 265 canine blood samples were collected through mass vaccination in Mazabuka and Monze districts in Southern province. In Lusaka district, a total of 278 canine blood samples were collected. These samples are going to be measured the level of immunity against rabies virus which is considered to have been induced by previous rabies vaccine by the golden standard defined by the World Organization for Animal Health. The antibody titre in each canine serum will be collated with the information of the particulars of the dog and its owner including the information of vaccine history. Then, the latest time when the dog was vaccinated will be compared with the actual level of antibody titre. The status of vaccine coverage in the dog population and the antibody retention ratio will useful to consider the effectiveness of the vaccination programme and the effectiveness of the vaccines in the field. It may contribute to reconsideration of the vaccination programme to reach further high immunization coverage and it will finally attain the control of rabies outbreaks in domestic dogs. Achievement of the control of rabies outbreaks in domestic dogs will lead to the reduction of human rabies.

Picture 1. Mass vaccination

Picture 2. Blood sampling

2. *KAP survey in human*

A total of 224 questionnaire responses for KAP were obtained from dog owners in Lusaka district. Through the collection of the information of dogs and owners and the result of KAP survey, the features of dog population and dog owners were observed. The dogs were mainly kept without using collars and they were usually free-range except for the cases that the owner's house had complete fence to surround the house or the cases using the chains to hold the dogs. It was the first time for 34% of the dog population sampled blood to be vaccinated in their life at the time of sampling in Lusaka district. In Mazabuka and Monze district, 45% of dogs have never been vaccinated before at the time of the sampling. Besides, the situations for safekeeping of the vaccination records by the owners were different between in urban area and rural areas. The dog owners in the urban area were more likely to keep the previous vaccine certificate for their dogs properly compared with the dog owners in the local areas. The further analysis of these data is now in progress.

Picture 3. KAP survey in Lusaka district

3. *Collection of the information regarding rabies cases in Zambia from relevant authorities*

Various types of information about e.g. the annual number of bite case in humans, the annual number of rabies cases in animals and humans, respectively, the annual use of vaccine against rabies in animal, etc., were collected through the relevant authorities. The data is going to be utilised to develop the risk analysis model and to consider overall situation in rabies in Zambia. The risk analysis will show the risk factors (areas, behaviour or phenomena etc.) for rabies occurrences and it will be also useful to estimate the real number of cases of rabies. The outcomes of the risk analysis will make the cost-effective control measures against rabies possible to be carried out with limited resources for obtaining the maximum effectiveness. The outcomes may be able to be proposed to the Ministry of Agriculture and Livestock and the University of Zambia. The control measures found to be effective and necessary will be implemented collaborating with the DVO which has the jurisdiction including the high risk areas of rabies.

Discussion

The samples and data from the field activities in Zambia have still been in the process of experiment in the

laboratory and in the analysis. They are unfortunately unable to be shown in this report so that it is impossible to discuss the study outcomes, however, only the current situation of dog population and on rabies in Zambia is able to be mentioned here.

Firstly, through the collection of the information on dogs and owners and the results of KAP survey, the features of dog population and dog owners were observed. According to the primary analysis of the result, it implied that the features of dog population and dog owners would be different between in the local areas and the urban area. It means that the fully effective approaches to enhance the control of rabies might not be entirely same between these areas. The results of the analysis for the features of the dog population and the owners between in the local areas and the urban area will be completely disclosed in the doctoral dissertation in 2017 with the result of the assay of canine blood samples detecting antibody titre level against rabies virus in the dog population.

Secondly, through the information from the relevant authorities, the features of rabies control and rabies cases in Zambia were cleared step by step. Rabies cases in humans and animals have been occurred regularly in Zambia. Munang'andu et al. reported a total of 98 cases in humans, 747 cases in dogs, 139 cases in cattle, 49 cases in the other domestic animals and 37 cases in wildlife were confirmed as rabies from 1985 to 2004 in Zambia. Rabid dogs constituted 69.9% (747 samples) of the total rabies positive cases from humans, domestic animals and wildlife during the period. The monthly distribution of dog rabies reflects an average of 2.93 (95% CI: 2.59-3.29) positive cases per month for the period 1985-2004 (Munang'andu et al. 2011). Furthermore, an association was found between the provincial human population density and dog rabies cases (Munang'andu et al., 2011). In another report, a total of 46, 36, and 24 cases were confirmed as dog rabies cases in 2010, 2011, and 2012, respectively. The total fatal numbers of human rabies diagnosed on clinical grounds were 2 cases in 2010, 3 cases in 2011 and 5 cases in 2012. The numbers of people bitten by dogs were 620 in 2010, 732 in 2011 and 595 in 2012 in Zambia (Kayesa, 2012). Considering these information from the previous literature, rabies infection occurrences have been maintained with the regular number of cases in humans and animals since several decades before, particularly cases in dogs have occupied the big numbers of rabies infection in Zambia, it implies that the control of rabies infection in dog population will highly contribute to the control of rabies infection in the nation. Regarding the human cases, they have been reported mainly according to the clinical signs-based diagnosis because the family of deceased are likely to decline the laboratory diagnosis. Considering the several hundreds of bite cases in humans per annum and inconvenient access to hospitals in rural areas, the number of rabies cases in human might be underreported. For controlling the situation of rabies in Zambia, the Ministry of Agriculture and Livestock, the Ministry of Health, the Zambia Wildlife Authority, the Central Veterinary Research Institute and the District Councils are mainly involved in the control of rabies in the country. They have regular meeting to share the epidemiological information in the different levels such as district level, provincial level or national level. However they also have other various public health problems like control of HIV/AIDS and Tuberculosis, diagnosis and treatment of Malaria or shoreline operation to prevent invasion of Ebola virus infection so that sometimes the prioritisation in infectious diseases field under public health issues may be difficult in the limited sources. Furthermore, as the previous literature reported, wildlife has been considered

to be important to maintain rabies virus in the ecosystem of Zambia (Röttcher and Sawchuk, 1978, Berentsen *et al*., 2013,). As Röttcher and Sawchuk observed that outbreaks of bovine rabies coincided both seasonally and geographically with outbreaks in Jackals on the Kafue flats where geographically overlapped between the national parks, game management areas and the wetlands which allows for the co-existence of domestic animals and wildlife (Röttcher and Sawchuk, 1978), rabies occurrences in Zambia are said that they mostly begin where the National Park locates near. However, the observation conducted by Munang'andu *et al.* indicated that the domestic dog was a more likely candidate to transmit rabies to cattle than the jackal (Munang'andu *et al*., 2011). More investigations are expected to be performed about rabies situation and circulation in the ecosystem in Zambia. Moreover, the control of rabies in wild animals is more complicated than domestic animals. It has been attempted mainly in Europe and North America and the attempts have given the consistent results in those areas. However the trials mostly needed organized strategies beyond the administrative jurisdictions. Cooperation and big driving force among the relevant agencies may be required to conduct the projects in wild animals. As the conclusion, vaccination of dogs is much easier and primary measure to prevent the outbreak of rabies compared with the control of rabies in wildlife, so promotion of vaccination in domestic dogs and education about rabies is highly important.

Future plan

  The experiment and analysis are still in progress. The primary outcomes of the field activities in this programme are now under the next step of analysis for developing an advanced risk analysis study. The information on the dog and its owner which collected at the same time with the blood sampling have been added and statistically analysed. Now the experiment to measure the antibody titre against rabies virus in the canine sera is under progress. The results of all the data of antibody titre level in the canine sera will be collated with the basic information. It will take approximately 1 year to complete all of step of this study as the doctoral dissertation.

  Unfortunately the detailed outcome data is not able to be shown here, however, the outcomes which obtained in the field activities will contribute to build up the risk analysis model of rabies in Zambia. The risk analysis will reveal the burden of rabies in the society as visualised and the high risk factors or control points to prevent the outbreak based on cost benefit. The outcome of the risk analysis should be utilized to promote risk management of rabies in Zambia. It will be introduced to the University of Zambia and the Ministry of Agriculture and Livestock, Zambia. The outcomes of the study will be utilized to take the risk based cost effective control measures for particularly in the high risk areas for rabies occurrences collaborating with each DVO under the Ministry of Agriculture and Livestock.

Reflection on the GLTP in Africa

  The five months period of the activities in Zambia were seriously challenging, however, it were also heavily valuable as the precious opportunity to develop the capability on how to plan and how to conduct the research and how to affect the supporters to make the united team to conduct the research. The experiences earned in this programme will contribute to my future as the researcher to be involved the activities for

promoting humans and animals' health and well-being in the African countries. I also hope that the outcome of the study will contribute to promotion of the activities for the public health on-site in the future.

Acknowledgement

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Annex: Identification of the clusters to be surveyed

Cluster Survey in LUSAKA district
Data: 2010 CENSUS OF POPULATION AND HOUSING PRELIMINARY REPORT (Cenral Statstial Offce, 2011)
Boldface and red : selected area as cluster to be surveyed

Constituency	No.	Ward	Number of household	Cumulative number of household	Cluster Number
Chawama Constituency	1	**Nkoloma**	16,501	16,501	**1**
	2	Chawama	15,264	31,765	
	3	**John Howard**	6,093	37,858	**2**
	4	Lilayi	3,050	40,908	
Kabwata Constituency	5	Kamwala	10,049	50,957	
	6	**Kabwata**	4,835	55,792	**3**
	7	Libala	4,487	60,279	
	8	**Chilenje**	10,521	70,800	**4**
	9	Kamulanga	5,185	75,985	
Kanyama Constituency	10	**Kanyama**	36,834	112,819	**5,6**
	11	**Harry Mwaanga Nkumbula**	35,989	148,808	**7,8**
	12	Munkolo	6,172	154,980	
Lusaka Central Constituency	13	Silwizya	1,595	156,575	
	14	Independence	3,638	160,213	
	15	**Lubwa**	7,635	167,848	**9**
	16	Kabulonga	12,704	180,552	
Mandevu Constituency	17	**Roma**	14,120	194,672	**10**
	18	Mulungushi	2,950	197,622	
	19	**Ngwerere**	14,164	211,786	**11**
	20	Chaisa	4,566	216,352	
	21	**Justine Kabwe**	8,560	224,912	**12**
	22	**Raphael Chota**	18,999	243,911	**13**
	23	**Mpulungu**	11,490	255,401	**14**
Matero Constituency	24	Muchinga	8,202	263,603	
	25	**Kapwepwe**	10,952	274,555	**15**
	26	Lima	13,195	287,750	
	27	**Mwembeshi**	13,016	300,766	**16**
	28	**Matero**	11,688	312,454	**17**
Munali Contituency	29	Chainda	8,485	320,939	
	30	**Mutendere**	22,729	343,668	**18**
	31	**Kalingalinga**	8,714	352,382	**19**
	32	Chakunkula	6,647	359,029	
	33	**Munali**	9,335	368,364	**20**
Sampling Interval				18418	
Random Number				15120	