3. Results of rabies diagnosis and molecular epidemiology of rabies virus circulating in Vietnam in the period of 2006-2009

3.1. Human rabies diagnosis

Table 6: Result of human rabies diagnosis by RT - PCR

Sort of samples	RT – PC	Total		
	Positive	Negative	Total	
CSF	4	2	6	
SLV	6	0	6	
CSF/SLV	13	6	19	
Total	23	8	31	

From 2007 – 2009, 31 rabies suspected patients admitted to Bach Mai hospital and Institute for Tropical and Infectious Diseases. From these patients saliva (SLV) and or cerebrospinal fluid (CSF)were taken. Twenty three out of 31 of these rabies suspected patients (74%) were confirmed to be infected with rabies virus by RT–PCR.

3.1. 1. Distribution of confirmed human rabies according to the days after onset

Table 7: RT – PCR results by the time of samples collection

Time after onset		1 day	2 days	3 days	4 days	> 5 days	Total
Results	positive	3	2	5	3	10	23
Results	negative	0	1	0	0	7	8
To	Total		3	5	3	17	31

Table 7 shows the results of viral RNA detection in the samples which were taken from patients at various time after onset of signs and symptoms. The results indicated that our RT–PCR system could detect RNA of rabies virus in intra samples taken shortly after onset. However, the samples taken as earlier as the more sensitive of RT–PCR can be achieved.

3.1. 2. History of exposure to rabid animals among the rabies confirmed patients

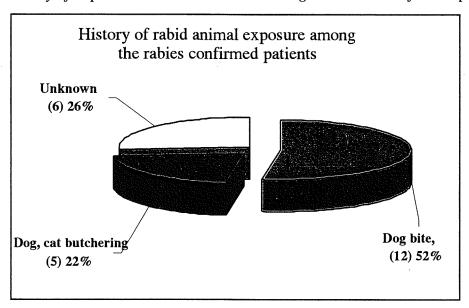


Chart 3: History of rabid animal exposure among rabies confirmed patients

In this study, 12 out of 23 patients who were confirmed to suffer from rabies had been exposed to rabid dogs or cats (52%); 5 of 23 had butchered sick cats or dogs (22%) and 6 patients did not have any history of dog bite or butchering sick animals (26%).

3.1. 3. Contribution of months to the number of confirmed rabies patients

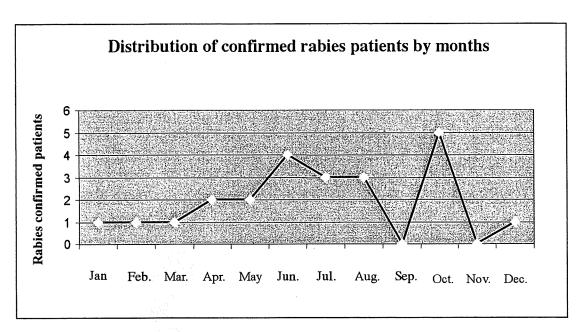


Chart 4: Distribution of confirmed rabies patients by months

The human rabies endemic occurs around year, but the peak is in the summer season (May, June and July).

3.1. 4. Distribution of rabies confirmed patients by age group

Twenty one out of 23 rabies confirmed patients are in the labour age group 16 - 60 (91,3%).

Table 8: Distribution of rabies confirmed patients by age group

Age group		0 - 15	16 – 60	> 60	Total
RT -PCR	Positive	0	21	2	23
result	Negative	2	3	3	8
Total		2	24	5	31

3.1.5. Distribution of rabies confirmed patients by geographical region

Table 9: Distribution of rabies confirmed patients by Northern geography region

Provinces	RT- PC	Total			
	Positive	Negative			
На Тау	10	2	12		
Phu Tho	4	0	4		
Bac Ninh	1	0	1		
Hoa Binh	3	0	3		
Yen Bai	1	0	1		
Son La	1	0	1		
Nghe An	1	0	1		
Lang Son	1	0	1		
Tuyen Quang	1	0	1		
Vinh Phuc	0	1	1		
Thai Binh	0	1	1		
Gia Lai	0	3	3		
Unknown	0	1	1		
Total	23	8	31		

According to the report of human rabies surveillance of Ministry of Health, most of human rabies deaths in recent years are occurring in the North and highland. So, in this study, we only focused on human diagnosis in the north and highland. The table 9 shows the human rabies epidemic occurred in many northern provinces, especially in Ha tay and Phu tho where there are the highest rabies deaths in Northern Vietnam in recent years.

3.2. Results of animal diagnosis

3.2. 1. Distribution of rabies suspected animals by geographical region

Table 10: Distribution of rabies suspected animals by geographical region

		Results					
Region	Provinces	Sample annalized	Positive				
North	На Тау	72	2				
	Ha Noi	04	0				
	Hoa Binh	10	0				
	Lang Son	06	0				
	Ninh Binh	06	0				
	Son La	01	0				
	Vinh Phuc	01	0				
South and	Gia Lai	03	03				
Higland	Lam Dong	01	01				
	Ho Chi Minh city	53	05				
	Long An	04	01				
	Soc Trang	03	02				
	An Giang	01	01				
	Dong Nai	04	01				
	Tay Ninh	01	01				
	Binh Duong	03	0				
	Tra Vinh	01	0				
	Tien Giang	01	0				
	My Tho	01	0				

Total	176	17

In the north, 100 sick dogs in slaughterhouses of different provinces were collected and annalised by RT – PCR, two among them (2%) were confirmed to be infected with rabies virus (table 10). Both rabies virus-infected dogs had the symptoms such as refusal of eating, increasing secretion and cage attack, and they are both in Hatay province where 10 confirmed humanrabies were reported. This result is the evidence which proves that rabies virus infection is most probably due to butchering sick animals (dog or cat).

In the South and Highland, from 2006 - 2009, a total of 73 rabies suspected dogs were collected and annalised by FAT and RT - PCR. Twelve out of 73 dog samples were confirmed rabies infection, the positive persentage equals to 16,4%. In this study, the average rate of rabies infection among sick dogs in Vietnam was 17/176 (9,7%).

3.2. 2. Rabies confirmation by methods used

Table 11: rabies confirmation of animals by diagnosis method

Methods	Positive	Negative	Total	Positive percentage
FAT	15	161	176	8,52%
RT – PCR	17	159	176	9,66%
Virus isolation on cell culture	0	109	109	0%

One hundred and seventy six samples were determined by three methods; FAT, RT-PCR and virus isolation on NA cell line. Only 109 samples were subjected to virus isolation (table 11). Rabies antigen was found in 15 dog samples by FAT (8.52%) while RNA of rabies virus was detected in 17 samples by RT-PCR (9.66%). No false positive and negative results of FAT was found. FAT is considered as the gold standard technique for rabies diagnosis, although its sensitivity is less than molecular biology technique.

3.3. Molecular epidemiology of rabies virus isolated in Vietnam from 2006 – 2009

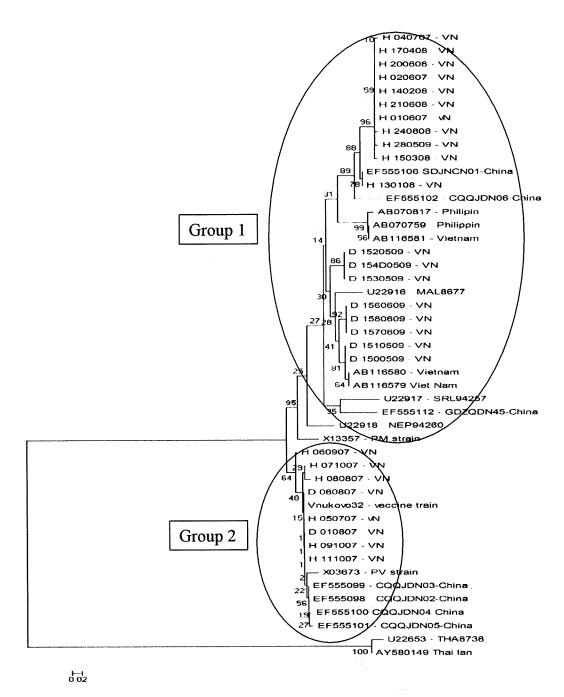


Figure 3 : Phylogenetic tree of 29 wild type rabies viruses isolated in Vietnam from 2006-2009

Phylogenetic tree of 29 rabies viruses isolated from dogs and human constructed on 387 nucleotide sequence of N gene (nt 85 - nt 387) shows that

rabies virus strains circulating in Vietnam can be divided into 2 groups. The first group, Vietnamese rabies viruses were closely related to strains isolated in China, Sri-Lanka, Nepal, Malaysia and the Philippines; the second group contained rabies viruses isolated from human and dogs in Northern Vietnam and Southern China.

Rabies group 1 may be branched off into 4 clusters, A cluster is from H040707 to EF555102, B is from AB070759 to AB116581, C1 is from D1520509 to D1530509, C2 is from MAL8677 to AB116579 and D is from H06907 to EF555101.

3.3. 1. Circulation of rabies virus group isolated in Vietnam from 2006 – 2009 by geographical region

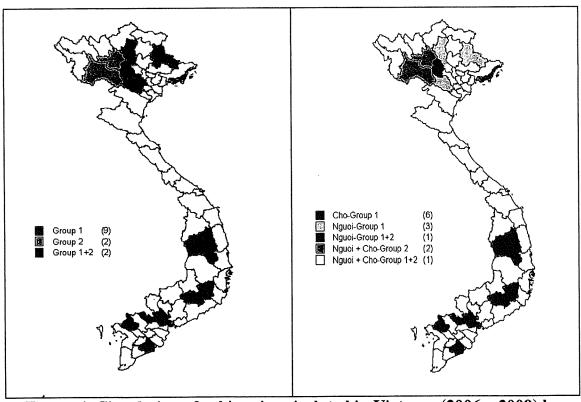


Figure 4. Circulation of rabies virus isolated in Vietnam (2006 – 2009) by

geographical region

The phylogenetic tree divided rabies virus isolated in Vietnam into two groups (fig. 3). Rabies virus group 1 circulated in 9 cities/provinces of all regions (North, South and highland), while the rabies group 2 was only found in northern Vietnam, and both rabies virus group 1 and 2 were present in two close provinces in the northern Vietnam (figure 4).

3.3.2. Amino acid sequences of rabies virus strains isolated in Vietnam

Figure 6: Alignment of nucleoprotein amino acid sequence of rabies viruses isolated in Vietnam with those of other rabies viruses

ARKGDKITPGSLVEIKRTDVEGNWALTGGMEL	O H	S	D 0 0	, A. D.) O O		D	D			00	000		2222
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25		U22627EGY8692 U22637ETH8807	U22641GUI9024 U226633AFS821 U22666RUS9141			AB116581Vietnam . H010607-VN .	H040707-VN H071007-VN						H_280509VN D_1500509VN	

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| 129 | 135 | 157 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150 | 150
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The nucleoprotein amino acid sequence of 29 rabies viruses isolated in Vietnam were aligned with those of 19 reference isolates. The numbering of each aa position was based on the PV strain (acc.no X03673). Amino acid substitution at position 84 specificially found in 6 rabies virus strains which were isolated in the south and highland Vietnam are indicated in the box. We also found that amino acid substitution of D for E at position 110 appeared in 13 rabies virus strains isolated from humans in the North Vietnam and that P at position 135 was replaced by A in 10 rabies virus strains isolated in the South Vietnam.

The nucleotide and amino acid of partial N genes of Vietnamese strains had high identity among themselves (86.7 - 100% nt and 96.6 - 100% aa), followed by rabies isolates in South China and The Philippines (86 - 100% nt and 95.5 - 100% aa) and lowest identity with those of West Asia (81.8 - 88.6% nt and 92 - 98.9% aa).

4. Discussion

Ability of rabies virus RNA detection by RT - PCR in intra vitam samples:

In this study, RNA of rabies virus in the saliva and CSF could be detected by our RT – PCR system on the first day of onset of symptoms and signs. The rabies positive percentage met 92.7% when samples were collected in the four first days of the illness while rabies virus RNA were only found in 58.8% samples which were taken after 5 days of the onset. According to Hervee Bourhy (2008), the detection percentage of RNA rabies virus in intra

samples by RT-PCR and nested RT-PCR was from 57.5% to 70.2% depending on the time of sample collection. So, it is recommended that collection of the samples should be done as early as possible and the second sample should be taken when the previous sample had shown the negative result.

Distribution of human and animal rabies by geographical regions:

According to the annual report of human rabies surveillance program. The human rabies cases mainly occured in northern and highland provinces of Vietnam in recent years. In this study, because of finance limit, the human rabies diagnosis were only focussed on northern and highland provinces. Thirty rabies suspected human who belongs to 12 provinces in northern and highland were taken samples and annalized by RT – PCR, twenty three patients were confirmed to be infection to rabies virus. Ten among 23 rabies confirmed patients live in Hatay province where there were two percent dogs in slaughterhouses infected with rabies virus that was found in this study. In Vietnam, the roaming dog raising is main practice and the dog vaccination coverage was only achieved 10 - 20%, so rabies in animals is easy to transmit from this region to others. For this reason, the control of rabies is recommended that not only focusing on the rabies epidemic regions, but also in whole country.

Molecular epidemiology of rabies viruses isolated in Vietnam

Phylogenetic tree of 29 rabies viruses isolated from dogs and human shows that rabies virus strains circulating in Vietnam can be divided into 2 groups. The first group, Vietnamese rabies viruses were closely related to strains isolated in China, Sri-Lanka, Nepal, Malaysia and the Philippines, the second group contained rabies viruses isolated from human and dogs in Northern Vietnam and Southern China. The rabies virus group 1 could be

found in whole country, meanwhile the second group only found in northern Vietnam, and both rabies virus group 1 and 2 were present in two close provinces in the northern Vietnam (figure 4). Those results suggested that may be there was a rabies transmission from to Vietnam – China.

There were 4 clusters which were branched off in the group 1 suggest that the history of (figure 3) the import – export from, to China, Philippines, Malaysia and Vietnam.







Final Report of the

Workshop on Policy Advocacy Strategy Development on Rabies Control and Prevention among ASEAN+3 countries

Venue: Hanoi, Vietnam

Organizer: NIHE and ASEAN+3 EID program

11 - 13 May 2009

Submitted by Dr. David Buchanan, DrPH

August 1, 2009

Rabies Control & Prevention Policy Advocacy Framework

Introduction

This report presents a framework for conducting advocacy activities aimed at increasing support among key public officials for eliminating rabies in ASEAN member states. It has been produced in follow-up to the <u>CALL FOR ACTION</u>, "Strengthening Cooperation and Information Sharing on Rabies among ASEAN plus Three Countries," developed at the 23–25 April 2008 workshop in Ha Long, Viet Nam. It was produced as a result of a second workshop held on 11-13 May, 2009 in Hanoi. Participants in this workshop included representatives from Viet Nam, Thailand, the Philippines, Myanmar, Cambodia, Laos and China. Technical experts from the WHO, CDC, WSPA and ASEAN Secretariat also participated in the meeting.

Advocacy is defined as the attempt to persuade or convince; it is intended to put pressure on people with power to change and improve a situation. To create an Advocacy Plan, three critical questions must be addressed:

- WHAT is the desired goal or action that you are seeking to achieve? What are you trying to convince people to do?
- WHO are you trying to convince? Who are your primary and secondary target audiences?
- HOW are you going to convince them to support the desired goal? What is your strategy?

This report provides recommendations on each of these key points.

What? Goals

The health goal of Rabies Control and Prevention is to eliminate rabies in each ASEAN country by the year 2020.

To achieve the health goal, the advocacy goal is to secure the resources needed to eliminate rabies.

To achieve the advocacy goal, three main resources are needed: (1) financial, (2) policy/legislation, and (3) leadership. Significant improvements in decreasing rabies incidence can be achieved without new money or legislation, but they require leadership. Leadership is defined as the ability to inspire others to adopt one's values, in this case, the value of eliminating rabies. By speaking out strongly in favor of eliminating rabies, leaders can persuade others to take step to eliminate rabies, even without additional financial resources. All of the action steps described below are directed towards achieving the advocacy goal of gaining the resources necessary to eliminate rabies.

Who? Target Audiences

To achieve the advocacy goal, the action plan presented here is designed to generate active support in three target audiences: (1) policymakers, (2) relevant government officials (health and animal control officials) and (3) the general public. Each action step is designed to contribute to the goal of increasing support in these three groups. Effective advocacy strategies are often designed to persuade one target audience to lobby another group, for example, to ask

community members (general public) to talk to their government representatives (policymakers) about the need to eliminate rabies, in order to prevent the terrible pain and suffering caused by the disease.

How? Strategy

After identifying the goals and target audiences, the next step in creating an advocacy plan is to identify strategies that will effectively persuade, convince and put pressure on policymakers, government officials and the general public to provide the resources needed to eliminate rabies by 2020. To develop an effective strategy, one starts by analyzing their concerns and priorities, their incentives and barriers: What is important to them? What are their priorities? Why should they care about rabies? How does taking the action that you advocate regarding rabies benefit them? What are the barriers (or costs) that they face in taking the desired action? This analysis is essential to inform one's advocacy strategy.

Strategy has two major elements: (1) messages and (2) delivery channels. In general, advocacy messages highlight the benefits and minimize the costs of taking the proposed action (appropriately tailored to each audience). In addition to the message content, people are also influenced by the process, the delivery channel: how the message is delivered to them. People are more likely to respond in the desired direction to (a) sources that they think are credible, and (b) their superiors, people who have power over them. When implementing an advocacy plan, it is important to choose the most effective "communication channel," to carefully select how the message is delivered to the intended target audience.

Messages: As a starting point for developing messages, people, in general, respond to rational appeals. They are more likely to take action where the benefits obviously outweigh the costs; conversely, it is very difficult to convince people to do anything where the costs are perceived to outweigh the benefits. Therefore, for our purposes, it is essential to show that it is feasible to eliminate rabies with modest, affordable increases in funding and that eliminating rabies (through dog vaccinations) will, in short time, save the government money relative to the high costs of administering PEP to people who have been exposed. Most importantly, eliminating rabies will save lives. (This point is sometimes stated in economic cost-benefit terms, e.g., eliminating rabies will reduce lost productivity due to premature death, etc.).

The biggest challenge in securing greater financial support for rabies control and prevention is the general lack of concern about rabies -- rabies is the "invisible" disease, the "neglected" disease - especially in the face of competing pressures on the use of scarce public resources. As a rule, putting additional funding into rabies control and prevention means taking money out of other potentially worthwhile projects (at least in the short run). To be successful in conducting advocacy activities, it is therefore important to remember that the perceived costs and benefits must be expanded beyond mere financial considerations to include more emotional appeals.

One important benefit to key target audiences is *fame*. One major cost is *shame*. Supporting the effort to eliminate rabies will enable contributors to claim credit for achieving a most praiseworthy goal; they can say that, due to their support, rabies has been (or is on the way to being) eliminated in their country. They have saved lives. They have put their country ahead of other countries that have failed to prevent unnecessary human suffering. Conversely, failing to support the effort to eliminate rabies would make them culpable, would make them responsible for each unnecessary and preventable rabies death that occurs in their country each year. They would be responsible for making their country one of the last on earth to stop rabies. Dying from rabies is a horrifying, painful way to die, and it predominantly affects children. They should be ashamed.

Delivery Channels: There are many different ways of getting one's messages to identified target audiences. Each action step described below offers ideas about the best way to get your message across to the audience that you are trying to reach. Even if the content of the message is quite similar, how the message is delivered needs to be tailored to the respective target group in order to maximize its impact.

Actions steps

The action steps that follow are designed to raise public awareness about (a) the worldwide goal of eliminating rabies, (b) the ready availability of existing scientific technologies for accomplishing this goal, and (c) the tremendous value of preventing unnecessary human deaths due to rabies. They are designed to take rabies from being the "invisible," "neglected" disease to putting it on the national agenda as an important priority needing attention and action today. The objectives for the three major target audiences are:

- For policymakers, the action steps are designed to put pressure (1) to enact model legislation to provide state officials with the power and authority to compel dog vaccinations (and other steps, such as regulations regarding stray dog control); and (2) to increase funding for rabies control and prevention programs.
- For government officials, they are designed to convince them (1) to provide leadership in making rabies control and elimination a priority, demonstrated by (a) allocating sufficient human and financial resources within their discretionary budgetary authority to reduce rabies incidence, and (b) holding responsible parties accountable for achieving measurable progress in eliminating rabies; and (2) to demonstrate inter-sectoral collaboration between health and animal control officials, as evidenced by joint reports and joint testimony on the need to increase funding for dog vaccination programs.
- For the general public, they are intended to generate broad public support for the call to eliminate rabies, as evidenced by their support and participation in activities aimed at influencing policymakers to increase funding for rabies control and prevention.

In this Advocacy Strategy Plan, ten recommended action steps are:

- 1. Produce Country Report
- 2. Develop Policy Brief
- 3. Convene National Advisory Board (for external public relations purposes)
- 4. Convene National Steering Committee (for internal planning purposes)
- 5. Develop National Plan for the Control and Elimination of Rabies
- 6. Develop media strategy
- 7. Issue Annual Progress Reports
- 8. Enact (or improve) national legislation
- 9. Provide leadership on increasing dog vaccination rates
- 10. Establish Provincial Coordinating Committees

NOTE: The countries involved in the Hanoi workshop in May 2009 are at different stages in implementing effective rabies prevention and control programs. Several Action Steps presented in this report have already been completed by some countries, and hence, may be skipped depending on one's current situation.

Action Step #1: Produce Country Report

The first step is to produce a Country Report that describes the current disease burden of rabies and prevention and control programs in one's country.

Strategy:

Purpose (what): The purpose of the Country Report is to raise awareness about rabies, to increase its visibility, and to put its elimination on the national agenda. The Country Report provides the fundamental justification to the National Government, the Department of Health (DOH), and Department of Agriculture (DOA, or wherever responsibility for Animal Health resides within the government) for making rabies a priority health problem.

Target Audiences (who): The primary target audiences for the Country Report are the Ministers of Health, Agriculture (or Animal Control), and Finance. Other important audiences might include the Minister of Tourism and Minister of Economic Development, in order to enlist their support in reinforcing the need for increasing funding for rabies control and prevention programs (e.g., they can tell the Minister of Finance how much rabies costs the country from their perspective each year).

Key themes (message): The message of the Country Report must be blunt and direct: Rabies can be eliminated; any deaths from rabies are unnecessary and preventable; dying from rabies is a horrifying experience that predominantly affects children. Each section of the report should support and reinforce this key theme.

Delivery Channel: To persuade the targeted government officials, the Country Report should be presented as an official report by national authorities on rabies control and prevention. (In most cases, this means the national representatives who participated in the Hanoi workshop on Policy Advocacy.)

Recommendations: Producing the Country Report should not be unduly burdensome. To make writing the Country Report manageable, authors can start by building on the presentations that they (and others) made at the Hanoi workshop. A suggested outline of the Country Report is presented in Annex I. To produce this report, authors are encouraged to make use of existing data (from the WHO, CDC and other sources), rather than expending significant time and resources in collecting new data or assembling and analyzing a lot of data from different domestic sources (see, for example, WHO Expert Consultation on Rabies, 2005, Technical Report Series 931; available at:

http://whqlibdoc.who.int/trs/WHO_TRS_931_eng.pdf.)

The most difficult part of the Country Report is estimating the amount of new resources that are needed for eliminating rabies. There is no simple formula here. Increases in funding are most likely going to come in annual incremental steps, so it may be helpful to think about what an effective system for rabies control and prevention system must include by the year 2020 and work back from there.

Next steps: Participants in the Hanoi workshop will aim to have their respective Country Reports completed by the follow-up meeting in September.

Action step #2: Develop Policy Brief

A policy brief is a short 1-3 page (maximum) statement that highlights the key points contained in the Country Report. Policy Briefs are necessary to produce because policymakers do not