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

Session 1: Overview of rabies in Asia : Country and sub-continent reports

Chair person: **Dr. M.K. Sudarshan,** *President, Rabies in Asia Foundation, India*

Co-Chair person: Prof. Dr. Quing Tang, *CDC China*

Report by Dr. M.K. Sudarshan

9 - Sep - 09	09:30 - 10:00	Rabies in Asia : evolution of the situation since the Asian rabies meeting, Hanoi 2001	**Dr. Betty Dodet** *Representative of AREB*
	10:00 - 10:30	Call for Action and Advocacy strategy "towards the elimination of rabies" in Asean +3 countries	**Dr. Luningning Villa,** *Programme Facilitator ASEAN Plus Three EID Programme Phase II. Health and Population Unit, Bureau for Resources Development, ASEAN Secretariat*
	10:30 - 11:00	Rabies Program in Brazil	**Dr. Marcelo Wada** *National Rabies Control Brazil Ministry of Public Health, Brazil*
	11:00 - 11:30	Research on Rabies: where are we and what more is needed?	**Dr. Franka Richard** (CDC/CCID/NCZVED)" *Rabies Program National Center for Zoonotic, Vector-borne, and Enteric Diseases Centers for Disease Control and Prevention, US*

❖ ***Rabies in North-Eastern Asian countries (15 minutes each)***

Chair person : Dr. F.X. Meslin, *World health organization*

Co- Chair person : Dr. Betty Dodet, *Representative of AREB*

Report by Dr. Betty Dodet

9 - Sep - 09	11:30 - 11:45	Rabies in China	**Dr. Quing Tang** *Head of Rabies Section Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, China CDC*
	11:45 - 12:00	Rabies in Japan	**Dr. Satoshi Inoue** *National Institute of Infectious Diseases*
	12:00 - 13:30	**Lunch break**	

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Date: 5th February, 2010

To: Dr. Satoshi INOUE
Chief,
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Department of Veterinary Science,
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Dear Dr. Satoshi Inoue,

On behalf of National Institute of Hygiene and Epidemiology, Hanoi Viet Nam, we express our sincerely thanks to your interest and collaborate with our institute to develop the new techniques for rabies diagnosis and set up the rabies laboratory net work in Vietnam for better rabies surveillance, diagnosis, control, prevention and research.

We would like to invite you to our institute, the National Institute of Hygiene and Epidemiology, Hanoi, Vietnam from 13 – 21 March 2010 to discuss detail on this project. During your visit to Vietnam, we will organize a field visit to Ha Giang province to investigate the laboratory capacity and rabies situation in both human and animals.

We would like to take this great opportunity to thank you for your support and looking forwards to the fruitful collaborations with you and your institute

Yours sincerely,

Assoc. Prof. Nguyen Thi Hong Hanh, MD., MPH., PhD.
Vice Director
National Institute of Hygiene and Epidemiology

厚生労働科学研究費補助金（インフルエンザ等新興・再興感染症研究事業）
分担研究報告書
アジアの研究機関との連携におけるラボラトリーネットワークの強化に関する研究
狂犬病の免疫組織診断系の検証と確立
（ウサギとニワトリ卵黄で作製した抗狂犬病ウイルス蛋白特異抗体の特性等に関する比較検討）

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研究要旨　狂犬病の確定診断には、蛍光抗体法、ELISA、ウイルス分離、PCR、電子顕微鏡および免疫組織化学的手法が用いられている。この中で、免疫組織化学的手法は特別な設備投資の必要性がなく、安価で確定診断が可能である。
今回、狂犬病の免疫組織診断系の検証と確立を目的に、ウサギとニワトリ卵黄で作製した抗狂犬病ウイルス蛋白抗体の特性等についてホルマリン固定材料を用いて比較検討した。その結果、最も染色感度が高いのはウサギおよびニワトリ卵黄由来の抗P蛋白抗体であり、ニワトリ卵黄由来のP抗体では非特異反応が殆どみられないことが明らかになった。動物種間の染色態度の比較では、ヒツジ由来の野外毒に感染したマウスの脳脊髄において大型な封入体が観察されるのに対し、固定毒CVS-11やコウモリ由来株では比較的に小型であり、HE標本では観察困難であることが判明した。今後は今回確立した免疫染色の手法を自然感染例に応用し、各種抗体の染色感度について比較検討する必要性が残っている。

A.　研究目的

狂犬病の確定診断には、蛍光抗体法、ELISA、ウイルス分離、PCR、電子顕微鏡および免疫組織化学的手法が用いられている。

今回、狂犬病の免疫組織診断系の検証と確立を目的に、ウサギとニワトリ卵黄で作製した抗狂犬病ウイルス蛋白抗体の特性等についてホルマリン固定材料を用いて比較検討した。特に、ニワトリ卵黄抗体はこれまでの抗ウイルス血清に比べ

て、安価でかつ大量生産が可能であり、狂犬病確定診断に対する有用性が注目されている。

B. 研究材料および方法

一次抗体として、固定毒CVS-11由来蛋白をウサギに免疫して得られた抗ウイルス血清3種類(anti-N9-5, anti-P13-31, anti-G (pc DNA-G)およびCVS-11のリコンビナント核蛋白を大腸菌で発現させ、その蛋白を産卵ニワトリに免疫させて得られた抗ニワトリ抗体5種類
(anti-N9-5IgY, anti-P13-31 IgY, Vaccine IgY 9～10 IU/ml, Vaccine+Adjuvant IgY 0.5～1 IU/ml, anti-G-F2 IgY 3～4, 9～10 IU/ml)を使用した。

研究材料として、固定毒CVS-11を感染させたマウスとサル、羊由来の野外毒(P-18)およびコウモリ由来の野外毒(MPIV)を脳内あるいは筋肉内接種したマウスの脳脊髄のホルマリン固定材料である。パラフィン包埋ブッロクから3μm厚の組織標本を作製し、以下のプロトコールに従って免疫染色を実施した。

抗ウサギ抗体

anti- P (P13-31)

1. 脱パラフィン、親水化
2. 0.25%トリプシン、室温30分
3. PBS洗浄(5分×3回)
4. 0.3%過酸化水素加メタノール、室温、60分
5. PBS洗浄(5分×3回)
6. 10%ヤギ血清、室温60分
7. 一次抗体(1200倍希釈)、4度、overnight
8. PBS洗浄(5分×3回)
9. 二次抗体(anti-rabbit IgG、200倍希釈)、室温30分
10. PBS洗浄(5分×3回)
11. ペルオキシダーゼ標識ストレプトアビジン、室温30分
12. PBS洗浄(5分×3回)
13. DAB発色(20~30秒)
14. 核染、色出し
15. 脱水、透徹、封入

anti-N (N9-5)

1. 脱パラフィン、親水化
2. 0.25%トリプシン、室温30分
3. PBS洗浄(5分×3回)
4. 0.3%過酸化水素加メタノール、室温、60分
5. PBS洗浄(5分×3回)
6. 10%ヤギ血清、室温60分
7. 一次抗体(1200倍希釈)、4度、overnight
8. PBS洗浄(5分×3回)
9. 二次抗体(anti-rabbit IgG、200倍希釈)、室温30分
10. PBS洗浄(5分×3回)
11. ペルオキシダーゼ標識ストレプトアビジン、室温30分
12. PBS洗浄(5分×3回)
13. DAB発色(20～30秒)
14. 核染、色出し
15. 脱水、透徹、封入

anti- G (pc DNA-G)

1. 脱パラフィン、親水化

2. オートクレーブ処理、121度、15分
3. PBS洗浄（5分×3回）
4. 0.3%過酸化水素加メタノール、室温60分
5. PBS洗浄（5分×3回）
6. 10%ヤギ血清、室温60分
7. 一次抗体（200倍希釈）、4度、overnight
8. PBS洗浄（5分×3回）
9. 二次抗体（anti-rabbit IgG、200倍希釈）、室温30分
10. PBS洗浄（5分×3回）
11. ペルオキシダーゼ標識ストレプトアビジン、室温、30分
12. PBS洗浄（5分×3回）
13. DAB発色（約30秒）
14. 核染、色出し
15. 脱水、透徹、封入

抗ニワトリ抗体

anti- P (P 13-31 IgY)

1. 脱パラフィン、親水化
2. 0.25%トリプシン、室温、30分
3. PBS洗浄（5分×3回）
4. 0.3%過酸化水素加メタノール、室温、60分
5. PBS洗浄（5分×3回）
6. 10%ウサギ血清、室温60分
7. 一次抗体（1000倍希釈）、4度、overnight
8. PBS洗浄（5分×3回）
9. 二次抗体（HRP anti chicken、200倍希釈）、室温30分
10. PBS洗浄（5分×3回）
11. ペルオキシダーゼ標識ストレプトアビジン、室温30分
12. PBS洗浄（5分×3回）
13. DAB発色（40秒～4分）
14. 核染、色出し
15. 脱水、透徹、封入

anti-N (N9-5 IgY)

1. 脱パラフィン、親水化
2. 0.25%トリプシン、室温30分
3. PBS洗浄（5分×3回）
4. 0.3%過酸化水素加メタノール、室温60分
5. PBS洗浄（5分×3回）
6. 10%ウサギ血清、室温60分
7. 一次抗体(150倍希釈)、4℃、overnight
8. PBS洗浄（5分×3回）
9. 二次抗体（HRP anti chicken、200倍希釈）、室温30分
10. PBS洗浄（5分×3回）
11. ペルオキシダーゼ標識ストレプトアビジン、室温30分
12. PBS洗浄（5分×3回）
13. DAB発色（2分～2分30秒）
14. 核染、色出し
15. 脱水、透徹、封入

anti-G (3～4IU/ml)

1. 脱パラフィン、親水化
2. 0.25%トリプシン、室温30分
3. PBS洗浄（5分×3回）
4. 0.3%過酸化水素加メタノール、室温60分
5. PBS洗浄（5分×3回）
6. 10%ウサギ血清、室温60分
7. 一次抗体(400倍希釈)、4℃、overnight
8. PBS洗浄（5分×3回）
9. 二次抗体（HRP anti chicken、200倍

希釈)、室温30分
10. PBS洗浄（5分×3回）
11. ペルオキシダーゼ標識ストレプトアビジン、室温30分
12. PBS洗浄（5分×3回）
13. DAB発色（2分30秒～3分）
14. 核染、色出し
15. 脱水、透徹、封入

anti-G (9～10IU/ml)

1. 脱パラフィン、親水化
2. オートクレーブ処理、121℃、15分
3. PBS洗浄（5分×3回）
4. 0.3%過酸化水素加メタノール、室温60分
5. PBS洗浄（5分×3回）
6. 一次抗体(200倍希釈)、4℃、overnight
7. PBS洗浄（5分×3回）
8. 二次抗体（HRP anti chicken、200倍希釈)、室温30分
9. PBS洗浄（5分×3回）
10. ペルオキシダーゼ標識ストレプトアビジン、室温30分
11. PBS洗浄（5分×3回）
12. DAB発色（約30秒）
13. 核染、色出し
14. 脱水、透徹、封入

anti-Vaccine IgY、9～10 IU/ml

1. 脱パラフィン、親水化
2. オートクレーブ処理、121℃、15分
3. PBS洗浄（5分×3回）
4. 0.3%過酸化水素加メタノール、室温60分
5. PBS洗浄（5分×3回）
6. 一次抗体(200倍希釈)、4℃、overnight
7. PBS洗浄（5分×3回）
8. 二次抗体（HRP anti chicken、200倍希釈)、室温30分
9. PBS洗浄（5分×3回）
10. ペルオキシダーゼ標識ストレプトアビジン、室温30分
11. PBS洗浄（5分×3回）
12. DAB発色（約30秒）
13. 核染、色出し
14. 脱水、透徹、封入

anti-Vaccine+adjuvant IgY、(0.5～1 IU/ml)

1. 脱パラフィン、親水化
2. オートクレーブ処理、121℃、15分
3. PBS洗浄（5分×3回）
4. 0.3%過酸化水素加メタノール、室温60分
5. PBS洗浄（5分×3回）
6. 一次抗体(200倍希釈)、4℃、overnight
7. PBS洗浄（5分×3回）
8. 二次抗体（HRP anti chicken、200倍希釈)、室温30分
9. PBS洗浄（5分×3回）
10. ペルオキシダーゼ標識ストレプトアビジン、室温30分
11. PBS洗浄（5分×3回）
12. DAB発色（約30秒）
13. 核染、色出し
14. 脱水、透徹、封入

関連参考文献

Inoue S, Sato Y, Hasegawa H, Noguchi A, Yamada A, Kurata T and Iwasaki T. 2003. Cross-reactive antigenicity of nucleoproteins of lyssaviruses recognized by a monospecific antirabies virus nucleoprotein antiserum on paraffin sections of formalin-fixed tissues. *Pathol. Int.* 53: 525-533.

Motoi Y, Inoue S, Hatta H, Sato K, Morimoto K and Yamada A. 2005. Detection of Rabies-specific antigens by egg yolk antibody (IgY) to the Recombinant Rabies virus proteins produced in *Eschericha coli*. *Jpn. J. Infect. Dis.* 58: 115-118.

C. 研究結果

ウサギ血清3種類(anti-N9-5, anti-P13-31, anti-G (pc DNA-G)およびニワトリ卵黄抗体5種類 (anti-N9-5IgY, anti-P13-31 IgY, Vaccine IgY 9～10 IU/ml, Vaccine+Adjuvant IgY 0.5～1 IU/ml, anti-G-F2 IgY 3～4, 9～10 IU/ml)のいずれも染色性が観察された。特に、ウサギ血清 (anti-N9-5, anti-P13-31)およびニワトリ抗体 (anti-N9-5IgY, anti-P13-31 IgY, Vaccine IgY 9～10 IU/ml, Vaccine+Adjuvant IgY 0.5～1 IU/ml)を用いた場合に染色感度が高いことが明らかになった。また、染色像が非特異反応ではないことを透過型電子顕微鏡観察でウイルスの蛋白とウイルス粒子の確認によって確かめた。

以下に抗体陽性像の一部を示す。

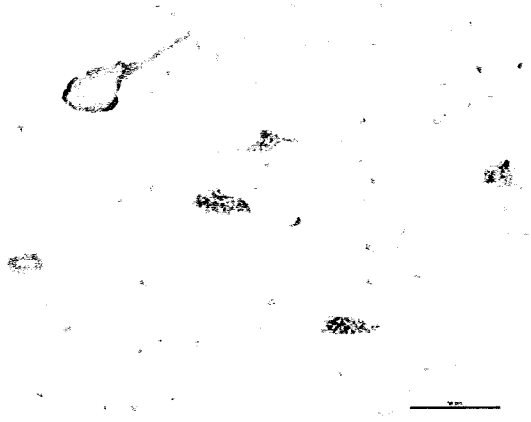

Fig. 1: anti-rabbit-N9-5、アカゲザル、CVS-11接種。小脳脚の神経細胞の細胞質が鮮明に染色されている。

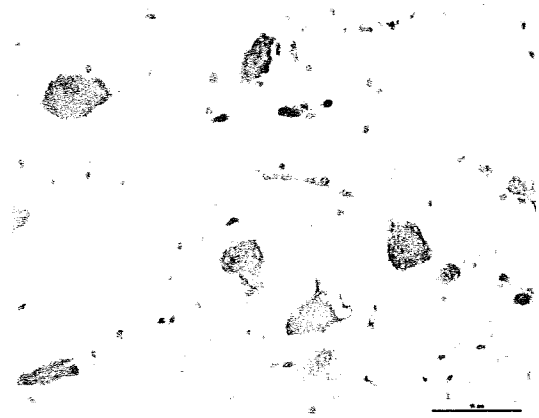

Fig. 2: anti-rabbit-P13-31、アカゲザル、CVS-11接種。小脳脚の神経細胞の細胞質および樹状突起が鮮明に染色されている。

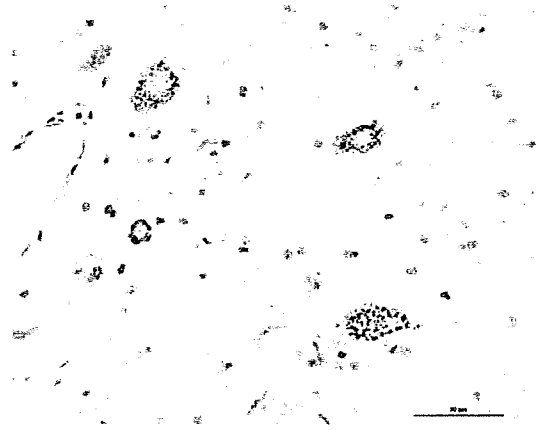

Fig. 3: anti-chicken-N9-5IgY、アカゲザル、CVS-11接種。小脳脚の神経細胞の細胞質が鮮明に染色されている。

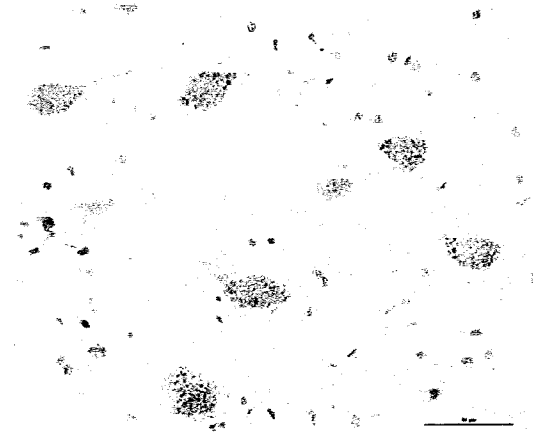

Fig. 4: anti-chicken-P13-31 IgY、アカゲザル、CVS-11 接種。小脳脚の神経細胞の細胞質が鮮明に染色されている。

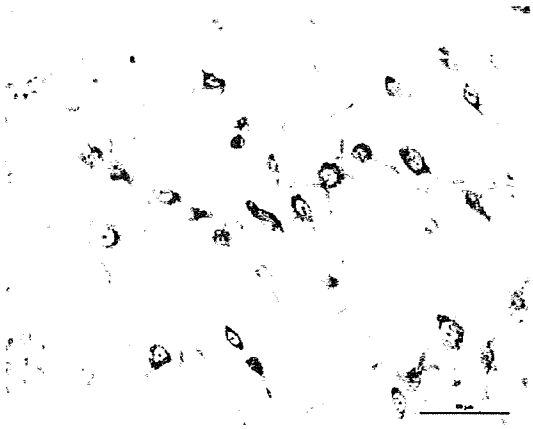

Fig. 5: anti- chicken-P13-31 IgY、マウスの視床、CVS-11 接種。視床の神経細胞および樹状突起が鮮明に染色されている。

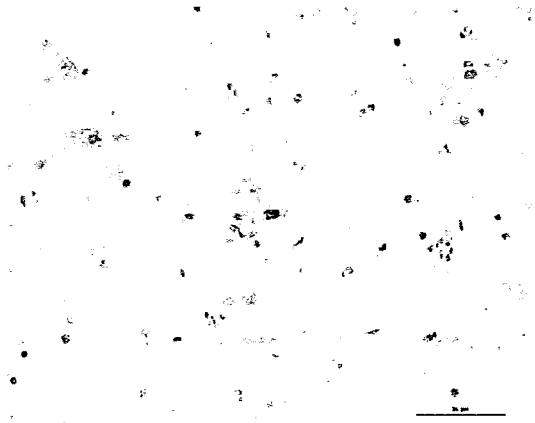

Fig. 6: anti-chicken-P13-31 IgY、マウス脊髄、ヒツジ由来の野外毒（P-18）接種。脊髄運動神経の細胞質に球状の抗体陽性象（封入体）が多数観察される。

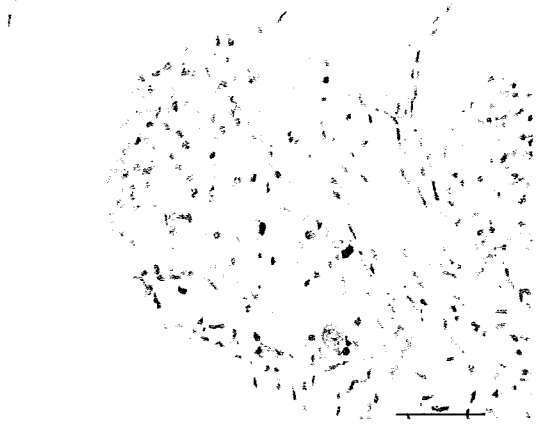

Fig. 7: anti- chicken-P13-31 IgY、マウス脊髄神経節、ヒツジ由来の野外毒（P-18）接種。
脊髄神経節細胞の細胞質に球状の抗体陽性象（封入体）が鮮明に染色されている観察される。

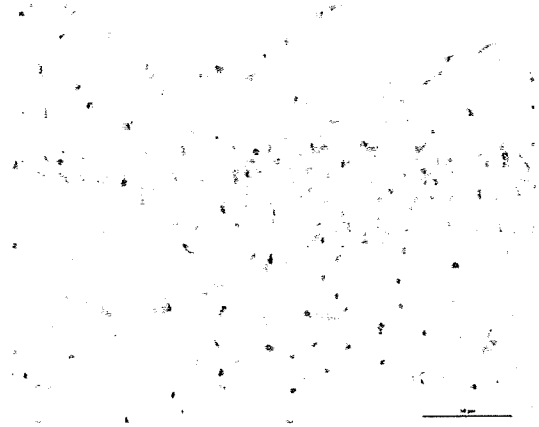

Fig. 8: anti-rabbit-N9-5、マウスの海馬、コウモリ由来の野外毒（MPIV）接種。海馬錐体細胞層に小型でスポット状の抗体陽性像が観察される。

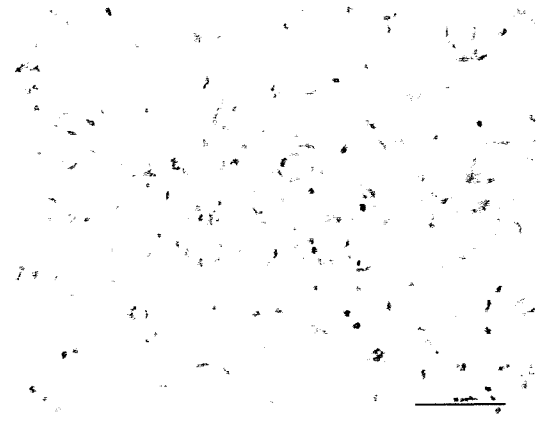

Fig. 9: anti-chicken-P13-31 IgY、マウスの海馬、コウモリ由来の野外毒（MPIV）接種。海馬錐体細胞層に小型でスポット状の抗体陽性像が鮮明に染色されている。

D. 考察

今回、狂犬病の免疫組織診断系の確立を目的に、各種一次抗体を用いて染色条件を検討した。今回の染色条件において、最も染色感度が高かった抗体はウサギおよびニワトリ卵黄由来の抗 P 蛋白であることが示された。特に、ニワトリ卵黄由来の P 抗体では他の抗体に比べてバックグラウンドが出にくく、非特異反応も殆

どみられないことから、今後、狂犬病ウイルスに感染した多種の動物の確定診断に充分応用できると思われた。また、ウイルス株の違いによる染色態度については、
ヒツジ由来の野外毒に感染したマウスの脳脊髄において大型な封入体が観察されるのに対し、固定毒CVS-11やコウモリ由来株では比較的に小型なスポット状を呈しており、ヘマトキシリン・エオジン標本では観察困難であることが判明した。
今回用いた症例は全て実験動物（サル、マウス）の脳脊髄であり、自然発症例との間に染色感度の違いがあるかどうかについては不明な点があり、今後は自然発症例との比較検討が必要と思われる。

E.　結論

本研究は、アジアのCDC機能を持つ国立の研究機関等との連携による狂犬病ラボラトリーネットワークの構築が到達目的である。安価で大量生産が可能なニワトリ卵黄抗体は狂犬病の多発地域であるアジア諸国において狂犬病の確定診断系として大変有効である事が示された。従って、今後、狂犬病ラボラトリーネットワークの構築に活用できるものと期待された。

F.　健康危機情報

特になし

G.　研究発表

1　論文発表

(1) Kojima D, Park CH, Satoh Y, Inoue S, Noguchi A, Oyamada T. Pathology of the spinal cord of C57BL/6J mice infected with rabies virus (CVS-11 strain). *J Vet Med Sci.* 71(3):319-324.

2　口頭発表

(1) 小嶋大亮, 朴天鎬, 小原慶子, 杉浦尚子, Boldbaatar Bazartseren, 佐藤豪, 野口章, 畑井仁, 小山田敏文, 井上智. 狂犬病ウイルス（CVS-11）を後肢筋肉内に接種したC57BL/6JおよびヌードマウスのCNSに関する比較病理学的研究. 第148回日本獣医学会学術集会。2009. 9. 25-27、とりぎん文化会館、鳥取

H.　知的財産権の出願・登録状況

1　特許取得　なし

2　その他　なし

Title: Construction of laboratory net work on the molecular epidemiology and the development and standardization of Rabies diagnostic methods

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Epidemiology of rabies in China in 2009.

A total of 14,065 human rabies cases were reported in China from 1996 to 2006, with an average of 1,297 cases each year and a rate of 0.0991 per 100,000, the fewest cases (159) occurred in 1996, after which the number of reported cases increased each year until 2006(Fig 1), with 3,293 cases reported, 20.7 times as many as in 1996. Under this situation, several national rabies control programs have been issued and progressed by the Ministry of Public Health and China CDC, which include: newly revised national criteria for human rabies diagnosis; revise "Rabies prevention and control manual"; national guideline for rabies post-exposure prophylaxis; expand the central fund for rabies prevention and control program in provincial health departments to support the surveillance, field investigation and management, technical training and health education etc. All those programs are very helpful and supportive for effective rabies surveillance, prevention and control in China. In 2008 the number of reported human rabies cases is 2373 and the incidence is 0.1796/100000, which is the beginning of rabies decline after ten years increasing, the high epidemic areas in 2008 are: Guangxi, Guangdong, Guizhou,

Hunan provinces and Chongqing city and the low risk area located in the northern and western provinces as well as the big cities(Fig 2, Fig 3). In 2009 human rabies was continuing decrease and the total reported cases is 2103 and it decreased about 10% compared with the case number in 2008, rabies cases occurred in all four seasons but with an autumnal peak(Fig 2, Fig 3).

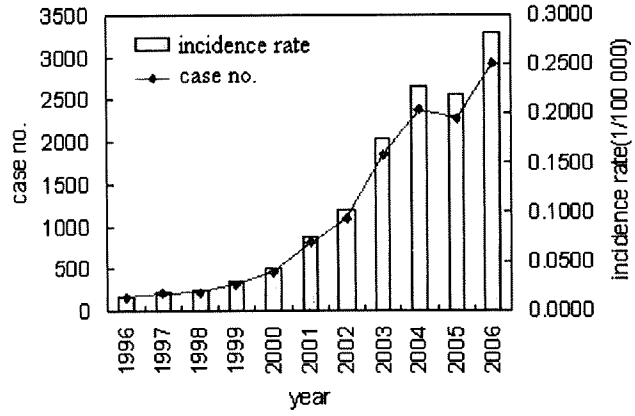


Fig 1 Human rabies in China, 1996-2006.

The high case number reporting provinces are almost the same as that of before, but nearly every province has fewer case reporting in 2009 compared with the year of 2008 (Fig 2).

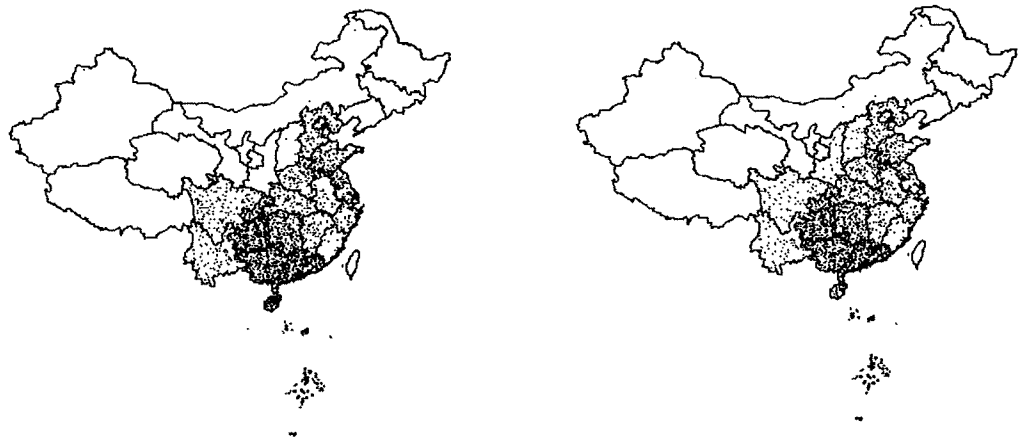
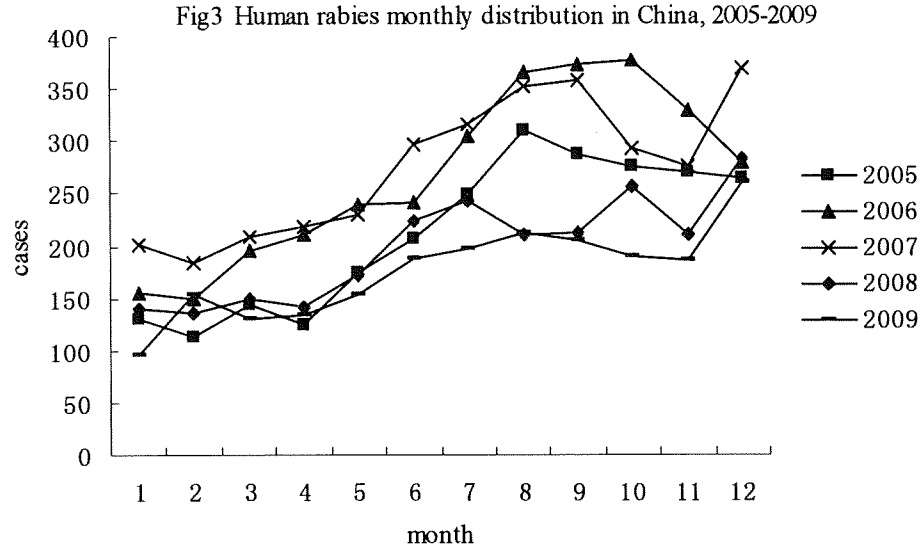


Fig 2 Human rabies geographic distribution by case in China in 2008(left) & 2009(right)

Fig3 Human rabies monthly distribution in China, 2005-2009



Molecular epidemiology investigation of rabies in southern China

Since 2003, there have been more than 2000 human rabies cases annually, of which most occurred in Guizhou, Guangxi and Hunan provinces. Therefore, it is necessary to take investigations on molecular properties about dogs infected with rabies virus in high-incidence regions, to discuss properties、origins and variations of rabies virus, and so on. From a total of 2887 dog specimens randomly collected from domestic apparently "healthy dogs", 66 were positive for RABV, yielding a positive rate of 2.3%. Details are given in Table 1, Figure 4. In regards to the 7 additional specimens obtained from 4 suspect rabid dogs and 3 deceased patients, all were positive. Dogs were confirmed as the primary rabies reservoir in China responsible for human infections.