have time to read long reports. They need a concise "bullet-point" fact sheet that makes the case for the recommended actions: (1) allocate more money, and (2) enact better legislation.

Strategy:

Purpose (what): The Policy Brief serves two purposes: first, to raise awareness about the value and feasibility of eliminating rabies; and second, to present a request for the resources necessary to do the job. The policy brief is used to enlist the support of potential allies among the national policymakers for increased funding. It also lays the foundation for enacting new or revised national legislation defining policies for rabies control and prevention.

Target Audience (who): The target audiences for the Policy Brief are those national and regional legislators who are likely to be sympathetic to the cause of eliminating rabies (for example, health care committee members and policymakers who are physicians or veterinarians). Another key target audience for the Policy Brief is staff members who work in the Finance Ministry with responsibility for overseeing the development of the Health and Agriculture (Animal Control) budgets.

Key themes (message): The paired, linked messages of the Policy Brief are: Rabies can be eliminated (any deaths from rabies are unnecessary and preventable, dying from rabies is a horrifying experience that predominantly affects children); and to eliminate rabies, the funding request herein is absolutely necessary.

Delivery Channel: To influence policymakers, the Policy Brief should be delivered by officials with the highest standing possible in the Ministries of Health & Agriculture, preferably by the Ministers themselves (or one of their staff). If possible, it is preferable to deliver the Policy Brief in person, meeting directly with targeted policymakers or their staff.

Recommendations: The Policy Brief should be based on the Country Report. Everything in the Policy Brief should be contained in the Country Report (which is where people who are interested can find greater detail and information). The funding request should provide an overview of the resources needed for scaling up specific areas including dog vaccination programs, PEP, labs, staffing, surveillance system, and training programs, and then it must prioritize the funding request: which projects are most important. The Policy Brief must be short, highlighting the major points that policymakers need to know to see the benefit of eliminating rabies and how it can be done.

It may also be helpful to prepare a separate fact sheet outlining the cost-effectiveness of vaccinating dogs versus providing PEP to humans.

Next steps: Participants in the Hanoi workshop will draft Policy Briefs completed by the follow-up meeting in September.

Action step #3: Convene National Advisory Board (for external public relations purposes)

The next two action steps are to convene a National Advisory Board and to convene a National Steering Committee. These two bodies have different purposes and different memberships. The National Advisory Board is composed of high profile, publicly well known opinion leaders, celebrities, luminaries and champions of the cause of eliminating rabies. Members of the National Advisory Board are invited based on what they can do to raise public awareness – people who can gain mass media coverage and the attention of policymakers by virtue of their public renown, not for their technical expertise (although it is important to have at least 1-2

rabies experts on the NAB). Their role is to put rabies on the national agenda. Such people might include minister's wives, religious leaders, business leaders, union leaders, celebrities, and people affected by problem.

Strategy:

Purpose (what): The two purposes of convening a National Advisory Board are: (1) to raise public and policymaker awareness of importance of eliminating rabies, and (2) to enlist the support of potential allies, such as pharmaceutical industry representatives, animal welfare NGOs, dog food industry representatives, professional organizations (e.g., veterinarians), child welfare groups, tourism, Red Cross, WHO and others who may have reason for speaking out on behalf of the cause of rabies control and elimination. Establishing the NAB is intended to raise the profile of rabies in the mass media; to influence policymakers to enact national legislation; and to build a broad base of public support for making rabies a priority health problem. The NAB is the public face of advocacy for increased support for rabies control and elimination, in general, and dog vaccination programs, in particular.

Target Audience (who): The NAB is set up to reach two target audiences: policymakers and general public. By garnering greater media attention, they put pressure on policymakers to do something about the problem of rabies.

Key themes (message): The message of the NAB is: Rabies can be eliminated; any deaths from rabies are unnecessary and preventable, dying from rabies is a horrifying experience that predominantly affects children.

Delivery Channels: The two most important delivery channels of the NAB are, first, press releases and press conferences, which are designed to gain widespread media coverage (TV, radio, newspapers, etc); and second, participation in public hearings and meetings with legislators. To attract greater media attention, it is important for NAB members to participate in public events such as World Rabies Day (see www.worldrabiesday.org), the signing of new legislation, or the release of the National Plan (see below).

Recommendations: Two challenges in setting up a NAB are to figure out whom to invite and how to get them to accept an invitation to sit on the Board. A starting point for identifying potential nominees is to contact the Alliance for Rabies Control (http://www.rabiescontrol.net/), which maintains mailing lists of people who have expressed interest and concern about rabies control and prevention by country. Depending on the quality and extent of the listing for your country, you may want to contact people on the list and ask them for additional nominations.

The second challenge is to get high profile public luminaries to accept the invitation. When possible, the best approach is to use personal contacts. The second best approach is to send a personalized invitation from the highest public official who is willing to help, such as the Ministers of Health and Agriculture. Likewise, if a well-known celebrity is already on board, he or she can be asked to send out further letters of invitation. The invitations need to be followed up with phone calls or other direct personal contact to urge them to accept.

Once a reasonable number of people have agreed to participate on the NAB, the next step is to organize a "launching event." At this media event, the NAB should prominently declare the national goal of eliminating rabies by 2020. For this media event, it will be necessary to prepare a press release that highlights the extent of the problem, the country's current standing relative to other countries, and the steps that are necessary to be taken to achieve the goal of eliminating rabies, *if* or when sufficient resources are provided.

After the NAB has been established, they will need to make plans about how they can best raise public and policymaker awareness of importance of eliminating rabies (for example, by establishing a media relations committee). Often, the NAB can capitalize on annual rabies awareness events, such as World Rabies Day, to call attention to the cause.

Next steps: Participants in the Hanoi workshop will bring a list of people who will be invited to join the NAB to the follow-up meeting in September.

Action Step #4: Convene National Steering Committee (for internal planning purposes)

The National Steering Committee is composed of key stakeholders in rabies control and prevention: representatives from the relevant government agencies and private organizations directly responsible for reducing and eliminating rabies. The primary function of the NSC is to develop and monitor a National Plan for the Control and Elimination of Rabies.

NOTE: Prior to proceeding with this step, it is important to find out whether there is an existing body, like a National Committee on Zoonotic Diseases (e.g., avian flu), in one's country, and if so, to assess the potential for putting rabies on its agenda. It is important is to avoid any duplication of efforts whenever possible.

Strategy:

Purpose (what): Establishing a National Steering Committee serves three purposes. The first is to give a group of prominent government officials direct and publicly proclaimed responsibility for making rabies control and elimination a visible national priority, for which they can be held accountable. The second is to have them initiate and oversee comprehensive coordinated national efforts to eliminate rabies. The third is to promote intersectoral collaboration, by bringing officials from the Ministry of Health and Ministry of Agriculture (or wherever animal control resides in the national government) directly together in regular face-to-face meetings.

Target Audience (who): The target audience of reports produced by the NSC is all national, regional and local personnel with direct responsibility for reducing rabies, including in particular, animal health and human health officers. The chair of the NSC can be expected to be called upon to testify at public hearings about current progress in eliminating rabies.

Key themes (message): The key messages that the NSC wants to promote are: 1) the elimination of rabies is feasible and a national priority; 2) the NSC is accountable for demonstrating measurable progress in achieving this goal, and 3) it is more cost-effective for the government to invest limited resources in dog vaccination campaigns than in providing costly post-exposure prophylaxis in humans.

Delivery Channels: The major communication channels for the work of the NSC are regular face-to-face meetings and the publication of public reports. The NSC is responsible for developing monitoring the implementation of a National Plan for Rabies Control & Prevention (discussed in Action Step #5). They will also be responsible for assembling and issuing Annual Progress Reports (Action Step #7). In coordination with the National Advisory Board, the first activity of the NSC may be to issue a joint declaration by the MOH and MOA pledging their collaboration and partnership in eliminating rabies by 2020.

Recommendations: The major recommendation here is to identify all relevant stakeholders involved in rabies control and prevention in one's country. In contrast to the National Advisory

Board, NSC members are people with technical expertise in rabies control and prevention, or responsibility for overseeing the work of those who do. The NSC includes representatives from the MOH (including Directors of Communicable Disease Control Bureaus), MOA, Veterinarian Organizations, relevant NGOs (like humane societies), and the WHO. Unlike the NAB, it should be relatively easy to convene the NSC because rabies control and prevention is already part of the existing responsibilities of those who will be invited. The ASEAN Call for Action can be used as a reminder that the Ministers of Health in each ASEAN member state have already pledged their commitment to achieving the goal of eliminating rabies. Thus, it would be appropriate and effective for the MOH to issue invitations to the identified stakeholders to join the NSC.

After convening the NSC, it may be helpful to set up a committee structure organized around the six areas identified in the ASEAN Call for Action: Policies/Legislation, Prevention and Control of Rabies in Animals, Prevention in Humans, Surveillance, Integration, Coordination & Partnership, and Public Awareness & Communication. (See next Action Step for further detail.)

Next steps: Participants in the Hanoi workshop will describe the steps they have taken and progress made in convening a National Steering Committee at the September meeting.

Action Step #5: Develop National Plan for the Control and Elimination of Rabies

The National Plan for the Control and Elimination of Rabies is a comprehensive guide that defines measurable objectives for achieving rabies control, the activities that will lead to the accomplishment of the objectives, and the parties responsible for carrying out each of the identified activities. The National Plan describes the current disease burden of rabies in the country, the status of current control and prevention efforts (baseline measures), and operational objectives that state what will be done, where, by how much, by when and by whom, to achieve the goal of eliminating rabies by 2020. (For example, dog vaccination rates in HaLong Province will be increased from 30% of all dogs in 2009 to 60% of all dogs by 2015, as administered and reported by district animal control officers.) The National Plan addresses the six areas identified in the ASEAN Call for Action: Policies/Legislation, Prevention and Control of Rabies in Animals, Prevention in Humans, Surveillance, Integration, Coordination & Partnership, and Public Awareness & Communication. The National Plan is the essential reference document for all rabies control and prevention personnel.

Strategy:

Purpose (what): The purpose of the National Plan is to direct and coordinate a comprehensive national effort to eliminate rabies. It tells responsible parties what to do and provides the fundamental yardstick by which progress will be measured. Another important function of the National Plan is to set priorities, for example, by targeting prevention & control efforts to high incidence areas (e.g., provinces/districts with high numbers of rabies deaths, or low dog vaccination rates).

Target Audience (who): The target audience for the National Plan is all public and private personnel at the national, regional and local levels with responsibility for rabies control and prevention.

Key themes (message): Rabies will be eliminated by the year 2020 by carrying out the activities presented in the National Plan.

Delivery Channel: The National Plan is an official report by National Steering Committee.

Recommendations: Many examples of National Plans are available. The WHO has a "model report" available at: http://www.who.int/rabies/en/. A suggested outline of the National Plan is presented in Annex II.

Next steps: The National Plan will be developed by the National Steering Committee within one year of its inauguration.

Action step #6: Develop media strategy

Because rabies is the "invisible" "neglected" disease, it is crucial to address this problem by developing an effective media advocacy strategy. Every Action Step in this report needs to include a media component, a media plan. Advocates need both to create and to take advantage of special events, such as World Rabies Day. You also need to be creative and resourceful in taking advantage of other news events and newsworthy happenings. The key strategy here is to use the media in ways that can benefit policymakers and government officials: politicians like positive media coverage and seek to avoid negative reviews.

Note: The framework presented in this report focuses on achieving the *advocacy goal* of gaining the resources necessary to eliminate rabies. This Action Step is not intended to educate the public about rabies prevention (while acknowledging that public education is critical to achieving rabies control and elimination and that public education is an inevitable and valuable by-product of gaining any media coverage about rabies). Rather, the strategy described here focuses on building support for gaining the financial resources necessary to mount a public education campaign.

Strategy:

Purpose (what): The aims of the media advocacy strategy are to put the control and elimination of rabies on the public agenda, to raise public awareness about the feasibility and value of eliminating rabies, to put pressure on policymakers to pay attention to rabies and demand measurable progress in achieving its elimination.

Target Audience (who): The primary target audience of the media strategy is news media reporters and editors, who are targeted in order to reach the secondary audiences of policymakers, government officials and the general public who consume mass media reports.

Key themes (message): The main message is that rabies is now taking a terrible and unnecessary toll on lives of people in your country. It would be a great accomplishment to eliminate rabies here. It would be truly horrible if we remained one of the few countries left on earth where rabies is still prevalent.

Delivery Channels: The delivery channels include all of the standard ways that one works with the mass media, including press releases, invitations to the press to public events (e.g., opening of new regional lab, regional mass dog vaccination campaign, etc.), press conferences, letters to the editor, contacting reporters to provide background materials on the problem, responding to interview requests, writing feature articles, participating in audience phone-in radio talk shows, and so forth.

Recommendations: The first recommendation is to find out if you have a media relations unit in the MOH and MOA and work with them. Then, to gain media coverage, it is important to capitalize on existing events, such as World Rabies Day, where one can create photo opportunities for high profile celebrity members of your National Advisory Board to appear with policymakers who sponsor needed legislation or promote increased funding. Whenever

any death from rabies appears in the news media, it is an occasion for contacting the reporters to encourage them to frame the story in the context of what needs to be done to eliminate rabies and stop these tragic deaths, and for writing letters to the editor, or editorials about the worldwide effort to eliminate rabies. The press generally likes "human interest" stories, so it is helpful to include people who have been personally impacted by rabies (e.g., survivors, parents of children who have died, etc.). One recommendation is that the National Advisory Board and the National Steering Committee appoint a joint sub-committee responsible for planning 2-4 major national media events each year to keep rabies control and prevention on the national agenda.

Next steps: Participants in the Hanoi workshop will contact their respective media relations units and report on their advice for developing a media plan at the September workshop. Participants will also be responsible for making sure that the NAB and NSC set up a joint committee on media relations.

Action step #7: Issue Annual Progress Reports

Annual Progress Reports help to insure that the issue of rabies remains visible in the public eye and they serve to put pressure on policymakers and government officials to demonstrate consistent progress in moving towards the goal of eliminating rabies by 2020.

Strategy:

Purpose (what): The two key purposes served by issuing Annual Progress Reports are to maintain (and increase) the visibility of rabies as an issue of public concern; and to track progress in implementing the National Plan. Annual Progress Reports highlight successes, which are highly important to recognize publicly, and identify areas that need greater attention.

Target Audience (who): The primary target audiences are the National Advisory Board and key supporters among policymakers and legislators. It is the responsibility of the NAB to develop a plan to maximize media coverage of the Annual Reports, towards the goal of building public support among policymakers to provide the resources necessary to eliminate rabies. Secondary audiences include the personnel within MOH and MOA who are responsible for conducting rabies prevention and control activities as part of the regular work.

Key themes (message): One important message to be delivered in the Annual Reports is the number of rabies deaths averted: for example, "If the National Plan had not been implemented, XX [number] people would have died last year. Because the National Plan was eliminated, only YY [number] people died. Rabies prevention and control efforts saved ZZ lives last year alone. Since the release of the National Plan in 2010, we have saved a total of MM lives in this country."

Delivery Channel: The delivery channel of the Annual Reports is an official report issued by the NSC. The NAB will be responsible for developing a media plan around the release of the report, including, at a minimum, a press release highlighting the progress made and the challenges that remain.

Recommendations: The quality of the Annual Report depends directly of the quality of the national surveillance system. If the National Plan has been well written with measurable objectives and clear links between the activities and objectives, then producing the Annual Report will be a straightforward task.

Next steps: Annual Reports are produced by the National Steering Committee and issued every year after the National Plan has been finalized.

Action Step #8: Enact (or improve) national legislation

Effective rabies control and prevention programs require enabling legislation that provides health and animal control officials with the legal power and authority to take those actions essential for achieving the goal of eliminating rabies. Compulsory dog registration & vaccination laws are a high priority.

Strategy:

Purpose (what): The purpose of enacting new regulatory policies is to provide the legal authority for enforcing measures that are instrumental in eliminating rabies, such as compulsory dog registration & vaccination laws. Such legislation may also be a vehicle for gaining additional funding, or for arguing for the need for more money.

Target Audience (who): Staff members of sympathetic policymakers are provided with drafts of model legislation.

Key themes (message): To eliminate rabies by 2020, the rates of dog vaccination need to be increased to >70% of the total dog population. Hence, laws that require compulsory registration & vaccination are urgently needed.

Another issue to consider is the inclusion of PEP coverage in the national health insurance plan.

Delivery Channel: The delivery channel is model legislation provided to the staff of key policymakers.

Recommendations: Examples of model legislation from the WHO, Philippines and other countries are available for use and adaptation.

Next steps: New or revised legislation must be approved and passed by legislators. Model legislation can be provided to their staff by a committee of the NSC.

Action step #9: Provide leadership on increasing dog vaccination rates

In general, eliminating rabies by 2020 will require additional funding and, pos sibly, better legislation. However, progress can be made prior to gaining these new resources by exercising leadership. Responsible health leaders must step up and mobilize key personnel to enforce existing laws with current resources. In some countries, the problem is less one needing better laws than one of enforcing existing laws. Animal control officers need to see the importance of increasing rates of dog vaccination coverage and make enforcement one of their priorities. They need to be provided with incentives for improving rates and applauded for their successes.

It may be helpful to identify "demonstration areas" where the cost-effectiveness of control in dogs versus providing PEP in humans can be shown (i.e., documenting the number of PEPs that were ***not*** necessary because the dog was known to be vaccinated). To call attention to the issue, it may be helpful to organize mass annual vaccination campaigns, potentially in conjunction with child immunization campaigns.

Strategy:

Purpose (what): The purpose of providing leadership is to make progress in achieving the objective of attaining >70% dog vaccination coverage in the interim period before gaining additional resources. It is also important to identify areas with low dog vaccination rates, determine the causes and correct problems that can be fixed without new money.

Target Audience (who): The target audiences for providing leadership of dog vaccinations are the providers and recipients of dog vaccinations: animal control officers; vets & para-vets; and dog owners. They need to see and recognize the value and importance of their contributions to eliminating rabies.

Key themes (message): Dog vaccination rates must be improved in order to eliminate rabies in an affordable, cost-effective manner. Where applicable, current laws need to be better enforced.

Delivery Channel: The most effective delivery channel here is personal meetings between the national bureau chief responsible for rabies control and the regional, district and local animal control officers, both public and private. Another important venue is delivering addresses at regional meetings. The Annual Report should also highlight those districts that are most successful and those most in need of improvement.

Recommendations: Any and every rabies death is an occasion for reminding people of their shared responsibility in preventing these unnecessary and preventable tragedies. Barriers to obtaining adequate vaccine supplies need to be lowered and achievements rewarded (e.g., provide official awards, letters of commendation and recognition to district animal control officers who have achieved the goal of >70% vaccination rates, most improved over the previous monitoring period, most innovative campaign, etc., at regional and national meetings). One incentive might be public recognition and declaration of "rabies-free" zones as they are achieved.

Next steps: Responsibility for providing leadership on improving dog registration and vaccination rates lies with the bureau chief responsible for the National Rabies Control and Prevention Program. This is an on-going responsibility and needed to be conducted continuously.

Action step #10: Establish Provincial Coordinating Committees

In many countries, provincial governments exercise significant control over major fiscal resources and have considerable autonomy in developing and enforcing policies. Therefore, depending on the situation in your country, it may be helpful to establish Provincial Coordinating Committees (PCCs) who will be responsible basically for replicating the Action Steps enumerated above at the provincial level. Also, because resources may vary and it is often difficult to monitor progress at the local level, PCCs can play an important role in assisting the NSC in collecting data, setting priorities, organizing media events, implementing annual mass vaccination campaigns, and so on.

NOTE: Like the National Steering Committee, it is important to avoid duplication of effort, and hence, to determine whether there are any existing bodies at the provincial level that can assume responsibility for coordinating rabies prevention & control activities there.

Strategy:

Purpose (what): The purpose for establishing PCCs is to provide a focal point for advocacy activities decentralized below the national level, towards the goal of increasing dog vaccination rates and eliminating rabies.

Target Audience (who): The composition of PCC parallels the composition of the NSC: key stakeholders in rabies control and prevention such as representatives from the relevant government agencies and private organizations directly responsible for reducing and eliminating rabies.

Key themes (message): Key messages in this Action Step are that eliminating rabies is a shared responsibility and this province ranks above/below average in comparison with other provinces. Local prevention and control efforts need to be strengthened and costs needs to be shared.

Delivery Channel: The delivery channel is an official letter from the National bureau chief responsible for Rabies Control and Prevention Programs to his or her counter-part at the Provincial level.

Recommendations: Rather than trying to set up PCCs all over the country at the same time, it is preferable to start by identifying high priority areas, provinces with a high incidence of rabies deaths and/or those with low vaccination rates, get them up and running, and incrementally establish new PCCs over time.

Next steps: The bureau chief responsible for National Rabies Control and Prevention Program

Title: Enhancement of Rabies Surveillance by Strengthening Rabies Research in the Philippines

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INTRODUCTION

Although efforts to eliminate rabies proved to be successful in developed countries, it remains a major public health problem in developing countries. More than 99% of the 55,000 human rabies deaths per year occur in developing countries, 56% of which are estimated to occur in Asia and 44% in Africa (WHO 2005). Disease control and prevention programs implemented by non-government organizations and national health departments of endemic countries continue as the battle against this disease is far from over. Factors leading to the failure of effectively implementing control measures in developing countries range from economic, social and political (WHO 2005).

Laboratory confirmation of rabies is essential for a country desiring to eliminate rabies. It facilitates the definition of current epidemiologic patterns of rabies and is of great value in planning and monitoring control programs. Timely availability of the result may aid the physician in decision making regarding the proper management of exposed persons. Moreover, a negative result may save a patient from unnecessary physical and psychological trauma, and financial burdens.

In this report, epidemiology of rabies, status of rabies laboratories, and on going laboratory researches in the Philippines will be described. This will give an overview how far have we gone in terms of rabies diagnosis. Furthermore, gaps will be identified and corrected and improvements will be implemented.

BACKGROUND

I. Epidemiology of rabies in the Philippines

In the Philippines, rabies is not a leading cause of disease and death but it has become a significant public health problem because of the following reasons. First, as a zoonosis, the control of human rabies rests mainly on animal rabies control which is far from ideal. Secondly, more than the 200-300 human rabies deaths every year, around 180,000 animal bite victims seek treatment for rabies exposure, which constitute a significant drain on the government's resources. The Philippines has always been among the top ten countries with the highest reported incidence of human rabies in the world (HPN 2008, NOHP 2005-2010).

The Department of Health (DOH) monitors human rabies cases in the country through its regional rabies coordinators in the Centers for Health Development (CHD). The diagnosis of rabies is made clinically and is rarely laboratory confirmed. In 2008, there were a total of 250 deaths; majority were children under the age of 15 with more males than females (Deray 2009). There were 181,253 animal bite patient consultations for that year with 51.4% were older than 15 years old, and a male to female ratio of 1:2. Most patients were bitten by dogs (88.5%); 9.1% were bitten by cats and 2.3% by other animals such as monkey, rats, horses pigs. For 2009, partial results as of February 2010 show that there were 125 human rabies deaths with incomplete reports from several regions (DOH, 2010).

The objective of the National Rabies Prevention and Control Program (NRPCP) is to reduce the incidence of human rabies cases to less than 3 per million population by 2010 from a baseline of 5 per million population in 1997 (NRPCP 2009). In 2007, there were 286 human rabies cases which is 3.23 per million population based on the 88,574,614 population of August 2007 (National Statistics Office, 2007 Census of Population). In 2008, the incidence of human rabies was 2.78 per million population based on 2008 NSO projected population (NSO, 2006).

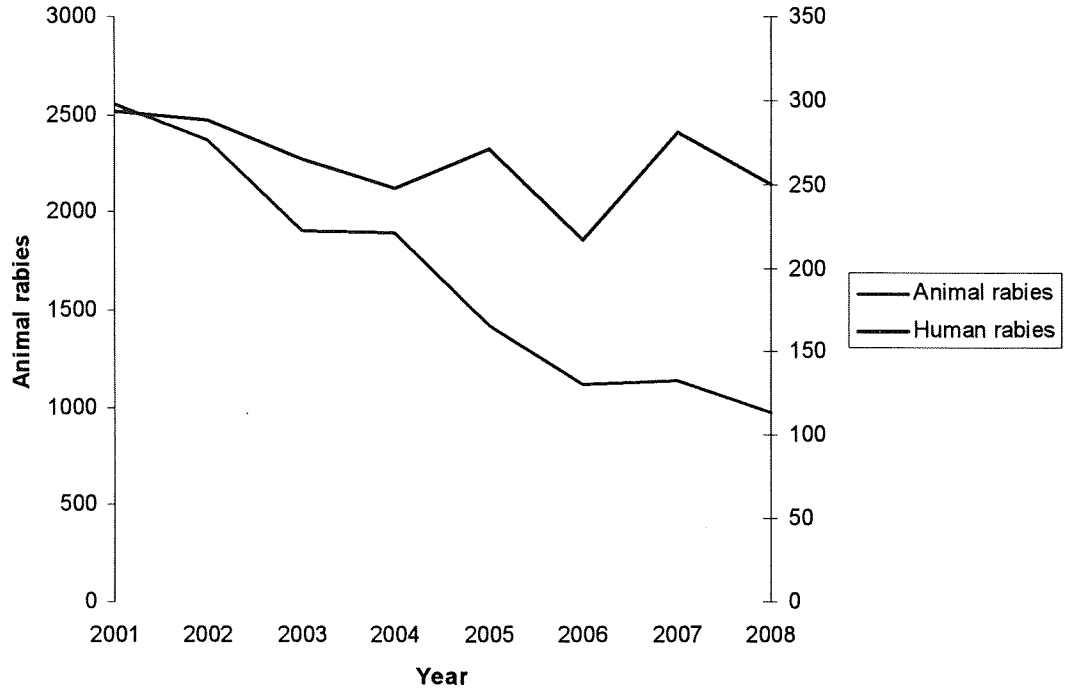

Figure 1. Human and animal rabies cases in the Philippine, 2001-2008

Figure 1 shows the number of animal and human rabies cases from 2001 to 2008. The number of animal rabies cases showed a downward trend from 2001 to 2008 despite a consistently high number of human rabies cases. This does not show the real picture of the disease in the animal population since only laboratory confirmed cases are counted. Many animals die without the benefit of laboratory testing.

The Bureau of Animal Industry (BAI) of the Department of Agriculture (DA) performs routine animal rabies diagnosis and animal rabies monitoring. From 2001-2005 a total of 10,119 or 32.39% out of 31,240 samples submitted were confirmed positive for rabies with an average of 2,023 samples per year (Atienza, 2009). Among the positive cases, 98.37% were dogs 1.52% cats and 0.01 % other domestic animals (swine, bovine, bubaline, goats and horses but were all bitten by rabid dogs). There were no reported cases of wild animals and in bats (Arguin 2002, Beran 1970). The objective of the National Rabies Prevention and Control Program is to reduce the incidence rate of dog rabies to 10 per 100,000 dog population from 28 per 100,000 dog population in 1997 (Atienza, 2009).

From 2001 to 2008, there was a decreasing trend in submission of samples to the rabies laboratories. These can be attributed to several factors: cost of the test, lack of awareness regarding the need for testing and the change in the guidelines on the management of animal bite patient wherein vaccination is started regardless of the status of the biting animal. In this case, bite victims may not care about the animal since they are already undergoing vaccination.

The rabies laboratory of RITM had received 13,673 animal specimens from 1995 to 2009. However, 210 (1.5%) of these were unfit for examination. They were either burnt or decaying specimen. From 1995-2009 there were 4, 852 that tested positive for rabies with a proportion of 36% from RITM rabies laboratory and more than 98% of which were dogs (figure 2).

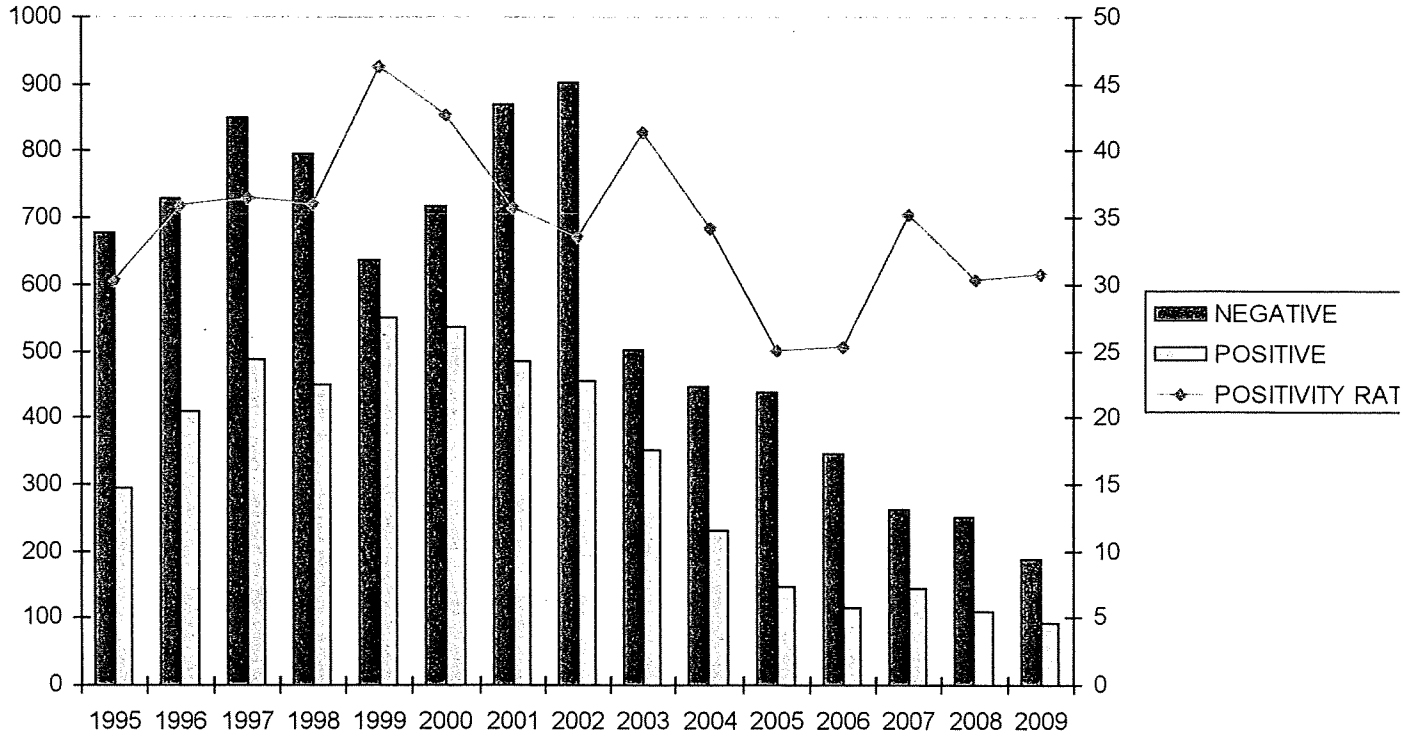

Figure 2. Animal Rabies Tested at RITM, 1995-2009

II. Rabies Diagnosis in the Philippines

Currently, there are 17 rabies laboratories in the country (figure 3). The Regional animal disease diagnostic laboratories (RADDL) are under the Bureau of Animal Industry of the Department of Agriculture (BAI-DA). Four of the laboratories are under the local government unit but still with the supervision of the BAI-DA and one (RITM rabies laboratory) is under the DOH. Only RITM laboratory performs both antigen [dFAT, reverse transcriptase polymerase chain reaction (RT-PCR)] [ELISA and rapid fluorescent focus inhibition test (RFFT)] for animal and human specimens. The rest of the laboratories do only antigen detection for animal specimens

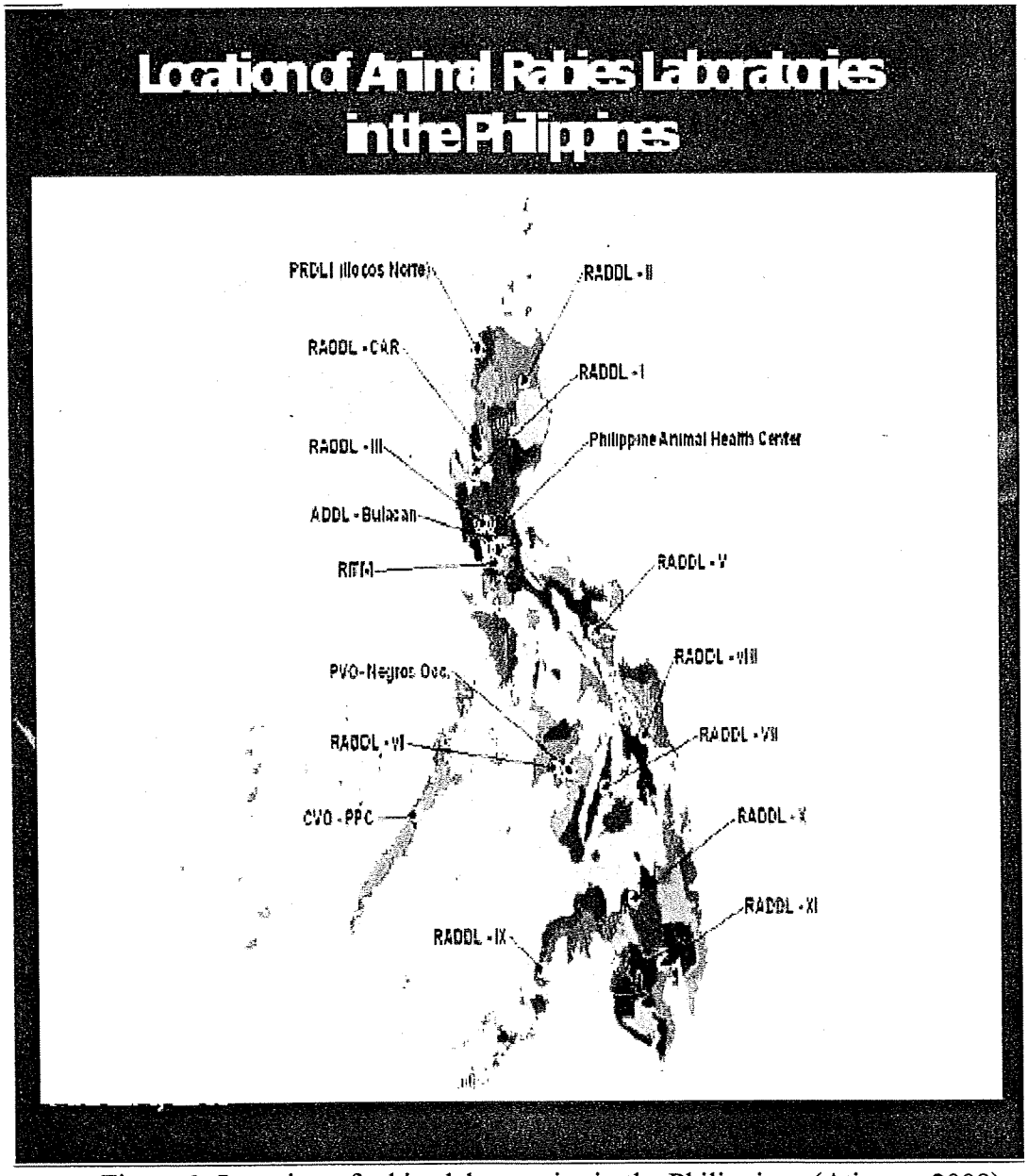

Figure 3. Location of rabies laboratories in the Philippines (Atienza, 2009)

The RITM rabies laboratory is the rabies reference laboratory in the Philippines. It regularly conducts quality assurance and control testing for selected

rabies laboratories in the country. In addition, it conducts proficiency training for rabies diagnosis and updates on new rabies diagnostic methods. The RITM rabies laboratory is also involved in the conduct of clinical, diagnostic, operational and field researches, providing input to the NRPCP and the DOH.

Rabies laboratories in the country use dFAT for rabies diagnosis. This test was developed in the 1960s and is already considered as the gold standard test for rabies diagnosis by the World Health Organization (WHO) provided that the three standards are met, namely: quality of the microscope, quality of fluorescein isothiocyanate (FITC) conjugate and skill of the microscopist (WHO 2005). It is comparable to Mouse Inoculation Test (MIT) in terms of sensitivity and specificity (Robles & Miranda, 1992). The drawback of the test in the Philippines is the lack of availability and affordability of the FITC conjugate and the expensive fluorescent microscope. Some of the rabies laboratories in the country have stopped testing because of inaccessibility of the conjugate. Specimens are then brought to the central laboratory, the Philippine Animal Health Center (PAHC) rabies laboratory, under the BAI-DA.

The RADDLs use rabies virus antinucleocapsid antibody, FITC conjugate (Biorad) supplied to them in a limited amount by the BAI-DA. There are also RADDLs that do not receive any conjugate from BAI-DA. RITM, on the other hand, uses FITC anti-rabies monoclonal globulin (Fujirebio) and oftentimes provides FITC conjugate to RADDLs upon request.

The direct microscopic examination (DME) or Seller's staining for Negri bodies, is still performed by some rabies laboratories. Although obsolete and low in sensitivity (Velleca, 1981, Robles and Miranda, 1992), the test is still being used because of budgetary constraints especially in the maintenance of immunofluorescent microscope and availability of FITC for dFAT. Twenty-eight percent (28%) of the seven rabies laboratories assessed still perform DME along side dFAT, where samples are initially screened using DME, and only the negative samples are confirmed with dFAT (RITM project report, 2009).

OBJECTIVE:

Rabies epidemiology and status of the rabies laboratories in the Philippines have been described above. This paper aims to discuss the ongoing rabies research activities that will fill in the gaps in rabies surveillance and improve the capacity of the rabies laboratories in the Philippines.

METHODS

Molecular Techniques

A. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and Nested Polymerase Chain Reaction (Nested PCR)

The target region tested was the rabies nucleoprotein (N) gene. Serially collected saliva was pooled and RNA extraction was performed using Qiagen™ RNeasy Mini Kit. After total RNA extraction, RT-PCR was done using Invitrogen™ Superscript III One-Step RT-PCR with Platinum™ Taq and the primers N12 (5' GTA

ACA CCT CTA CAA TGG 3') and N40 (5' GCT TGA TGA TTG GAA CTG A 3') with expected amplicon size of 1.2 kb. The 25 µl RT-PCR reaction mix consisted of the following: 8.5 µl ddH_2O, 12.5 µl 2x reaction mix (Invitrogen), 0.25 µl for each of the primers at 50 µM, 1 µl RT/Platinum Taq Mix (Invitrogen) and 2.5 µl RNA template. One step RT-PCR was done using TaKaRa PCR Thermal Cycler Dice with the following thermocycling conditions: reverse transcription at 50°C for 30 mins, denaturation at 95°C for 5 mins, 40 rounds of 94°C for 1 min, 48°C for 1 min and 72°C for 1 min, 30 s and a final extension of 72°C for 10 mins. Generated amplicons were then analyzed in 1% agarose gel, prestained with SYBRSafe® DNA Gel Stain (Invitrogen), along with 1 kb DNA ladder (Invitrogen) as marker. QuantityOne (BioRad) software was used to visualize and document the gel.

To complete the procedure, nested PCR which targets the internal region of N gene, was performed. Invitrogen™ Taq DNA Polymerase and the primers N1 (5' TTT GAG ACA GCC CCT TTT TG 3') and N2 (5' CCC ATA TAG CAT CCT AC 3') were used for nested PCR. The expected amplicon size is 0.49 kb. The 25 µl nested PCR reaction mix consisted of the following: 18.925 µl ddH_2O, 2.5 µl 10x reaction mix (Invitrogen), 0.875 µl of 50 mM $MgCl_2$, 0.5 µl of 10mM dNTP mix, 0.5 µl for each of the primers at 50 µM and 0.2 ul Taq DNA Polymerase (5U/ul) (Invitrogen). Nested PCR was done using TaKaRa PCR Thermal Cycler Dice with the following thermocycling conditions: denaturation at 95°C for 5 mins, 30 rounds of 95°C for 1 min, 50°C for 1 min and 72°C for 1 min, 30 s and a final extension of 72°C for 10 mins. Generated amplicons were then analyzed in 2% agarose gel, prestained with SYBRSafe® DNA Gel Stain (Invitrogen), along with 100 bp (Invitrogen) as marker. QuantityOne (BioRad) software was used to visualize and document the gel.

B. Reverse Transcriptase Loop-Mediated Isothermal Amplification (RT-LAMP)

Total RNA was extracted from brain tissues of dog and cat from the Philippines using RNEasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions; and from human tissues using TRIzol reagent (Invitrogen, Carlsbad, Calif., USA). All infectious materials were handled in BSL2 and BSL3 laboratories.

Primer design: A total of 10 primers including 4 outer primers 2 loop primers and 4 inner primers were designed using the LAMP primer designing software Primer Explorer V3 (Eiken, Tokyo Japan) based on nucleotide sequence of the CVS-11 strain (GenBank accession no. AB069973) or on the DNA fragment amplified from the human rabies patient.

RT-LAMP was carried out using the Loopamp RNA amplification kit protocol (Eiken).

Reaction mixture

40 pmol FIP and BIP primers
5 pmol F3 and B3 primers
20 pmol of the BLP primers
1.0 ul of enzyme mixture containing avian myeloblastosis virus

AMV) reverse transcriptase and *Bst* DNA polymerase
12.5 ul 2X reaction mixture (40 mM Tris-HCI, 20 mM KCl, 16 mM $MgSO_4$, 20 mM of each dNTPs)
5 ul RNA sample

The mixture was incubated at 63 °C for 1 hour, and the reaction was stopped by heating at 80^0 C for 5 minutes. The RT-LAMP product was visualized under a UV transilluminator after 1.5% agarose gel electrophoresis with TAE buffer followed by bromide staining.

The restriction enzyme digestion of the RT-LAMP: The restriction enzyme digestion was carried out with 2 ul of the RT-LAMP product and 0.5 ul f *Rsal* enzyme (Toyobo, Tokyo, Japan) at 37°C for 1 hour.

Nucleotide sequence analysis of the RT-LAMP product: The fastest migrating band of the RT-LAMP product was cut out from the gel and purified by Qiaquick column (Qiagen). Sequencing was performed using the Genetic Analyzer 3130 (Applied Biosystems, Foster City, Calif., USA) with the FIP or BIP primers (Boldbaatar, 2009).

All primers used in the molecular diagnosis of rabies were designed by and obtained from the National Institute of Infectious Diseases (NIID), Japan.

Immunological Technique

A. Evaluation of the Direct Rabies Immunohistochemistry Test (DRIT) as a Diagnostic Assay for Animal Rabies Infection

Known positive specimen of both medulla and hippocampus were incubated at 2-6 °C and 26-31 °C. Every eight hours, the brain tissue was smeared and fixed with cold acetone. DRIT was performed. All incubation steps were at room temperature. Briefly, the slides were washed by dip-rinse method with tween-phosphate buffered saline (TPBS). The slides were immersed in 3% hydrogen peroxide for 10 minutes and incubated in a humid chamber with primary antibody-biotinylated anti-rabies polyclonal antibody (NIID Japan). After incubation, the excess conjugate were washed off and the slides were dip-rinsed with TPBS. The slides were incubated with strepavidin-peroxidase complex for 10 minutes in humid chamber and washed with TPBS. Peroxidase substrate, amino-ethylcarbizole (AEC) were added dropwise to cover the whole impression smear in a humidity chamber for 10 minutes. The slides were dip-rinsed in deionized or distilled water. The counter stain which is Gills hematoxylin was added and incubated for 2 minutes. Immediately, the slides were dip-rinsed twice in deionized water. Slides were mounted and covered with cover slip. The slides were examined using light microscope.

B.Development of local fluorescent isothiocyanate conjugate (FITC) for direct antigen detection by fluorescent microscopy and evaluation of the direct rabies immunohistochemistry test as a diagnostic assay for human and animal rabies infection

All procedures involving the street virus were done in BSL 2 laboratory and

with personnel protection equipment (PPE).

Preparation of the antigen for antibody monoclonal production

A +4 fluorescence rabies positive dog's brain was chosen for the brain suspension preparation. It was macerated and 10% (m/v) was suspended in Dulbecco's Modified Eagle Medium (DMEM) buffer. It was centrifuged at 200 x *g* for 5 minutes. About 0.3 ml brain suspension was inoculated intracerebrally to each suckling mice. Mice were checked daily for 21 days. The brain of these mice (dead or sick which were sacrificed) were collected and inactivated with 0.5 mg/ml ascorbic acid and 5 ug/ml $CuSO_4$ and incubated for 72 hrs at 4°C. After 3 days of incubation, mouse inoculation test was done to determine viral infectivity.

The brain suspension was concentrated using low speed centrifugation. Supernate was discarded and pellet was collected and resuspended in NTE buffer. The concentrated rabies virus was purified by isopycnic centrifugation in a sucrose gradient (15%, 20%, 25%, 30%, 35%, and 50%). Protein concentration was also determined for all samples using the bicinchoninic acid (BCA) Protein Assay. Samples were stored in -20°C freezer.

To confirm the presence of antigen, RT-PCR of rabies glycoprotein (G) gene was done. Briefly, total RNA was extracted from the sucrose gradient purified samples using Roche High Pure Viral RNA Kit. RNA was eluted in DEPC-treated water and stored in -70°C until further processing. PCR amplification of the G gene was done using primers RV-7F (5' CTA TGG TCT GAC ATG TCT CTT CAG 3') and RV-9RPh (5' TCA ACC GGG TCA TCA TAG ACC using Superscript III™ One-Step RT-PCR with Platinum® *Taq* High Fidelity Polymerase (Invitrogen). The 25 µl RT-PCR reaction mix consisted of the following: 9 µl ddH_2O, 12.5 µl 2x reaction mix (Invitrogen), 0.25 µl for each of the primers at 50 µM, 0.5 µl Superscript III™ ® *Taq* High Fidelity enzyme mix (Invitrogen) and 2.5 µl RNA template. One step RT-PCR was done using TaKaRa PCR Thermal Cycler Dice with the following thermocycling conditions: reverse transcription at 50°C for 30 mins, denaturation at 95°C for 5 mins, 25 rounds of 94°C for 1 min, 65°C for 1 min and 68°C for 1 min, 30 s and a final extension of 68°C for 10 mins. Generated amplicons were then analyzed in 0.8% agarose gel, prestained with SYBRSafe® DNA Gel Stain (Invitrogen), along with TrackIT™ λ DNA/Hind III fragments (Invitrogen) or 1 kb DNA ladder (Promega) as markers. QuantityOne (BioRad) software was used to visualize and document the gel.

Mouse immunization and antibody titer screening

The antigen (sucrose-purified rabies virus) was immunized to three (3) mice with 100 µg per dose, intraperitoneally. Immunization of mice followed the standard procedure of initial immunization at day 0 and boosting every 2 weeks at days 14, and 30. Sera were collected at baseline (D0), D14, and D30 to determine antibody titers.

Enzyme-linked immunosorbent assay (ELISA) was used to determine antibody titers of mouse immunized with street rabies virus. Briefly, different ELISA plates were coated with challenge virus standard (CVS), purified street rabies virus (PSRV) and multiple freeze-thawed PSRV. The coated plates were blocked with 5% milk-PBST solution. The plates were washed with PBST and 50 µl of mouse serum (1:1 dilution in 1% milk-PBST) were added.and incubated for 2 hours at 37°C.

Secondary antibody conjugated with alkaline phosphatase enzyme was added, then incubated for 1 hour at 37°C. The slides were washed again and substrate (pNPP) solution was used to develop color reaction.

RESULTS

Molecular Technique

A. RT-PCR and nested PCR

A total of 9 human specimens were tested for PCR from 2009 to present; four tested positive. All of the specimens were collected before the patient expired.

Table 1. Human specimen tested for rabies using PCR technique, 2009-present

Specimen type	No. tested	Positive	Negative
Saliva	8	4	4
Cerebrospinal fluid	1	0	1
TOTAL	9	4	5

B. RT-LAMP

Total RNA obtained from the clinical samples (12 dogs, 2 cats and 2 humans) subjected to RT-LAMP using the CVS primer set did not work. However, when the Philippines primer set was used instead of the CVS primer set, there was successful amplification of the rabies virus gene fragment. Unequivocal amplification was seen if there were more than 1000 copies which corresponds to approximately 5 fg of RNA in the reaction. There was a ten-fold higher sensitivity when compared with conventional RT-PCR. Similarly, RT-LAMP can detect the virus if titer was greater than 10^{-1} FFU.

All the street viruses from clinical samples tested positive when subjected to RT-LAMP with the Philippine primer set whereas, three of them were negative by conventional PCR (Boldbaatar, 2009).

Immunological Technique

A. Evaluation of DRIT as a diagnostic assay for animal rabies infection

Table 2 shows the comparison of DRIT and dFAT results on samples incubated at different temperature and time.

Table 2. Result of DRIT and dFAT on brain specimens at different conditions

Temperature	Exposure Time (hrs)	DRIT		dFAT	
		Hippocampus	Medulla	Hippocampus	Medulla
2-6°C	8	Positive	Positive	Positive +4	Positive +
	16	Positive	Positive	Positive +4	Positive +
	24	Positive	Positive	Positive +4	Positive +
	32	Positive	Positive	Positive +4	Positive +
	40	Positive	Positive	Positive +4	Positive +
	48	Positive	Positive	Positive +4	Positive +
	56	Positive	Positive	Positive +2	Positive +
	64	Positive	Positive	Positive +2	Positive +
	72	Positive	Positive	Positive +3	Positive +
	80	Positive	Positive	Positive +2	Positive +
	88	Positive	Positive	Positive +2	Positive + 2
26-31° C	8	Positive	Positive	Positive +4	Positive +
	16	Positive	Positive	Positive +4	Positive +
	24	Positive	Positive	Positive +4	Positive +
	32	Positive	Positive	Positive +4	Positive +
	40	Positive	Positive	Positive +4	Positive +
	48	Positive	Positive	Positive +4	Positive +
	56	Negative	Positive	Positive +2	Positive +
	64	Negative	Positive	Positive +2	Positive +
	72	Negative	Negative	Positive +2	Positive +
	80	Negative	Negative	Negative	Negative
	88	Negative	Negative	Positive +1	Positive + 1

Results showed that within 88 hours of specimen exposed to 2-6°C and within 48 hours exposed to 26-31°C, the DRIT and dFAT results were comparable. Hippocampus gave a negative result through DRIT if exposed at 56 hours or more at 26-31°C, whereas in dFAT it tested positive. Both medulla and hippocampus exposed for 72 hours or more at 26-31°C showed negative results by DRIT but still positive by dFAT. At 56 hours or more on both 2-6°C and to 26-31°C, dFAT results were positive but with +1 to +3 fluorescence, and with only few fields with fine dust-like fluorescence. Results showed that medulla is a more appropriate specimen for both DRIT and dFAT.