Proceedings of

The National Workshop on Rabies Prevention and Control in Ethiopia



The Ethiopian Health and Nutrition Research Institute







Participants of the workshop,

October 18, 2012

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National Workshop on Rabies Prevention and Control in Ethiopia

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Acronyms

AAU/FVM - Addis Ababa University/ Faculty of Veterinary Medicine

- ABC Animal Birth Control
- APHRD Animal and Plant Health Regulatory Directorate
- BHK Baby Hamster Kidney Cell
- CCC Country Coordinating Committee
- CDC Centers for Disease Control and Prevention
- CSA Central Statistics Authority
- CVS Challenge Virus Strain
- dRIT Direct Rapid Immunohistochemical Test
- EA Enumeration Area
- EHNRI Ethiopian Health and Nutrition Research Institute
- EMLA Ethiopian Medical Laboratory Association
- EPHA Ethiopian Public Health Association
- EPHLA Ethiopian Public Health Laboratory Professionals Association
- ERA Evelyn Rokitniki Abelseth
- EVA Ethiopian Veterinary Association
- EWCA Ethiopian Wildlife Development and Conservation Authority
- EWCP Ethiopian Wolves Conservation Program
- FAO Food and Agriculture Organization
- FAT Fluorescent Antibody Test
- FAVN Fluorescent Antibody Virus Neutralization Test
- FMoA Federal Ministry of Agriculture
- FMoH Federal Ministry of Health
- GMO Genetically Modified Vaccine
- MHPR Members of the House of Peoples' Representatives

ID - Intra dermal

INDRD - Infectious and Non infectious Diseases Research Directorate

JU - Jimma University

MIT- Mouse Inoculation Test

NAHDIC - National Animal Health Diagnostic and Investigation Center

- NGO Non Governmental Organization
- NIH National Institutes of Health

NTV - Nerve Tissue Vaccine

- NVI National Veterinary Institute
- OHCEA One Health Central and Eastern Africa
- PANVAC Pan African Veterinary Vaccine
- PEP Post exposure Prophylaxis
- PV Pasteur Virus
- RFFIT Rapid Fluorescent Focus Inhibition Test.
- RT-PCR Revers transcrptase Polymerase Chain Reaction
- SAD Street-Alabama-Dufferin
- SEARG South and Eastern Africa Rabies Group
- SERO Scientific and Ethical Review Office
- SNNP South Nation and Nationalities People
- TCV Tissue Culture Vaccine
- **UN** United Nations
- WHO World Health Organization

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I. Acknowledgement

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We would like also to acknowledge the professional contribution of the Rapporteurs, Dr.Fisseha Abenet and Dr. Girma Zeleke. We thank Professor Getachew Abebe for writing workshop's highlights.

Our acknowledgement also goes to the Honorable Members of the House of Peoples Representatives, Ato Yosef Daemo and Ato Ojulu Gilo for their presence and directives given towards the Rabies prevention and control efforts.

We would like also to express our special thanks to organizations and individuals who contributed for the success of the National Rabies Survey, that includes the Central Statistic Authority (CSA) for its participation in the selection of Enumeration areas (EAs) and provision of information on the selected EAs, Regional Agricultural and Health Bureaus, Zonal Agricultural and Health Offices, District Agricultural and Health Offices and the Ethiopian Wild life Development and Conservation Authority (EWDCA) for their technical assistance and provision of information. We also thank survey participants, leaders and members of Peasant Associations, Health Extension Workers and Development Agents of the survey areas, and well wishers and friends for their encouragement and inputs rendered for the success of the survey.

Finally, our sincere thanks go to our partners; Centre for Disease Control (CDC) Atlanta, Rabies Section for their technical assistance and provision of Supplies, WHO- Ethiopia for their financial support for the rabies diagnosis scaling up project, and institutions who contribute for rabies vaccine production and surveillance.

The Workshop Organizing Committee, on behalf of the Institute

II. Introduction

A national workshop on rabies prevention and control in Ethiopia, which was organized by the Ethiopian Health and Nutrition Research institute (EHNRI), was held from October 18 to 19, 2012 at Adama town, Ethiopia. The workshop was officially opened by Dr. Amha Kebede; Director General of ENHRI. On his speech, Dr. Amha emphasized that the objective of the workshop is aimed at networking stakeholders and creating strong collaborative platform for the prevention, control and eventual eradication of the disease from Ethiopia.

The Consultative workshop on rabies prevention and control was the first of its kind to be organized at this level. This national workshop, that attracts prominent professionals of the field, has also helped to form a national task force, which is in charge of enhancing collaboration and coordination among the concerned stakeholders.

A. Objectives of the workshop

The objectives of the workshop were to:

- Identify the role and responsibility of stakeholders in rabies prevention and control activities
 Disseminate major preliminary findings of the national rabies baseline survey
- Officially launch the published rabies educational material
- Sensitize establishment of rabies laboratory diagnosis service at regional level
- Commemorate the world Rabies Day and
- Promote the contributions of the EHNRI and its collaborators towards rabies prevention and control in Ethiopia

B. Content of the workshop

Generally, the two days stay of the workshop provides an exciting and proactive opportunity for the exchange of ideas related to rabies status countrywide as well as worldwide. Technical activities carried out by the Ethiopian Ministries of Health and Agriculture was discussed. The existing challenges and gaps also have been professionally assessed and the way forwards have been suggested.

C. Methodology

The workshop used several Presentations on different topics of rabies. Questions and General discussions and suggestions were an approaches used in the workshop.

D. Focus areas

The workshop was focused on several issues of Rabies prevention and Control in Ethiopia that includes; National rabies base line survey preliminary findings of Household and facility based assessments and the overall rabies situation in the world with special emphases on some Asian and African countries. In addition to that , human rabies surveillance and animal rabies situation in Ethiopia were also well addressed, and Anti-rabies vaccine produced by EHNRI which is sheep brain derived and current situation of tissue culture vaccine production at EHNRI were also presented and discussed by respective presenters and participants.

Regarding the current and future prospective of rabies diagnosis situation in Ethiopia, rabies legislations and best practices from Addis Ababa city Administration has been presented and discussed. Public educations on Rabies were also one of the focus areas of the workshop that followed by the official inauguration of the educational material. The major challenges and gaps in rabies prevention and control in Ethiopia and the way forward were also one of the main focus areas of the discussion and the workshop. As a focus area the workshop has also discussed and formatted a taskforces that contains stakeholders under the leadership of EHNRI.

E. Participants

The workshop was attended by over 80 participants delegated from stakeholders, partners, staff members and Media organizations group that include representatives from FMoA, FMoH, EHNRI, NVI, NAHDIC, EVA, EPHA, Regional Public and Veterinary Laboratories, and representatives from Amhara, Oromia, Tigray, Benishangul Gumuz and Somali regional states Health Bureaus, Federal and Regional public and veterinary offices, universities, research institutes, UN organization, and two members of the House of Peoples' Representatives.

Day one

1 Opening session

1.1 Speech by Dr. Amha Kebede, Director General, EHNRI

Your Excellencies Members of House of Representatives Representatives and Staff members of Stakeholder organizations Members of the Management Committee of the Institute Ladies and Gentlemen

The nation's health sector is performing several interventions in order to prevent and control and reduce the burden and occurrence of major diseases. To improve



the interventions and promote the health service delivery, numerous policies and strategies that have registered encouraging and hopeful achievements have been deployed.

However, to make the registered achievements more reliable and sustainable and ensure efficiency of the health service delivery, we still need to work so hard as quite a lot challenges are ahead of us.

As part of sustaining the registered achievements, the Ethiopian Health and Nutrition Research Institute, as a public health research arm of the health sector, is conducting several problem solving researches that are designed to address priorities of public health problems.

One of the major research focuses areas of the Institute is the researches on the Infectious and noninfectious diseases. Under this research block, we have also Zoonoses Research Team that conducts different researches on Rabies, which is the cause for our gathering today.

Our Institute is conducting researches using the advantage of its long standing experience in Rabies prevention and control and well educated and experienced professionals is producing vaccines against animal and human rabies and working on awareness creation programs on Rabies.

In order to make our strategies and policies on rabies are evidence based and policy makers' friendly, the Institute has undertaken a national level baseline survey recently at a cost of over 2 million ETB, which allocated from the government budget. Allocating this much money directly from the government budget is at least a prominent signal that rabies has been in the priority list of the Ethiopian government health affairs.

The finding of this survey, I believe will provide us relevant data on the annual incidence of animal bites and human rabies deaths, annual consumption of post exposure treatment, time trends of rabies in human and animal populations, the level of rabies control and prevention efforts and the awareness level of the population concerning Rabies. The preliminary report of this survey will be disseminated after a while.

Your Excellencies Members of the Parliament

Invited Gusts

Ladies and Gentlemen

Although our Institute is working as the only Institute in the field of rabies since many years, we call you today to strengthen and harmonize that effort and maximize the rabies prevention and control interventions. This workshop is also a forum conducted to coordinate and integrate efforts that all concerned are making separately.

In addition to coordinating the disease burden reduction effort, in this workshop, we will also discus on the facilitation of rabies diagnosis service at Regional level.

I am hopeful that our rabies prevention and control efforts will be successful if professionals of the field and other concerned organizations coordinate their effort and perform tasks in an organized manner.

I wish your discussions, professional imputes and initiatives to work together with in these two days are fruitful.

I finally would like to thank all partners and staff members of the Institute for the contribution you made for the success of the workshop

Thank you

1.2 Speech by Dr. Almaz Abebe, Director, Infectious and Non-infectious Disease Research Directorate



Your Excellencies Members of House of Representatives! Your Excellency, Dr. Amha kebede, Director General of EHNIR! Representatives and Professionals of Stakeholder organizations! Members of the Management Committee of the Institute! Ladies and Gentlemen,

I first of all would like to welcome you all to this national workshop that is organized by EHNRI to bring all stakeholders of the rabies disease prevention and control together to discuss on this important issue and suggest possible ways that helps to reduce the disease burden sustainably.

As you all know, the rabies disease situation in our country is getting very worse from time to time. Considering this, several

interventions have taken place by all concerned government bodies. Among the interventions, I can mention the suggested strategies for canine rabies control that includes placing rules and regulations which enforces dog ownership and vaccination, controlling dog movement and reducing stray dog population.

Besides this, our institute is providing rabies vaccination and other related services for the last half a century.

However, those efforts don't seem to satisfy all the vital issues of rabies to its satisfactory level. In other words, the effort of the disease prevention and control doesn't meet the expectation.

For this, several reasons can be sighted. But, the major reason for the disease situation getting worse from time to time is that, it is only the Ethiopian Health and Nutrition Research Institute, which is the only institute in the country working in rabies diagnosis, human exposure counseling and anti-rabies vaccine production for animals and human.

Ladies and Gentlemen,

That is why; our institute, as it always takes the lead, conducts this workshop today for all concerned bodies to discus on possible ways of coordinating the rabies prevention and control effort.

This workshop, as one of the means of maximizing the effort towards the disease prevention and control and crating cooperation and collaboration between GOVs, NGOs, Research institutes, universities, professional associations, partners and professionals, is also accompanied by several events.

The dissemination of the preliminary report of the national rabies baseline survey findings is one of the events we have and it will be presented by my colleagues after a while based on the program schedule.

The survey is conducted to generate a comprehensive data on rabies and its related aspects in the human and animal populations in Ethiopia. By the time that we complete the data processing and come with the final findings, I believe, it will contribute a lot for our national rabies prevention and control interventions.

In addition to that, we will have also an event in which the rabies educational material, which is prepared to create awareness among the selected target groups and human and animal health professionals, will be officially launched.

Dear participants,

Ladies and Gentleman

I once again would like to welcome you all to the workshop that deals with the national agenda.

2. Presentations

During the day one of the workshop, seven presentations were delivered by different professionals. Name of presenters and Chair persons and an overview of the presentations presented below.

Presenters: Dr. Almaz Abebe(EHNRI), Dr. Eshetu Yimer (EVA), Ato Haftom Taame(EHNRI), Dr. Melaku Assefa (MoARD), Ato Abebe Mengesha (EHNRI), Dr. Abraham Ali (EHNRI), Dr. Hailu Mamo (EHNRI)

Chair Persons: Ato Kelbessa Urga, Dr. Sheferaw Jenberie, Dr. Eshetu Lemma, Dr. Kefle Weldemichael

2.1 An Overview of the Workshop, by Dr. Almaz Abebe

Dr. Almaz presented that rabies is one of the most important but most neglected diseases in Ethiopia. Owing to this fact, EHNRI has organized the workshop with an overall objective of establishing strong collaboration / networking among concerned stakeholders and creates holistic approach for rabies prevention and control in Ethiopia. Dr. Almaz explained the objective of the workshop and discussed the expected outcomes of the workshop that include:

- Increased collaboration/ networking among concerned stakeholders on rabies control and prevention
- Increased awareness on the current status of human and animal rabies in Ethiopia
- Sensitization of regional laboratories on the diagnosis of rabies
- The launching of published rabies educational material / booklet for increased public education and awareness on rabies
- The dissemination of Preliminary findings/ Results of the national survey on rabies in Ethiopia
 The Roles and responsibilities of stakeholders in rabies prevention and control identified

Dr. Almaz, subsequent to giving a clear overview of the objectives and expected outcomes of the workshop, has winded up her presentation by introducing the workshop schedule that is annexed in this document.

2.2 Overview of World Rabies situation, by Dr. Eshetu Yimer

This presentation focused on the overall rabies situation in the world with special emphases on some Asian (India and Bangladesh) and African countries (South Africa, Sudan, Mozambique, Uganda, and Kenya).

The presentation addressed in detail the gaps and challenges on rabies prevention & control, best practice and current intervention method and the way forward on rabies prevention and control. The presenter also described that India is the first country in the world in Human rabies death and also Ethiopia is one of the countries where the high number of deaths is reported.

2.3 Human Rabies Surveillance in Ethiopia, By Ato Haftom Taame

Ato Haftom presented that Rabies was first reported in Ethiopia (Addis Ababa) in 1903 and currently human rabies is an immediately reportable disease with case definition. He has presented that the surveillance data compiled since 2007 indicates that 15,178 exposure cases (3.4 /100,000 populations), 272 fatal cases with more than 88% of the exposure cases were due to dog bites. Among the exposed people 59.2% of the cases are males while 40.8% females. Out of the total exposure 98.9% (15,008) of exposure cases and 97.1% (264) of fatal cases were from Addis Ababa, Oromia, Amhara, SNNPR, Tigray regions.

He has also indicated that the national data indicates an exposure of 2.6/100,000 people. The highest incidence is registered from Tigray (11.4/100,000), followed by Oromia (3.5/100,000), Benshangul (3.3/100,000), Amhara (1.5/100,000), SNNPR (1.2/100,000) and Addis Ababa (0.8/100,000).

2.4 Animal Rabies in Ethiopia, Presented by Dr. Melaku Asseffa

In his presentation Dr. Melaku indicated the situation of rabies in domestic and wild animals, gaps and challenges, and best practice and current intervention method put in place as well as the way forward on domestic/ wild animal rabies prevention and control.

The presentation was depended on the rabies surveillance data that is generated by the EHNRI and the monthly disease reports of Regions to the FMOA. Dr. Muluken reported that Rabies is a problem in domestic animals and wildlife. The Federal Ministry of Agriculture, Animal and Plant Health Regulatory Directorate is working on rabies control strategy and Addis Ababa city administration drafted rabies control regulation. The Ethiopian Wolf Conservation Program (EWCP) through its strategic planning for Ethiopian wolf conservation has started its rabies activities with the objectives to reduce dog populations living in and around the wolf habitat and the incidence of rabies among the wolves in 5-12 years to zero (no out breaks). The activities being performed so far are vaccination of domestic dogs in and around the Ethiopian wolf habitat and trial of oral rabies vaccine SAG2 on wolves and domestic dog.

2.5 Production of Cell culture based Anti rabies vaccine in Ethiopia, Presented by Ato Abebe Mengesha

The presenter highlighted the vaccine produced by EHNRI which is sheep brain derived. The vaccine has a neuroparalytic reaction that occurs 1 in 200 - 600. The production of the Fermi type vaccine started in Ethiopia since 1944 E.C. and even though it is not recommended by WHO the vaccine is still in use due to the lack of modern vaccine production plant. Annual consumption of the vaccine is more than 36,000 doses for human and 12,000 doses for animals. Currently, EHNRI is working on tissue culture vaccine. The objectives of the technology transfer in manufacturing of improved Anti-rabies vaccine as recommended by WHO has three phases: -

- I. Production of appropriate primary or continuous cell-cultures must be adequate for vaccine production.
- II. The use of seed virus and tissue cultures to produce sufficient amount of rabies vaccine for animal use.
- III. The use of seed virus and tissue cultures to produce sufficient amount of rabies vaccine for humans use.

Currently, the institute has already produced over 100,000 doses of cell culture based vaccine for animal use. This bulk vaccine will be handed over to the National Veterinary Institute for quality assurance, packaging and distribution for veterinary use. Cell culture based vaccine production for humans is under way and shortly the institute will replace the Fermi vaccine with the aim of increasing safety and potency.

2.6 Discussions on the above presented topics

The following questions and Issues were raised:

- In the presentation of Human rabies surveillance by Ato Haftom, he mentioned that low case report but higher vaccine request from Benishangul Gumuz region; what is the justification to this apparently paradoxical situation? In connection to this, what does EHNRI is doing to regulate the vaccines and the vaccination process at regional levels?
- It has been said that destroying stray dogs is part of the rabies control and prevention process on one hand. On the other hand, this is severely refused by animal welfare and animal right activists. These are opposing ideas and thus what is the explanation and the rationale behind recommending the destruction of stray dogs?
- In the Human rabies surveillance, how much sensitive is the 'case definition' of human rabies cases?
- In the presentation addressing animal rabies in Ethiopia, it is mentioned that there is no rabies diagnosis service facility in the animal health sector particularly at NAHDIC. So why?
- Dr. Kifle Woldemekael of Jimma University who raised the above two questions also added that there are already best practices in the country that we work with a strong collaborative platform at the ministerial level. He mentioned, as an example, the DRM-FSS, a multi-stakeholder unit over seen and chaired by the Deputy Prime Minister. He also briefed the participants about the One Health Central and Eastern Africa (OHCEA) secretariat and its country coordinating committee (CCC) in Ethiopia. The CCC includes: Jimma University school of Veterinary Medicine, School of Public Health, Mekele University School of Veterinary Medicine, MoA, MoH, EVA, EPHA, EHNRI, EPA, EWLA. He underscored that rabies is a very important entry point for appreciating the importance of "one health approach".

- Dr. Eshetu Lemma of EHNRI in his part said that rabies remained a very big agenda and its public health importance is on the rise in Ethiopia due to a number of factors. One of the issues he emphasized is the lack of competent institutional set up for 'zoonosis' control and prevention. The Ministry of Health focuses on diseases affecting the human race while the FMoA focuses on disease affecting animal species and trans-boundary diseases affecting international animal trade. Most of the zoonotic diseases like rabies which fall at the cross roads of both human and animal concerns are often neglected. Efforts to prevent and control them are very weak while they continue to threaten both humans and animals at an alarming rate.
- Rabies, on top of its significance to human and animal health, has a big deal to do with the national heritage of the country with regard to its impact on the endemic species of wild animals in different national parks.
- There is a program in EWLA, which is called Ethiopian Wolves Conservation Program (EWCP). In this program we are using GMO vaccine in form of oral bait. Is this work being controlled by MoA?
- Is there any regular surveillance system for animal diseases by the FMoA that compares with the PHEM in FMoH?
- In most of the recommendation made by the presenters, mass destruction of stray dog population has been forwarded as a means to prevent and control rabies. But, this is quite a backward recommendation as it has been done for the past so many decades and has not yielded sustainable solutions to the problem. So how long should we continue killing dogs of brutally? There are other recommended means that were found and proven effective in other parts of the world. These can be regulatory tools, to implement responsible dog population management and licensing of dog ownership, continuous public education and sterilization of females dogs (neutering).
- In the presentation of the cell culture vaccine production process, it has been inquired 'what is the advantage of 10X titration?' Also, it seems from the presentation that the presence of immunogenicity of the vaccine suspension is taken as a grant for immunity of the animal; but caution has to be taken as this is not always true.
- Honorable participant from the Federal House of People's Representatives, Social Affairs Standing committee appreciated the progress made in producing the cell culture vaccine by EHNRI. He also appreciated the youth driven activities of the institute.

- He further wondered why there is a lack of regulation needed for the control and prevention of rabies while we used to have so many proclamations and regulation. He seriously questioned, "Is it because of reluctance by FMoA/FMoH to draft and present it to the Federal parliament or the parliament delayed the endorsement?"
- What is the reason why the FMoA and FMoH work in interface while their collaborative efforts and such arrangements matters a lot? We can take this to the attention of the Council of Ministers to achieve a senior buy in. As parliamentarians, it is easy for us to take up the necessary check list of activities to create awareness to the community and the sense of urgency at the level of decision makers.
- It is appreciable to see such supportive research evidences that are much needed to make the required decision to control the disease. We also advise to handle the animal welfare issue as it is very sensitive these days although we cannot care for animal rights by leaving humans to die from rabies.
- As far as we produce the vaccine here in our country, is it insufficient amount we produce? Do we produce the post exposure vaccine?
- We have only one laboratory of whose service remained suspended in Addis Ababa and vicinity. What is your plan to expand the diagnostic services to the regional states? You should capacitate them with technical backstopping, training and laboratory facilities.
- What are the natural reservoirs of rabies and what is their implication to our control and prevention efforts both in humans and animals?
- Regarding the Ethiopian Wolves Conservation Program of EWLA: The Ethiopian Wolves are endemic to Ethiopia and their number is less than 450 the world over. Rabies is the single most important threat to this endemic animal. The source is mainly domestic dogs which introduce in to parks. No wild animal has been reported to act as a reservoir to the wolves except the domestic dogs. EWCP is a program of EWLA, FMoA and sometimes EPA is involved.
- As we know Ethiopia has signed the biosafety protocol and thus we have taken this in to consideration when we import the oral rabies vaccine after a long debate whether to vaccinate the wild life or not and whether oral or injectable form of vaccines to use. What we are routinely doing is capture, vaccinate and release them. The oral vaccine is being used as a pilot project and the results to date are very encouraging. Oral vaccination seems to be a best alternative for conserving of the wild canids of the country in general.

The following responses were given for the above mentioned questions and reflections by the presenters:

- In order to have a balanced solution to animal welfare issues and rabies control and prevention, the best way out is putting in place responsible dog ownership pattern. Mass destruction of dogs is neither cost effective nor sustainable in terms of addressing rabies. Killing of dogs with strychnine or bullets is also incompatible with international norms and animal welfare regulations which the country has to comply with. We should strictly put for regulatory and birth control alternatives that will bring us a long term solution to the problem.
- In Ethiopia domestic dogs play an important role in the transmission and epidemiology of rabies in wild animal. Extensive research has been done in bats but no lyssa virus was detected and they are ruled out from the epidemiological picture of the diseases in Ethiopia.
- The most important issue with stray dog destruction is not the killing of the dogs which are ownerless, but the way we do so. We should avoid barbarian way of killing them!
- Rabies control is being done effectively by EHNRI. So why should we establish additional federal level diagnostic facility at NAHDIC? It is like wastage of resources to duplicate diagnostic facilities of EHNRI in FMoA. What is better is to scale up the activities of the EHNRI diagnostic facilities to make them available at regional levels to the minimum.
- FMoA seems uncomfortable with the idea that zoonotic diseases are neglected in Ethiopia as he believed that the FMoA has a unit handling issues of zoonotic diseases and public health concerns especially focusing on the import-export regulation by the quarantine unit. But, he still indicated that rabies is a low priority disease in the Federal Ministry of Agriculture.
- EWCP, the vaccination/conservation program on Ethiopian wolves is regularly checked/ well monitored by the FMoA, Animal and Plant Health Regulatory Directorate and the possible risks from the Genetically Modified Vaccine (GMO) vaccine which is thermo stable and is given with oral bait will not pose any problem feared from GMO.
- The PHEM template of FMoH is found in the FMoA for emergency purposes simple in the emergency preparedness plan. But here, there seems to have a misconception of disease reporting and active surveillance; what the FMoH does is active disease surveillance while FMoA passive surveillance.

- The issue with regard to lack of relevant proclamations, it has its own root with the decision making bodies, mainly the Federal Parliament and the Council of Ministers. Due to the endorsement process is very lengthy and other priority task load, draft proclamations and regulations are not yet in place.
- Antibody titer mentioned in the presentation of the cell culture vaccine is 0.5IU/ml 100IU.ml which is obtained after the experimental animals are immunized with the vaccine suspension produced by EHNRI. And this is within the recommended levels by WHO.
- With regard to working in interface, there has been bilateral discussion between EHNRI and the National Veterinary Institute, NVI. The vaccine has been produced by EHNRI that is ready to provide 100,000 doses of vaccine suspension produced for animal use. It is possible to utilize this same vaccine for human vaccination after one step of production process which is called purification. Currently, EHNRI do not have a purification instrument and a chemical required to do so. We do not also have the necessary dispensing and packaging equipments, but NVI have these materials and we could work together.
- But until we produce the best vaccines from the cell culture source, we could not ban using the sheep brain derived vaccines although out dates and have so many complications.
- National Veterinary Institute (NVI) is not producing any animal rabies vaccine currently. But, it has already gone a long way to produce the vaccine. All necessary equipments for production are being installed and would being production in the short run. As a short-term solution, we have imported some 70 million doses of rabies vaccines for distribution throughout the country but only 40,000 is sold up to now. Although we do not produce it locally, there is no shortage of rabies vaccines for animal use.
- In the case definition of rabies, data might have a chance of misinterpretation due to lack of sufficient understanding of the case definition. We use a case definition which is so simple, understandable, translated in local languages. In this definition any bite, vaccination case of humans for rabies, are all considered as rabies/reported as rabies whether confirmed or not/ this make is the case definition very sensitive that we do not miss rabies cases. But due to poor reporting system in health facilities, there is under estimation of the situation.
- The sector ministries have to be able to draft all the necessary rules and regulations so that the parliament can endorse for them. Without them doing their part, the federal parliament cannot help them.

2.7 National Rabies Baseline survey preliminary report; household assessment, Presented by Dr. Abraham Ali

On his presentation, Dr. Abraham said that the survey was conducted with the aim of estimating annual exposure and death, identify the major source of rabies and estimate the number of dogs per household. All the regions were considered in the survey and households were used as sampling sites. The survey was done using a pretested questionnaire. The number of household reached were 4,972 (19.8% urban and 80% rural), 44% male and 56% female.

With regard to rabies exposure Dr. Abraham said that the result indicated 354 exposures and 45 deaths. In all the regions except Harar and Dire Dawa there were rabies deaths. The survey finding indicates incidence rates of 12 exposure /100,000 population and 1.6 death /100,000 population at national. India has got the highest death incidence (1.7/100,000). A proportion of exposure is high among young less than 15 years of age. 50% of the rabies victims were seeks support from traditional healers, 40% do not go anywhere and 10% of the victims is not unknown. Twenty one percent of the surveyed households own dogs (24% in urban and 21% in rural) out of which less than 19% are vaccinated. In the rural area it is only 13% of the dogs that are vaccinated. Stray dogs as well as owned dogs were equally responsible for human rabies cases in the surveyed areas. Density of stray dogs is high in urban (55 dogs per football pitch) and rural (6 dogs per football pitch) areas.

2.8 National Rabies Baseline survey preliminary report; Facility Assessment, by Dr. Hailu Mamo

According to Dr. Hailu's presentation, the survey was conducted along with the household study with the main objective of generating a comprehensive data on rabies and its related aspects in human and animal populations in Ethiopia. Specifically, estimate the incidence of animal and human rabies cases and deaths, identify the distribution of rabies among different animal species, measure the level of vaccination coverage, and assess the challenges on rabies prevention and control activity. Under this survey a total of 176 facilities each for public and veterinary health, and 15 national wildlife parks were considered. Data was compiled for exposure, number cases and vaccination coverage for public health and veterinary facilities while exposure/case, prevention and challenges for national wildlife parks.

From 176 facilities 135(79%) responded for questionnaire and 69% of them said that rabies is a public Health problem. Among the surveyed facilities only 46% of them had system for the data record. The last five years data record from 1999 to 2004 E.C. showed that High percentage of Human Rabies Death in Amhara (33.6) and Oromia (25.6) as well as High Human Anti-Rabies vaccination was covered by Amhara region, i.e. 57, followed by Oromia , SNNP and Tigray Regions i.e. 23,14, and 4, respectively.

Dr. Hailu has also mentioned how animals are important as the main source for the rabies cases in humans, because where there were high animal cases always exist human cases. The Distribution of Rabies case in different animal species was showed 67%, 11% and 10%, for Dogs, Cats and Cattles, respectively. It was also observed that different challenges on vaccination and management of dog were due to lack of fund, low level of awareness on rabies, lack of legal frame work, and poor dog management almost at all regions.

Both the national as well as regional parks are not protected from human and animal contact, Dwellers live and keeps dog inside the facility and also seen rabid animals with in the compound.

2.9 Discussions on the above presented topics

The following questions and Issues were raised:

- The Baseline survey study done by EHNRI is very important and may be a good indication of the increasing attention being given to the disease. Had this work been done some one year before, there would have been a project to implement control measures by this time. We know that the problem is there but we cannot tell the magnitude of the problem, the associated factors involved. This information gaps makes difficult to get any financial and technical support from partners for rabies prevention and control activities. The current survey will pave the way towards such developments. A question has been posed to the institution why the survey has been initiated at this time while it has been working for the last 60 years of age?
- Rabies is not a single agency problem/agenda. It needs a multi-disciplinary and multiinstitutional participation for effective work to be done. In the current survey some important institutions that have a big stake are missing.

For instance, the Ethiopian Veterinary Association (EVA) could have been including in the survey as the association has been implementing various project/activities regarding rabies. EVA has streamlined rabies in its activities mainly as it relates to 'one health'. Some of the activities that the association have done so far include; initiated the celebration of world rabies day for the first time, done extensive public awareness creation, enabled the drafting of rabies control and dog ownership regulation with the Addis Ababa city Administration.

- The simplest side of the disease is that its major transmission route is only animal bite that the prevention, control and eventual eradication are also very simple. But, we should look at the case beyond the horizons of public health; it is also an issue of high concern in connection with tourism and wild life heritage. How many of us know that rabies has also negative impact on the national economy? Its control and prevention requires a sustained political commitment as our country Ethiopia should not bear any negative impression from this easily preventable disease; as it is an African capital.
- How strong is the survey in its depth and width in addressing the rabies problem in different regions and contexts? Most often it is claimed that lack of information and poor reporting has been a problem. But why is this so? We do have health and animal health extension workers more than ever. I think the problem is not lack of reporting, it is not lack of information but it is lack of proper data analysis system.
- The death rate expressed in every 100,000 people is very small; why is that so? It has been also requested to get some explanation on what it means by responsible dog ownership pattern.
- Animal welfare is a matter and an indication of the people's civilization in any nation. It does not matter if stray dogs are destroyed in what is called 'humane' means. This has to be complemented with efforts to neuter the dogs.
- Practically speaking rabies is burning agenda in most urban centers of the country while it is paradoxically less understood by the urban citizens compared with rural people. We have not created sufficient sense of urgency and awareness in urban areas where we can easily do so. We have very big problems in institutional arrangement of our cities where even the presence of animals amongst the population is forgotten. Administrators do not allocate any budget for animal health related activities like rabies prevention and control. We cannot even get the money to carry out the stray dog destruction; we are rather sick of campaigning at times where the problem reaches at an emergency level.



Ethiopia does not have a zoonotic diseases profile at all; I think the current national survey on rabies undertaken by EHNRI is a very good kick start. How reliable is the case specific death report from rabies which has been presented in the survey? How do you see the feasibility of availing rabies diagnosis facilities at the grass roots level, as you recommended it in the survey report?

The following responses were given by the presenters:

- The motive behind initiating the current survey by EHNRI emanates from the institutional mission and mandate of EHNRI. It is one of the projects planned and incorporated in the five years strategic plan of the institute to generate a baseline data for rabies at national level. Besides, there is also request from various stakeholders and partners who are interested in taking actions against rabies for such data.
- EHNRI has taken advantage of its long standing experience in conducting similar surveys. To insure the data quality a lot has been done before, during and after data collection process. With regard to the survey instruments, we have developed the questionnaire and pretested in peri urban setting, Sululta town. Training has also been given for data collectors, supervisors and coordinators. The data entry, clearing and proper management has been done by the professionals of the institute.
- Recommending the inclusion of rabies public education in the curriculum of children and teenagers is crucial in order to address the knowledge gap on the prevention and transmission of the disease. This is especially true due to the fact that the survey and other similar studies have indicated that children are among the highly vulnerable groups.
- The recommendation of availing diagnostic facilities at the grass roots level is meant to say to avail rabies diagnosis at the level of the regions in the short/medium term.
- The comment to include some missing partners (like professional associations) while doing the survey is well taken.
- The strength/reliability of the survey results is the outcome of the study design. It is a well designed scientific approach we have used in getting the data. We have generated primary data by going down to the primary source as much as possible.

- Increased number of extension professionals both in public and animal health does not necessarily indicate the availability of required information. Of course we have high number of extension workers but still there is a gap in information and there is still poor reporting rate. The survey team has indicated that good response rate was obtained from rural health and veterinary offices as compared to the urban facilities.
- Explanation has been given what all it means by responsible dog ownership. In a nutshell, it is the compulsory registration, vaccination and management of dogs owned by the public. It may also further involve some legal requirements like payment of taxes.
- Many of the participants generally agreed that this work on human and animal rabies situation in Ethiopia is the first of its kind and should be well encouraged.

2.10 Rabies Educational Material, Presented by Dr. Fasil Mengistu

Dr. Fasil Mengistu in his presentation has indicated that the Ethiopian Health and Nutrition Research Institute (EHNRI) have been delivered information on rabies to the public through brochures, magazines, television and radio programs. In order to strengthen that effort, currently the EHNRI have developed an educational booklet on Rabies with the objective of increasing public awareness on the diseases that was already pretested using focus group discussions and individual interview method in selected target groups comprising of 31 participants from school children, health professional, veterinarian and members of media organizations to examine its effectiveness. The minimum of 81% for the convincingness of the booklet was observed in school children and where as a maximum of 95% for accuracy in journalists. In general, the educational booklet was given satisfactory level of understandability, convincingness, acceptability, accuracy, usefulness and attractiveness by the participants.

Day Two

3. Presentations

During the day two of the workshop, three presentations were delivered by different professionals. Name of presenters and Chair persons and an overview of the presentations presented below.

Presenters: Dr. Asefa Deresa, Dr. Zerihun Abegaz, Dr. Almaz Abebe **Chair Persons**: Ato Gonfa Ayana, Dr. Eshetu Yemer

3.1 Current Rabies Diagnosis in Ethiopia and future prospect, Presented by Dr. Asefa Deressa

The presentation focused on the commonly used OIE/WHO recommended tests for rabies and the existing facility in Ethiopia. According to Dr. Asseffa, currently there exists only one referral laboratory (EHNRI) for rabies diagnosis service, which is based on animal clinical observation under quarantine period & laboratory confirmation. The facility has got a post-mortem and incinerator. The laboratory confirmation is done using Fluorescent Antibody test (FAT), and Mouse Inoculation Test (MIT). Furthermore, the Institute has introduced Direct Immunohistochemistry Test (dRIT) that detects antigen. There is no established diagnosis on human rabies at the EHNRI except clinical observation and Pre Exposure Prophylaxis (PEP).

In order to strengthens the diagnosis facilities at regional levels EHNRI is working on technology transfer in introducing dRIT to selected Veterinary and Public Health laboratories in Amhara (Bahir Dar Veterinary & Public health laboratories, Kombolcha Veterinary laboratory, and Dessie Public health laboratory), Oromia (Asela Veterinary laboratory and Adama Public health laboratory) and SNNPR (Wolaita Sodo veterinary laboratory, Hawassa Public health laboratory) Regional States. The presentation concluded by highlighting the way forward in strengthening the rabies diagnosis through scaling up of rapid rabies diagnosis tests (dRIT) and other techniques supported with TOT on rabies diagnostic techniques and technologies.

3.2 Rabies Legislation; best practice from Addis Ababa City Administration, Presented by Dr. Zerihun Abegaz

On his presentation, Dr. Zerihun indicated that the retrospective information recorded at EHNRI during the period 1990-2000 indicates that canine rabies is well established disease in Addis Ababa with no decline in the annual number of confirmed rabid cases. Dog population in Addis Ababa is estimated to be around 230,000 - 300,000 out of which 30% are owned while 70% are ownerless (stray dogs). According to EHNRI, report, (1990-2000 E.C) about 22,219 people in Addis Ababa city and its surrounding received post exposure treatment following dog bite (about 2200 people annually).

As best practices the presentation highlighted the use of Animal Birth Control (ABC)) that was implemented in 2001/2002 E.C. Three NGOs (Best Friends Animal Society, human society international and Amsale Gusesse Memorial Foundation) sponsored spaying & neutering dogs. Out of the planned 1200 dogs for ABC 736 received the service in nine months. 37 Veterinarians and paraprofessionals were trained and equipped with surgical materials and medicines. Addis Ababa city administration rabies prevention and control regulation is under process and hopefully will soon be finalized.

3.3 Discussion on the above presented topics

3.3.1 Points discussed on Human and Animal Rabies situation, and control efforts in Ethiopia

- It has been discussed that why dog owners in Addis Ababa are not willing enough to accept the very humane way of dog population management techniques (Spaying and neutering). The presenter from Addis Ababa City Urban Agriculture Bureau elaborated the scenario; it is because of lack of awareness and mainly the cost of sterilization (176-200ETB).
- 2. Regarding the exploitation of best track records in disease control participants discussed thoroughly. The efforts exerted so far to contain avian influenza, the role FAO, OIE, WHO played, could be extrapolated for the control and prevention of rabies. More over the experiences of Europe, N. America and other successful countries should be considered as we strive for the containment of this dreadful disease.



- 3. Regarding the adoption of euthanasia for both animals and human rabid cases, participants agreed in principle; but it is elaborated that implementation may require to strictly evaluate cost and other implications.
- 4. Skilled manpower for the subcutaneous administration of post exposure anti-rabies vaccination around the navel area is becoming a challenge, as unveiled by Dr Eshetu Yimer of EVA, a senior veterinarian and years of rabies allied issues expert. He has elaborated the seriousness of the case exemplifying one important case, in that a rabid dog bitten individual (physician) has received a wrong subcutaneous administration of the vaccine and developed subcutaneous edema in due course he has experienced serious complication and died of it. Thus, he has emphasized the risk of miss-vaccination and remarked the importance of training to individuals engaged with the post exposure vaccine administration.
- 5. Efforts which are being attempted by the Addis Ababa city administration, EVA and relevant stakeholders are encouraged and believed to apprehend worthwhile changes. Particularly in realizing quality private veterinary service (in which rabies would be addressed).
- 6. It has been suggested by Dr Fasil Mengistu of EHNRI to capacitate the Addis Ababa and regional veterinary clinics in quarantine facilities to keep the suspected dogs for ten days and send the brain sample to EHNRI.
- 7. Participants suggested the need for strong collaboration among the veterinary and public health sectors for integrative action against the disease.
- 8. Presenter who delegated the Addis Ababa City Urban Agriculture, Dr Zerihun Abegaz remarked, the rewarding changes know the city administration is striving to bring about, considering the metropolis as a city of prominent international personalities and organizations. But it has been underlined that still the concern given for the veterinary service by the urban agriculture is very poor, clinics at the various sub-cities aren't coordinated, aren't well equipped, aren't important facilities fitted and incapable to offer full fledged pet animal diseases control and prevention service. Thus, it is highly stipulated



to bring about change in the awareness and commitment of the Urban Agriculture, the city dwellers and the city government.

- 9. It has been suggested by participants that up until we reach the level of managing the dog population in a very humane way, employing the stray dog intoxication and killing would be a choice of dog population management and rabies control in combination with vaccination and birth control.
- 10. Regarding the proper disposal of intoxicated and died bodies of dogs; it has been discussed that the current incineration facility at EHNRI couldn't go beyond serving the institute demand. Thus, it is a wiser approach to capacitate region to erect their incineration facility.

3.3.2 The effort of anti-rabies vaccine production and distribution in Ethiopia

Inquiry regarding the safety of the anti-rabies cell culture vaccine particularly from the perspective of inducing autoimmune reaction has been raised. Mr Kelbessa of the EHNRI has elaborated that the vaccine that have got the side effect of causing autoimmune reaction are nervous tissue vaccines, whereas the cell culture anti-rabies vaccines are protein vaccines and devoid of the such side effect.

3.3.3 Current rabies diagnosis capacity of Ethiopia

- Participants requested for the adoption of rabies diagnosis technologies to regional laboratories. With this regard Mr Kelbessa of EHNRI explained the costliness of fluorescent Microscope and conjugates to adopt techniques like FAT which EHNRI is utilizing as of now. Rather he discussed to turn for using dRIT, as it isn't costly but the kit availability is questionable. Anyhow it would be better to implement cost sharing by regional laboratories and EHNRI as cascading the diagnostic capacity is underway.
- 2. Concerning the unavailability of important reagent to run the dRIT ,Dr Abraham Ali elaborated that EHNRI has already triggered discussions with CDC and set long and short term plans so as to let EHNRI be self sufficient in the production of its own diagnostic kits.

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- 3. Moreover the director general of the EHNRI, Dr Amha Kebede emphasized that in short term CDC support of reagents will go for the selected pilot regions (Amhara, Oromiya and SNNPR) and in the long term CDC planned to be engaged in the technology transfer and capacitating Ethiopia (EHNRI) in the vaccine and diagnostic kit production. Besides Dr Almaz Abebe of INDRD/EHNRI disclosed the commercial unavailability of the dRIT diagnostic kit (In house Technology), and showed the prospect of the technology transfer in the near future due to the promising discussions going on as of now.
- 4. Regarding the empowerment of regional laboratories, Dr Abraham Ali of the EHNRI has elaborated the scenario. Regions have manifested enormous interests for the EHNRI's diagnostic capacity cascading plans, as per the information obtained in need assessment. Hence, in the very near future the engagement of EHNRI will be more of assistance than direct involvement.
- 5. Participants requested for the capacity building and standardization of higher learning institutions' labs, particularly of Jimma University and the respondent from the EHNRI, Mr Gonfa, recalled the technical, logistic support and collaboration so far had with the institutions and promised for further considerations.

3.3.4 Regulatory mechanisms for the control and prevention of rabies in Ethiopia

1. Inquiries regarding the rabies control and prevention regulatory document drafted by the Addis Ababa city administration from the point of concern given to puppies role in the transmission of the disease, other animals like cat and the registration of dog selling individual was raised. With this regard it has been discussed that the regulation addresses all animals including cats that have important role in the rabies transmission. More over dogs more than 3 months of age and all dog owners are mentioned on the regulation for obligatory vaccination and registration respectively.

2. Participants required meticulous elaboration regarding the eligibility of the drafted rabies control and prevention regulation and the process of producing such legal document. The person in charge from the Addis Ababa City Urban Agriculture Bureau (Dr Zerihun Abegaz) explained the process. "Basically regulations enacted spring boarding proclamations, for that matter this document has based the national enacted Animal Diseases Prevention and Control proclamation (Proclamation No. 267/2002)." Every articles of the regulation was consulted thoroughly with prominent figures and institutions of veterinary medicine, public health personnel, civic societies, animal welfare institutions and private practitioners. More over legal advisor has involved in the overall drafting and consultation process of the regulation. After successfully consulted wider stakeholders and rectified in accordance, the document passed to the Addis Ababa Justice bureau, there it has been again thoroughly investigated and confirmed for endorsement.

Recently the document has fulfilled every procedure and waiting for enactment at the Addis Ababa City council. Literally speaking it is requiring only authorized signature. The presenter, Dr Zerihun Abegaz delegating the Addis Ababa City Urban Agriculture bureau considerably dignified the technical, coordination and logistic support of EVA (Ethiopian Veterinary Association) for the production of such valuable document. In general this regulation spring boarded on the national/federal document, and would contribute as a template for regions to develop their own rabies control and prevention regulations.

3. Supervision regarding door to door dogs' anti-rabies vaccination was one of the concerns raised by participants. Dr Zerihun of the Addis Ababa Urban Agriculture bureau elaborated the scenario. Though still it isn't solved, the bureau has attempted to solve the situation through holding successive dialogues with private veterinary practitioners. It has been discovered that there are uncertified and untrained vaccinators who are vaccinating wrong dosage and vaccine. Thus, it shows that still there is enormous gap and the city administration in collaboration with relevant stakeholders needs to work vigorously.

3.3.5 Public awareness

1. Participant from the National Veterinary Institute (NVI), Dr Shiferaw, has raised the magnificent importance of using awareness materials in particular Videos to mobilize the general public and relevant decisive actors vigorously act against the dreadful disease rabies. With this regard Dr Assefa Deresa of the EHNRI elaborated the significance of videos as a best learning tool; but the scenes in rabies videos most of the time are very disgusting and aren't inviting to disseminate for the general public use, ethically questionable.

3.3.6 Best experience shared by stakeholders

Ethiopian Wild life conservation authority (EWCA)

The delegate of the authority has elaborated that rabies has a biodiversity implication on top of its impact on public health and socioeconomic aspects. Particularly the implication that the rabid dogs are exerting to the endangered Ethiopian Wolves which are very small in number, about 450 is extremely dangerous from the point of extinction and shocking ecotourism. With this consideration the EWCA through its Ethiopian Wolves Conservation Program (EWCP) has tried to address the challenges of rabies.

The authority conducted rabies vaccination to about 66,000 dogs, aware the community to the vicinity of the parks regarding dog handling and health management. Besides the authority conducted oral vaccination of wolves against rabies, it has been conducted in collaboration with the university of Oxford and Ministry of Agriculture. The oral vaccines are non-GMOs (not Genetically Modified types). Tentative dog control policy has also been applied by the authority with the intention of containing rabies. With this policy park rangers and scouts were permitted to kill any dog that pass the park area. It has been applied with check and balance of the loss and gain; through weighing the significance of such small numbered (450 wolves) over millions of stray dogs.

Amhara Regional State Bureau of Agriculture

Dr. Nuria Yedigu and Dr. Zeleke of the Amhara regional state agricultural bureau unveiled the efforts at the regional state. The Amhara bureaus of agriculture with collaboration of the health bureau organized consultative meetings to disclose the challenges of rabies prevention and control, its impact and develop way outs to counteract constraints. Thus, the regional bureau consolidated the specific areas of preventive and control obstacles and produced refined resolution as to manage the dog population, conduct mass vaccination of dogs and availing of post exposure prophylaxis. For the realization of the action points a task force was established.

Tigray Regional State Bureau of Agriculture

The regional agricultural bureau's effort in collaboration with the health bureau has able to strengthen awareness among the community, in that it has produced and disseminated awareness creation manuals. Besides the region coordinated unemployed vets, assistant vets and extension workers and delivered anti-rabies vaccination to about 6000 dogs. The person, who has delegated the regional bureau and unveiled this best track record, has also acknowledged the support of Mekele University for availing financial assistance during the aforementioned number of dogs' vaccination. Meanwhile it has been disclosed that during the indicated vaccination campaign more than 10,000 dog owners have shown their unwillingness to pay for the vaccine.

Mekele University

Since 2000 Ethiopian calendar the University has been engaged in various schemes of rabies prevention and control endeavors. The university participated in awareness creation programs through organizing dialogue forums/workshops, paying visit to schools that encompass students of 5-8 grades and educating them, transmission of rabies control messages via regional FM-radios, and organizing great run. More over the university has mobilized resources and technical inputs for mass vaccination of dogs, dog sterilization (spaying and neutering) and importation (from India about 76 doses) and delivery of pre-exposure vaccines.

Euthanasia using Xylazine infiltration (sedative) and injection of Thiophentol/MgSO4/Formalin was also a friendly dog depopulation strategy accomplished by the university.

3.4 Major Challenges and Gaps in Rabies Prevention and Control, Presented By Dr. Almaz Abebe

After all the deliberations, the major challenges clustered in four categories were presented. And these are:

- I. Low effort of animal rabies control (no defined legislation/guidelines, fragmented stakeholders efforts, lack of animal rabies surveillance, insufficient availability and misuse of vaccines, low vaccination coverage),
- II. Low effort in human rabies prevention (absence of modern tissue culture vaccine, low public awareness, limited PET service and in adequate skill on the use of PET, Weak human rabies surveillance),
- III. Limited rabies diagnostic capacity (rabies diagnoses technology, absence of trained manpower, only one laboratory facility, lack of inter-sectoral effort), and
- IV. Coordination (lack of strong collaboration/networking among concerned stakeholders, no defined role and responsibilities of stakeholders in rabies prevention and control activities, Limited awareness)

3.5 Panel Discussion

Chair Persons: Dr. Amha Kebede, Professor Getachew AbebePanelists: Dr. Daddi Jimma, Dr. Birhanu Admassu, Dr. Kifle Woldemichael, Dr. Melaku Assefa

Panel Discussion outputs:-

I. Low effort in animal rabies prevention and control

Since rabies is a zoonotic disease which requires collaborative engagement; in that the Federal Ministry of Agriculture and Health are required to work interactively.

The role of the Federal Ministry of Agriculture to halt the disease at the level of the animal and assist the preventive strategy of the Federal Ministry Health is underlined. The socio-economic and biodiversity significance of rabies is the other aspect of the disease that seeks government priority other than its dreadful feature and public health concern. Regional representations with this workshop trusted to play

a very crucial role to influence policy makers at all levels. The presence of rabies control strategy set by Federal Ministry of Agriculture and the trend of disease reporting (though it is very poor) should be appropriately exploited and strengthened.

The experience of other countries for instance the role Bill and Melinda Gates Foundation played in Philippines to establish a workable regulatory framework and collaboration should be contextualized to our country situation. Having a regulatory mechanism could be a good support of the prevention and control strategy success. A practical experience faced at EHNRI has indicated the significance; as individuals bitten by a dog and visit the institute and the owner of the dogs asked to present the dog, due to the awareness gap and the absence of a regulatory framework peoples avail a different dog or even did not totally become willing to bring the animal.

Strengths such as a well established rabies profile by the EHNRI, the regulatory document produced by the Addis Ababa City Administration and opportunities (like the animal disease eradication success with rinderpest) could be extrapolated to tackle the limitations and threats encountered.

SWOT FOR THE CONTROL OF ANIMAL RABIES AS POINTED OUT FROM THE PANELISTS' DISCUSSION

STRENGTH

- ✓ Well established rabies profile by EHNRI
- The regulatory document produced by the Addis Ababa city administration in collaboration with the magnificent assistance of EVA

WEAKNESS/LIMITATIONS

- ✓ Fragmented control efforts
- ✓ Absence collaborative rabies control
- ✓ Absence of sustainable control strategy
- ✓ Lack of ownership
 - Absence of well equipped and sufficiently budgeted veterinary clinics

OPPORTUNITY

- ✓ Ethiopian has best animal disease control track record, a historical success in the eradication of rinderpest
- Ethiopia is becoming a cosmopolitan country, due to the fact that Addis is the capital of Africa and the host of many prominent international organizations
- ✓ Endowed with enormous veterinary manpower (The nation has about 10 vet faculties with a rough production potential of 500vets/annum)
- \checkmark The availability of rabies prevention and control strategy by the Ministry of Agriculture
- \checkmark The availability rabies control strategy by the Ministry of Health
- \checkmark Best experience of Avian influenza and pandemic flu control could be adopted
- ✓ Production of animal anti-rabies vaccine by EHNRI
- ✓ Diagnostic capacity cascading strategy of EHNRI to regional veterinary and public health labs

THREAT

- Absence of policy framework, strategy, guidelines and structured (Relevant stakeholders synchronized) prevention and control document
- ✓ Less national concern and priority
- ✓ Poor dog vaccination custom
- ✓ Lack of awareness of the community and policy makers

II. Low Effort in Human Rabies Prevention and Control

Rabies has been discussed as one of the major public health concerns. It is one of the 20 reportable diseases in Ethiopia. In Ethiopia there are 13 diseases which require immediate reporting and 7 diseases out of the aforementioned 20 should be reported within a week time. Federal Ministry of Health requires the immediate reporting of rabies case unlike other diseases which may need to be reported within a week time for instance malaria. Federal Ministry of Health has already set the strategy of rabies control but what is lacking is implementation.

The awareness gap among the community and even medical personals is one of the prior areas that need to be addressed. Health extension workers are part and parcel of the disease control and surveillance programs; they should be introduced with rabies focused package, and it should be a future direction of Federal Ministry of Health.

Still Fermi-type vaccine is on use to save more lives .Production of a safer human anti-rabies vaccines is on progress, it may take 3-5yeras.In the mean time the EHNRI is running research to produce a more efficient and safer human anti-rabies vaccine. The production and distribution of human anti-rabies vaccine may require active participation of the private sector to full fill the growing demand of the community and assist efficient financial and technical focus of the government.

Strengthening the surveillance and reporting system is another focus that seeks considerable attention. Sometimes diseases outbreaks reported lately and that complicates measures and made the intervention success to be graver. Thus, the capacity of PHEM needs to be strengthened. Electronic reporting system that is installed and operational in Addis Ababa and Dire dawa will be expanded to regions; which hasten rapid information flow from health posts to EHNRI. Any dog bites recently are detected as rabies, unless otherwise diagnosed and proven, for that matter strengthening the diagnostic capacity and cascading the capacity to the regional level is a need.

Pre-exposure vaccine has been noted to be unavailable almost anywhere except at EHNRI, which the instituting is importing for its experts only and Embassies for their diplomatic members. The one which was available at ILRI previously was not available as of now.

Hence, concern given for it has shown to be very poor. Recently veterinarians working at "pet clinics", almost all over the nation, who have the risk of contracting the diseases are working at hazardous situation. Panelists and all participants of the workshop reached on agreement for the development of efficient strategy that could resolve the contracting of such deadly occupational hazard.

Regarding the importation of interadermal vaccine to save cost by reducing the full dose administration to at least 60ml and avail affordability, and diminish the Fermi-type vaccine implications described to complicate the health extension system. EHNRI is striving vigorously for the production a cell culture vaccine in the coming 3-5 years which is going to be safer and cost effective. Recently the institute is conducting purification process.

Care and support to the rabies symptom manifesting individuals and inducing euthanasia was one of the serious issues raised. In that it has been elaborated to be considered as far as the care takers / the family of the patient is willing and affordable. It is a matter of cost and willingness of the family in charge of taking care for the patient.

III. Limited Rabies Diagnostic Capacity

Recently EHNRI is the only center diagnosing rabies, but initiatives are to start cascading the capacity to regional labs. Currently cases of rabies from any corner of the country are subjected for the shipment of the brain sample travelling hundreds of kilometers holding the dog brain. Regional veterinary and public health laboratories are mentioned to take care of the diagnostic effort in adopting the technology. Capacities of university laboratories at various regions of the country has been tried to be built through several attempts of EHNRI, but with this specific rabies diagnostic capacity building endeavor primary concern has been given to government service offering veterinary and public health laboratories.

Strengthening of a one health concept and optimal exploitation of the available human, financial and physical resources has subjected EHNRI to focus in capacitating both veterinary and human laboratories, creating a very close and integrated working environment between the two sectors. Priority to pilot the capacity building effort has been given to Oromiya, SNNPR and Amhara regions based on the challenge of the rabies case.

The National Animal Health Diagnostic and Investigation Center (NAHDIC) have been serving as a good help for EHNRI even in providing a bio-safety level-2 laboratory. But regarding the diagnostic capacity building aspect EHNRI dedicates to capacitate the regions due to their responsibility and role to play with respect to the diagnosis and reporting of rabies.

IV. Collaboration Gaps

Gaps to work with integration between the veterinary and public health laboratories, regional labs and the EHNRI has been unveiled .In that regard it has been well discussed to consider referral linkage and collaboration.

There is considerable gap in collaborative commitment among relevant stakeholders particularly between the Ministry of Agriculture and Health to counteract rabies. It would be a good approach adopting previously established collaborative council against zoonotic diseases. The national zoonotic council in placed to control pandemic influenza and avian flu can be in use as a good template. The ToR structure, constituency, leadership structure, working procedures of such council could be adopted as a model for the formation of rabies control and prevention task force. The one health collaboration established by the public health teaching universities and relevant stakeholders could also serve as a good opportunity for exploiting technical working procedures. More over the forum between ministry of health, agriculture and education could be used as a fertile ground. Experiences of the existing and/or so far established collaborative efforts would in general help to extrapolate a well structured technical and leadership structure as a rabies prevention and control task force is set.

3.6 The Way forward

Participants of the workshop with panelists and chair persons playing the leading role thoroughly discussed and brought out the multifaceted challenges in rabies control and prevention attempts.

Based on the four major challenges indicated above and the participants agreed that the FMOA and FMOH need to work closely while undertaking their respective responsibilities with regard to rabies. The zoonotic nature of the disease subjected concerted effort among the veterinary and public health realm.

In order to safeguard the health of the public, rabies need to be control at the animal level and this responsibility lays on the veterinary service.

For the prevention and control of rabies the two ministries and other relevant stakeholder should collaborate and work closely. Experiences of the national council to control zoonotic diseases and the one health approach established among the public health teaching institutes and relevant stakeholders could be exploited. The Avian Influenza, Pandemic Flu and Rift Valley fever control attempts could also contribute paramount experience regarding the integrative work among the veterinary and public health spheres. In order to have a concerted effort on the prevention and control of rabies public awareness on the magnitude of the problem should be created from grassroots up to the highest government body/leadership. Once awareness is created at all level the commitments of government, non government organizations, civic societies and the community at large will be onboard. Thus, organizing a task force that constitutes relevant stakeholders found to be crucial. The task force is anticipated to relief the limitations and threats and at the same time exploit the strengths and opportunities available at a disposal.

As a way forward for Rabies Prevention and Control, National Taskforce is established. The proposed/ suggested members of the taskforce are:

Federal Ministry of Health, FMoH Federal Ministry of Agriculture, FMoA Ethiopian Health and Nutrition Research Institute, EHNRI Ethiopian Wild Life Conservation Authority, EWCA Regional Health Bureau Representatives Regional Veterinary Representatives National Veterinary Institute, NVI Addis Ababa University Faculty of veterinary Medicine, AAU/FVM Jimma University, JU Ethiopian Veterinary Association, EVA Ethiopian Public Health Association, EPHA Ethiopian Medical Laboratory Association, EMLA Ethiopian Public Health Laboratory Association, EPHLA Members of the house of peoples' representatives, HPR World Health Organization, WHO Food and Agriculture Organization of the United Nations, FAO Center for Disease Control and Prevention, CDC Clubs- Lions Club, Rotary International and others

Endorsement of the task force members, setting of duties and responsibilities and core task force selection will underway in due course. EHNRI is recommended to play the leading role in establishing the taskforce. In a month time TOR shall be developed and shared with participants along with the proceedings of the two days workshop.

3.7 Rabies Educational Material Inauguration, by Fekadu Beshah

The Rabies Educational Material official launching ceremony had an event of presenting the educational materials to the selected target audience groups. During the ceremony, Dr Amha Kebede, Director General of the Institute, Dr. Almaz Abebe, Director, Infectious and noninfectious Disease research Directorate have presented the material to the representatives of the Addis Ababa Health Bureau, Addis Ababa Bureau of Education and Ethiopian Wild Life Conservation Authority.

3.8 Reception and Recognition

Day one of the workshop was ended with the reception and recognition ceremonies. During the reception, certificates were awarded to professionals who contributed to the success of the workshop. Ato Yosef Daemo, Member of the House of Representatives awarded a certificate to staff members of the Institute who worked as members of conference organizing committee. The Institute's Director General, Dr. Amha Kebede has also awarded a certificate to presenters and panelists of the workshop

3.9 Closing Remark, by Dr. Amha Kebede

Dear, Participants and members of the House of Representatives

I would like to thank the workshop organizers and participants for making such a success. As you know Rabies is a very serious problem. It is clear that this problem is not addressed very well and now it is time to all stakeholders to come together depending on the one health approach to find a solution. This forum has given us an opportunity to exchange ideas and knowledge based experiences and so we must move to the next step for much action in prevention and control of rabies in Ethiopia.

Based on your suggestion given in this workshop, EHNRI would take the leadership role and act accordingly, in the Endorsement of the task force members, setting of duties and responsibilities and core task force selection as well as submission of workshop proceeding within a month period.

Thank you all again and I declare this workshop is officially closed.



4. Annexes

4.1 Overview of World Rabies Situation

Eshetu Yimer (DVM, MSc, MSc) Ethiopian Veterinary Association (EVA) E-mail: esyima_n@yahoo.com

Rabies as a disease

Rabies, a fatal but preventable zoonosis, is a major public health problem in developing countries. It is caused by a virus that belongs to the Family rhabdoviridae and Genus lyssavirus. In Ethiopia the disease burden is largely underestimated because patients with encephalitis following dog bites are rarely hospitalized and die at home. Virus is typically present in the saliva of clinically ill mammals and is transmitted through a bite. After entering the central nervous system of the next host, the virus causes an acute, progressive encephalomyelitis that is almost always fatal. The incubation period in humans is usually several weeks to months, but ranges from days to years.

The genus lyssavirus was at first divided into four serotypes (1-4) by antigenic cross-reactivity with sera and monoclonal antibodies, which correspond to the following species; rabies virus (RABV); 2, Lagos bat virus (LBV); 3, Mokola virus (MOKV); and 4, Duvenhage virus (DUVV). Further isolations of new bat lyssaviruses in Europe, then Australia and the progress in genetic characterization of several genes (N,P,



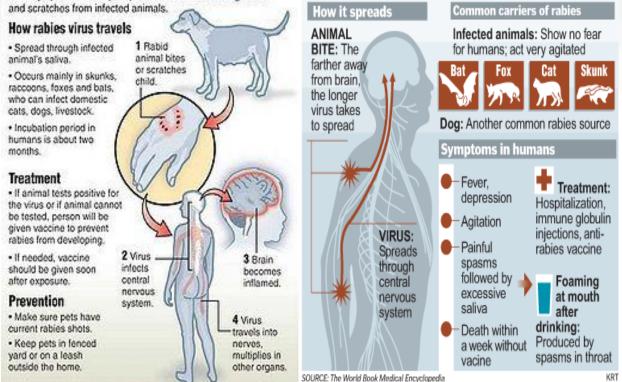
and G) supported the delineation of seven genotypes (1-7), confirming and expanding the antigenic data: 1, RABV; 2, LBV; 3, MOKV; 4, DUVV; 5, European bat lyssavirus 1 (EBLV-1); 6, European bat lyssavirus 2 (EBLV-2) and 7, Australian bat lyssavirus 1 (ABLV) (WHO, 2005).

Figure 1: Hospitalized rabid man



Rabies

Rabies, a virus that attacks the nervous system and is fatal once symptoms develop, is passed to humans through bites and scratches from infected animals.



Rabies

The incubation period of the disease varies from 2 weeks to 6 years depending on the amount of virus inoculum and site of inoculation and proximity of the site of virus entry to the CNS increases the likelihood of short incubation period. The estimated speed of virus migration is 15 - 100 mm per day. The virus then moves from the CNS via anterogradeaxoplasmic flow within peripheral nerves leading to



infection of some of the adjacent non-nervous tissues like the secretory tissues of the salivary glands.

Figure 2: Stray dogs. Rabies is seen in dogs especially Figure 3: Vaccination of dogs against rabies where there is a large population of ownerless dogs.

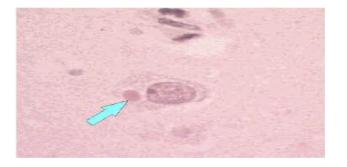


In Africa and Asia, dogs continue to be the main carrier of rabies and are responsible for most of the human rabies deaths worldwide. Humans most often become infected with rabies through the bite or scratch of an infected dog or cat (WHO, 2008). However, in developed countries rabies continues mainly in wild animals. The disease is transmitted to domestic animals and humans through exposure to infected saliva. In the past few years, bat rabies has emerged as a public health problem in the Americas and Europe. More people in South America died from rabies following exposure to wildlife, particularly bats, than from dogs for the first time in 2003.



Figure 4: Rabid dogs

Signs of hyperactivity (furious rabies) or paralysis (dumb rabies) dominate in the critical stage. In both furious and dumb rabies, some paralysis eventually progresses to complete paralysis, followed by coma and death in all cases, usually due to breathing failure.



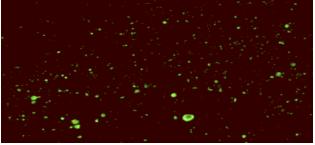


Figure 5: Positive Immunohistochemistry for rabies

Figure 6: Positive IF test for rabies antigen

Situation of Rabies in the world

Every year, more than 15 million people worldwide receive a post-exposure vaccination (WHO, 2012).Dog rabies potentially threatens over 3 billion people in Asia and Africa. People most at risk live in rural areas where human vaccines and immunoglobulin are not readily available or accessible. Poor people are at a higher risk, as the average cost of rabies post-exposure prophylaxis after contact with a suspected rabid animal is US\$ 40 in Africa and US\$ 49 in Asia, where the average daily income is about US\$ 1–2 per person. Children aged 5-15 years represent 40% of people exposed to dog-bites in rabies-endemic areas. Dog bites are the primary source of human infection in all rabies-endemic countries in the Region and account for 96% of human rabies cases.

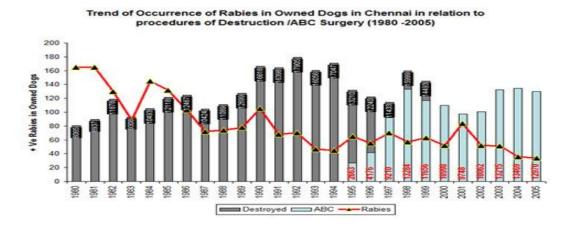


Figure 7: Map showing major animals involved in rabies transmission worldwide

A combination of large human & dog populations in congested habitable areas combined with widespread poverty has led to more deaths due to rabies in South-East Asia Region than in any other part of the world (WHO, SEARO 2011). WHO continues to promote human rabies prevention through the elimination of rabies in dogs as well as a wider use of the intradermal route for PEP which reduces volume and thereby cost of cell-cultured vaccine by 60 to 80%. WHO supports targets for elimination of human and dog rabies in all Latin American countries by 2015 and of human rabies transmitted by dogs in South-East Asia by 2020. In this latter region a five-year plan (2012–2016) aims to halve the currently estimated number of human rabies deaths in endemic countries.

Rabies in India

In India, each year approximately 7 million people undergo post exposure rabies treatment after a dog bite and the disease is common in most parts of the country. The dog population of India is around 25 to 30 million animals. Most of these are ownerless or stray dogs. The majority of the stray dog population is found in rural areas. These dogs play a major role in the spread of rabies. APCRI reported in 2004 that there were 20,565 reported human deaths over the period of one year (Underreported due to misdiagnosis). Nearly 96% of cases were due to bites from stray, ownerless dogs and about 70% of the victims were children younger than 15 years.



Findings of APCRI-WHO study on rabies in India;

- a) Approximately 17 million people are bitten by animals (80% by stray dogs) and need post exposure treatment, every year.
- b) 1% to 2% of the population suffers animal (mainly stray dog) bites each year
- c) Frequency of bite is one bite every 2 seconds
- d) One death from rabies every thirty minutes
- e) Annual man days lost due to animal bites 38 million
- f) Annual medical cost for animal bites treatment is Rs. 2 Billion
- g) India contributes the highest number (80%) of rabies death in the whole world, approximately 30,000 per year as per WHO/NICD sources.
- h) Of the 17 million victims bitten, only 3 million receive the post-exposure anti-rabies vaccine, leaving 14 million to survive by "Luck by Chance"

Rabies in Bangladesh

Every year human deaths due to rabies greater than 2,000 are reported in the country (Infectious Disease Hospital (IDH), Dhaka reported cases only), more than 300,000 people receive Post-exposure treatment every year and more than 85% of them come from rural areas. Although grossly under reported, large numbers of livestock also die.

Strategic Plan Goal: To prevent death from rabies and to control animal rabies by 2020 through GO-NGO collaboration

Short term plan 2010 - 2012

Baseline survey on animal bite, rabies cases and dog population

Registration, sterilization & vaccination of dogs

Introduction of Intra dermal (ID) TCV at national & divisional levels

Phase out NTV by 2011

Midterm target (2012-2016)

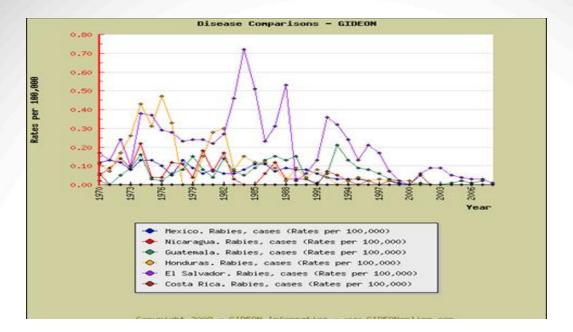
- Implementation of ID TCV at all levels
- Dog population control (euthanization, vaccination, registration, Animal Birth Control (ABC) etc.)
- Manufacture of TCV and immunoglobulin locally

Long term target (2016-2020) and onward

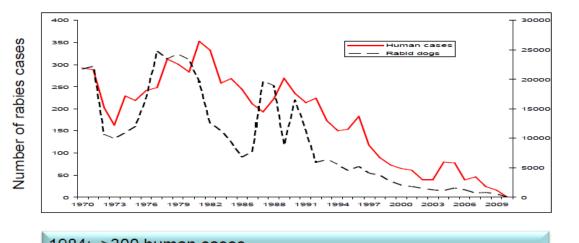
 Sustainability of the program through coordinated approach of Ministry of Health, Local Government & Livestock & NGOs.

Effective implementation requires

- Rabies control program should be an integral part of Ministry of Health, Livestock and Local govt. system
- Partnership with NGOs
- Sustained political commitment
- Adequate human and financial resources



Epidemiological Trends of Human and Canine Rabies Cases (N=7,228) Latin America, 1970–2009



1984: >300 human cases2009: 19 human cases; 95% reduction of human and dog cases

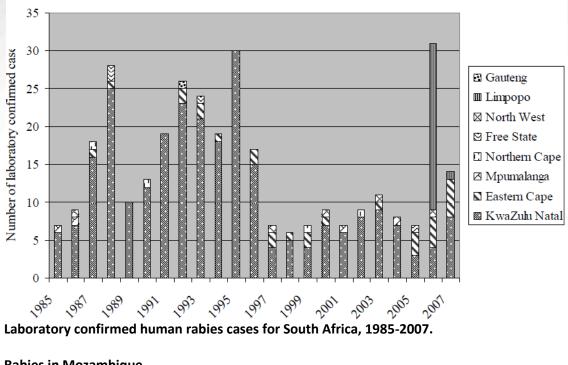
PAHO Rabies Information System, SIRVERA (www.panaftosa.org.br). Updated December 2010. Accessed Jan 18, 2011

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CDC

Rabies in South Africa (SEARG, 2008)



Rabies in Mozambique

	1998	1999	2000	2001	2002	Total
Human rabies	25	22	26	33	29	135
Animal rabies	24	31	17	22	33	127

Rabies in Namibia (confirmed)

	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002
Canine	15	7	17	18	23	32	30	49	59	47
Feline	1	-	1	1	-	1	1	6	10	7
Jackal	-	-	-	1	-	-	1	-	-	-
Bovine	1	4	2	2	-	2	4	14	21	15
Ovine / Caprine	2	-	1	2	-	-	-	4	7	4

Page 5C

Rabies in Sudan (suspected)

Laboratory diagnosis

				2001		2002	
	2001	2002	[positive	negative	positive	negative
Dog	233	314	Dog	7	8	2	2
Cat	27	18	Cat	9	4	4	1
Monkey	5	18	Monkey	-	-	-	-
Goat	58	31	Goat	11	2	2	2
Sheep	22	15	Sheep	2	-	-	-
Equine	78	195	Equine	6	3	2	-
Cattle	73	29	Cattle	3	-	2	-
Camel	19	16	Camel	-	-	1	-
TOTAL	485	636	TOTAL	38	17	13	5

Rabies (Human) in Uganda

Year	No. of post exposure treatments	ARV doses (imported)	No. of rabies cases	Estimated cost (USD)
1992	766	3976	50	35000
1993	1518	5720	23	52000
1994	2614	8298	15	74700
1995	3222	13623	14	122000
1996	1698	16000	9	144000
1997	2916	16000	10	144000
1998	3112	16000	10	144000
1999	4537	10000	5	90000
2000	5398	10000	9	90000
2001	5490	19570	16	119371
2002	4324	15133	12	98365
2003*	1543	8900		54290
TOTAL	31421	119630	173	955321

Animal rabies cases

Species	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002
Dog	243	228	376	252	38	298	61	1214	442	1841	1568
Cat	-	-	1	4	1	5	3	7	13	11	19
Cattle	1	2	-	4	1	30	5	6	8	1	4
Goat/Sheep	2	-	7	5	8	4	-	-	-	2	1
Fox	-	2	-	5	8	-	-	3	3	-	9
Jackal	3	6	-	4	2	-	-		4	1	-
Monkey	-	-	1	-	3	3	-	2	-		
Rabbit	-	-	-	-	2	2	-	-	-	-	-
Mongoose	-	-	-	2	-	-	-	-	-	-	-
Pigs								1	1	-	-
Leopard								1	-	-	-
Bush back									1	-	-
TOTAL	249	238	385	276	73	268	69	1234	472	1856	1601

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	19	998	19	99	20)00	20)01	20)02	TOTALS	
	Pos	Total	Pos	Total								
Dogs	42	50	41	55	28	61	27	56	52	76	190	298
Cattle	16	19	19	24	18	24	19	28	24	31	96	126
Cats	7	9	4	6	5	5	3	7	2	3	21	30
Equine	1	3	4	4	2	3	1	2	2	4	10	16
Goats	1	2	1	2	2	2	3	5	3	3	10	14
Sheep	2	2	0	0	2	2	0	2	3	5	7	11
Wildlife	0	4	2	3	0	2	0	12	0	41	2	62
Pigs	1	1	0	0	0	1	0	0	0	0	1	2
Human	0	0	0	0	0	0	0	1	0	0	0	1
Total	70	90	71	94	57	100	53	113	86	163	337	560

Rabies in Kenya Positive cases according to species, 1998 – 2002 (SEARG 2003)

Gaps and challenges on Rabies Prevention & Control

Four common reasons given for the lack of effective canine rabies control in Africa (Lembo, et al., 2010)

- 1. A low prioritization as a result of lack of awareness of the rabies burden;
- 2. Epidemiological constraints such as uncertainties about the required levels of vaccination;
- 3. Operational constraints including accessibility of dogs for vaccination and insufficient knowledge of dog population sizes; and
- 4. Limited resources for implementation of rabies surveillance and control.

-	
Reason	Explanation
LOW PRIORITISATION	- Lack of accurate data on the disease burden and low
	recognition among public health practitioners and
/ · · · · · · · · · · · · · · · · · · ·	policy makers;
	- lack of inclusion of rabies in global surveys of disease
	burden;
	- only recent recognition of rabies as a neglected
	tropical disease;
	-statements of rabies as an 'insignificant human
	disease;
EPIDEMIOLOGICAL CONSTRAINTS	- Abundance of wild animals and uncertainties about
	the required levels of vaccination coverage
OPERATIONAL CONSTRAINTS	- Perception of existence of many inaccessible
	stray/ownerless dogs
	- Owners unwilling or unable to bring dogs for
	Vaccination
	- Insufficient knowledge of dog population size and
	ecology
LACK OF RESOURCES	- Weak surveillance and diagnostic capacity
	- Insufficient resources available to veterinary services

Reasons for the lack of effective dog rabies control

Hence in general;

- (1) Rabies exerts substantial burden on human and animal health, local & national economies & wildlife conservation,
- (2) Domestic dogs are responsible for rabies maintenance & main source of infection for humans throughout most of Africa and Asia & control of dog rabies should eliminate the disease,
- (3) Elimination of rabies through domestic dog vaccination is epidemiologically feasible,
- (4) Domestic dog populations across sub-Saharan Africa are accessible for vaccination & communities education and awareness programmes enhances more coverage,
- (5) Improved local capacity in rabies surveillance and diagnostics will help evaluate the impact of control and elimination efforts and
- (6) Dog rabies control is affordable, but is likely to require intersectoral approaches for sustainable programmes that will be needed to establish rabies-free areas.

Challenges for Rabies control

- Achieving adequate immunity in owned-dog population
- Vaccination of free-roaming dogs
- Dog overpopulation may impede or prevent rabies control success
- Virus spillover at the dog-wildlife interface may confound success of dog and wildlife rabies control
- Low Prioritization of Rabies control (Less commitment given to rabies by both Health & Agriculture sectors
- No sector takes direct responsibility for rabies control
- For Health sector; Low priority compared to: Malaria, HIV/AIDS,TB
- High incidence but Low prevalence of rabies; rabies cases/deaths are fewer ("not seen")
- Lack of awareness
- Unavailability of appropriate Tissue Culture Vaccine (TCV)
- Use of toxic Nerve Tissue Vaccine (NTV) & Rx by traditional healers
- Large number of unsafe dog population
- No or limited effort for controlling animal rabies

For Agriculture sector; Low priority compared to:

- PPR, CCPP, FMD, CBPP; rabies has little impact on livestock production & marketing.

- Disease is associated with traditional beliefs/ remedies that delay in accessing medical treatment.
- No viable structures for effective Inter-sectoral collaboration (Lack of an institutionalized "One Health" approach)
- Rabies is not much of a problem of the rich but the poor (countries & individuals)

-inadequate funding

- Consequently, this translates into lack of interest in rabies making it a "forgotten" disease except for the victim & the relatives.

Under reporting/no reporting; <50% of WHO/AFRO countries respond to WSR (RABNET; closed), poor or no surveillance for rabies

Best practice and current intervention method

In 2009, the Bill & Melinda Gates Foundation, in cooperation with WHO, started a pilot programme to eliminate canine rabies in three candidate territories where the disease is endemic;

- KwaZulu Natal province in South Africa,
- south-eastern part of the United Republic of Tanzania and
- Visayas archipelago in the Philippines.
- The programme aims to demonstrate that human rabies can be prevented through the control of rabies in dogs and was also intended to support the "one medicine" paradigm shift in global approaches to public and veterinary health.
- Over the past three years, the project has progressively taken shape, as systems and logistics have been put in place and control campaigns started.
- The absence of reported human rabies cases over a 12-month period is highly significant for KwaZulu Natal and cause for celebration because:
- surveillance has always been of a relatively high standard and,
- Although undiagnosed cases may have occurred, this is the first time in 20 years that the province has not recorded a human death from rabies in a 1-year period.
- Much training and awareness has been conducted around the province over the past two years in both the health and public sectors, efforts will have improved the chances of identifying human rabies cases.
- Historically, human rabies cases have followed the trend of animal rabies cases. Cases of animal rabies have decreased steadily from 363 in 2007 to a projected 156 cases in 2011 that support reduction in the number of human rabies cases.
- The 5-year project aims to achieve elimination of human and dog rabies from KwaZulu Natal by 2014.
- The collective influence of the Gates Foundation's name, & technical input from WHO and other international experts from leading institutions around the world who have visited the project, have boosted the project's profile, brought sustainability and encouraged, motivated and influenced the direction and purpose of the efforts in KwaZulu Natal.

The way forward on Rabies prevention and control

- Redefine Institutional arrangements under One Health concept through promoting cross sectoral and multi-disciplinary approach
- Reliable long term Financing Strategy
- Building more robust public and animal health systems
- Strengthening the national emergency response capabilities
- Addressing the concerns of the poor by focusing on locally important diseases including rabies.
- Establish national rabies surveillance system
- Conduct assessment to fully understand burden of rabies and develop impact models that assess strategic interventions
- Production of vaccines and immunoglobulins
- Control of the disease in animals
- Establish effective diagnosis, prevention and treatment in man
- Conducting strategic research

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4.2 Human Rabies Surveillance in Ethiopia

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Introduction

Rabies is a fatal viral infection that is most commonly spread to humans through the bite of an infected animal which has the highest case fatality rate of any infectious disease. Rabies is the leading cause of death from zoonotic diseases with Case fatality rate of 100%. Rabies Causes 40,000 to 60,000 human deaths per year globally.

According to Professor Richard Pankhurst book called "The History and Traditional treatment of Rabies in Ethiopia", History of Rabies goes to the 19th century in Ethiopia. In the Early 19th century a French traveler Rochet D' Hericourt told that an apparently rabid dog bite three dogs and a soldier at Debre Tabur. The first rabies epidemic in Ethiopia was occurred in Addis Ababa in August 1903, and was reported by Lincon Decastro, a physician at the Italian Legacion.

Objective

- To detect epidemics/outbreaks
- To predict epidemics
- To monitor trends of priority diseases
- To evaluate an intervention

Case definition

According to WHO case definition?

- Human rabies is defined as:
 - Suspected: A case that is compatible with the clinical case definition
 - Probable: A suspected case plus history of contact with a suspected rabid animal
 - Confirmed: A suspected case that is laboratory-confirmed

- Human exposure to rabies
 - Possible exposure: A person who had close contact (usually a bite or scratch) with a rabies-susceptible animal in (or originating from) a rabies-infected area
 - Probable exposure: A person who had close contact (usually a bite or scratch) with an animal displaying clinical signs consistent with rabies at time of the exposure, or within 10 days following exposure in a rabies-infected area
 - Exposed: A person who has had close contact (usually a bite or scratch) with a laboratory-confirmed rabid animal.

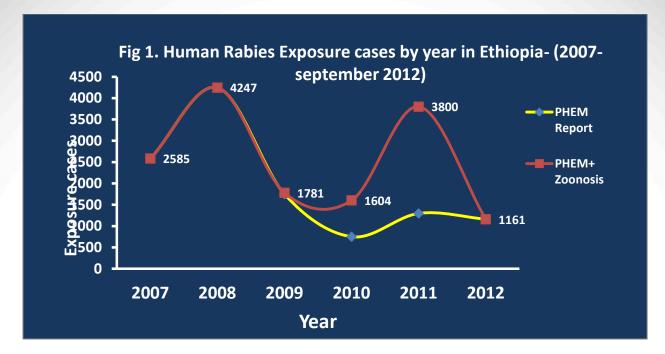
However the PHEM definition if a bit different from the WHO case definition. The PHEM case definition for Rabies aims at monitoring exposure cases so that victims could get early treatment before developing symptoms. The definition is stated as suspected case definition for human rabies case as follows:

- Suspected case is defined as a person bitten by suspected rabid animal presenting with fever, nausea, vomiting, agitation, pharyngeal spasms (hydrophobia/ aerophobia) or a person who is bitten by a confirmed rabid animal (national PHEM guideline)
- Death is defined as a person who died after being bitten by a rabid animal presenting the clinical signs of rabies.

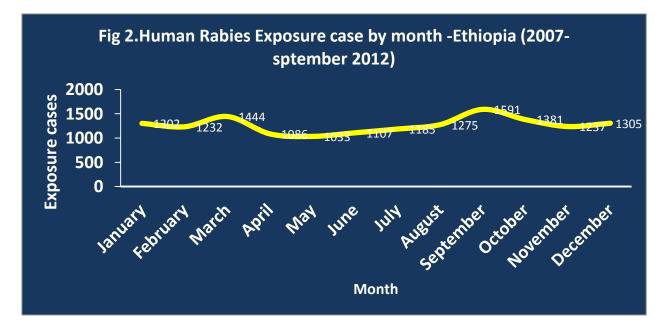
Result

From 2007-september 2012, a total of 15,178 exposure cases which is similar to 3.4 /100,000 populations and 272 fatal cases were reported nationally. More than 88% of the Exposure cases were due to dog bites and 59.2% of the Exposure cases were males and 40.8% females. More over 98.9% (15,008) of exposure cases and 97.1 % (264) of Fatal cases were from 5 regions only (AA, Oromia, Amhara, SNNP, Tigray).

As could be seen from figure 1 the highest numbers of exposure cases were reported in 2008 and 2011. The number of Exposure cases reported through the routine surveillance system didn't reflect the actual situation of human rabies in Ethiopia because many of the regions were not reporting all cases to the national PHEM. This is true that patients which came to the EHNR zoonosis research team for diagnosis were not reported from any of the region.

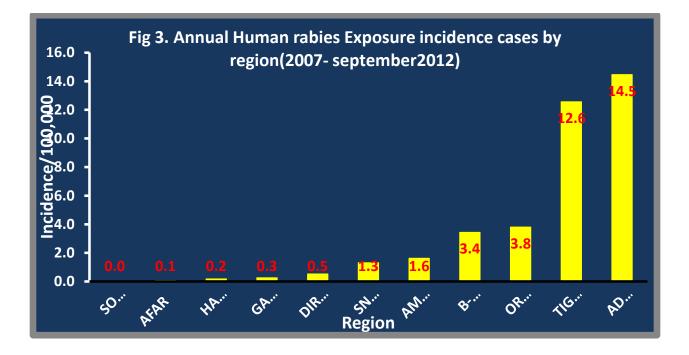


Larger numbers of cases were reported during March and September of the years. This is the time of mating for dogs and fights each other to get the female dog. Therefore transmission of rabies in the dog population is very common during this time increasing risk of human rabies exposure (fig 2).





As the national surveillance data from 2007- september2012 indicates, more cases of rabies were reported from regions with relatively good surveillance system and socio-economic development. The highest incidence was in Addis Ababa followed by Tigray, Oromiya, Benshangul Gumuz, Amhara and SNNP. This is due to the difference in surveillance system strength. Regions with strong surveillance system had reported highest number of cases compared to regions with poor surveillance system. More over the surveillance in some of the regions is very sensitive in which all suspected rabid dog bites are considered as confirmed exposure cases without proper follow up of the dog increasing many false positives (Fig 3).



Children of the ages of 1-14 years are at the greatest risk. Children have the tendency to play with dogs and are unable to identify rabid dogs and defend themselves from dog bites. This is similar to the case in South Africa in which children of fewer than 15 years accounts (30%-60%) of the total cases. Males were mostly affected than Females which is similar to other African countries like Ghana: 60% and Uganda 57% (Table 1).

Table 1. Inc	Table 1. Incidence of Human Rabies Exposure cases by Age group and Sex –Ethiopia(2007-2011)								
Age group	Male		Female						
	Number(Proportion)	Incidence per 100,000 population	Number(Proportion)	Incidence per 100,000 population					
1-14	881(43.5%)	18.9	528(37.5%)	6.4					
15-45	864(42.7%)	7	612(41.5%)	3.5					
>45	278(13.7%)	5.6	207(42.7%)	4.3					
Total	2023(60%)	31.5	1347(40.0%)	14.2					

Regardless of the number of rabies cases reported from each region, all have got a lot of doses of rabies post exposure vaccine every year. No or only few cases of rabies were reported from Afar, Somali, Gambela, Harari and Dire Dawa in the last six years (2007-september2012). However, a lot of doses of human vaccine were distributed to these regions. This indicates that there had been many but unreported cases.

Table 2. Human Rabies va	ccine Distribution to	Regions betweer	1 2007 and 2011				
Administrative Region	Year					Total	
	2007	2008	2009	2010	2011		
Tigray	1281	1133	33	1,300	1270	5017	
Afar	3	0	16	0	1	20	
Amhara	3,901	2,804	1,163	2,105	2,404	12,377	
Oromia	13,353	12,887	6,993	10,028	7,990	51,251	
Somali	88	1436	647	308	288	2,767	
SNNP	2,913	3,293	2,686	2,530	3,595	15,017	
Gambella	8	88	47	8	34	185	
B-Gumuz	341	306	118	226	378	1,369	
Harari	500	0	29	0	80	609	
Dire Dawa	1,079	1,078	71	57	22	2,307	
Addis Ababa	2,025	2,074	829	973	1,164	7,065	
Grand Total	25,492	25,099	12,632	17,535	17,226	97,980	

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Surveillance Completeness

The above data was analyzed based on the reports obtained from the following health facilities. According to WHO, for a surveillance to be considered as representative, at least 80% completeness should be achieved. However, the numbers of health facilities reporting were less than this standard although there was improvement from year to year (Fig 4).



Fig 4. Surveillance report completeness fro 2002-2004 E.C.

Challenge/Limitations

Most of the exposure cases and deaths were reported in a weekly summary report that was difficult to see by sex and age. More over these cases were not disaggregated as suspected, probable and confirmed cases as per the WHO standard and this difference in case definition compromises the acceptance of the data. All regions were not reporting all cases with the required reporting format and at the appropriate time.

Conclusion

Males were more affected than females and the young ages are vulnerable for rabid dog exposure compared to adults. The Surveillance system of Human rabies is very poor and all regions were not reporting cases and deaths with the recommended standards. A lot of doses of vaccine had been produced and distributed to all regions however the utilization is not known.



Recommendations

All relevant sectors should work in One Health Approach to mitigate the problem as it requires multi sectoral response and coordination. It is also necessary to have a regulation that enforces dog owners to Vaccinate their dogs regularly and imposes a responsibility for all damages that the dog might made on another person. Municipalities and the agriculture sector should also manage the stray dog population in the rural and urban areas. More over Health extension workers should also educate the community about rabies prevention and control. The EHNRI zoonosis unit should be considered as one of the reporting sites.

Acknowledgement

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4.3 Animal Rabies in Ethiopia

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Situation of Rabies in Domestic and wild animals

The rabies virus is present on all continents except Antarctica (OIE). Canine rabies are eliminated from North America, western Europe, Japan and many areas in South America; However, it is still widespread and occurring in over 80 countries and territories, predominantly in the developing world(WHO, 2004).

In Ethiopia rabies is an endemic disease, which affect domestic and wild animals and humans. The laboratory confirmatory test has been conducted in EHNRI, which is the only laboratory in the country for rabies diagnosis to date. In the laboratory test conducted from 1983-1992/1990-2000 in EHNRI, among domestic animals 1951 Dogs, 116 Cats, 63 Cattle, 10 Sheep, 3 goats, 8 Donkeys and 1 Horse, while among wild animals 9 hyena, 2 Monkey, 4 Jackal, 1 Mongoose, 1 Cerval cat and 1 Cheetah were positive for rabies (Eshetu et.al, 1990-2000). Clinical observation and quarantine of animals suspected of rabies from Addis Ababa and surrounding areas between 2001 and 2009 disclosed that 90% of biting dogs were been free from rabies(Asefa et.al, 2001-2009). This observation indicates the importance of quarantine and observation of the biting dogs before taking actions in bitted animals and man, since many of the biting dogs may be free of rabies infection (Table 1).

The last three years data compiled from animal disease outbreak report of APHRD/ FMOA showed that animal rabies were reported from all regions except Dire Dawa and Harare (Table 2-5) (APHRD/FMOA report). In addition to that there are reports, which showed that wolf population of the Bale Mountains National Park (currently <450) has been affected by rabies outbreaks in 1991, 2003 and 2008/09(EWCP, 2011).

Table 1: Clinical observation and quarantine of animals suspected of rabies from Addis Ababa andsurrounding areas between 2001 and 2009 (Asefa et al)

Species	Total number of animals underclinicalobservation&quarantine	Number and (%) of animals free from rabies after 10 days of quarantine	Number and (%) of positive for rabies
Dog	20113	18102 (90%)	2011 (10%)
Cat	265	125 (47%)	140 (53%)
Others (donkey, monkey, horse, cattle)	36	16 (44%)	20 (55%)
Total	20414	18243 (89%)	2171 (10.63%)

Table 2: Rabies outbreaks of 2010 by region (APHRD/MOA)

Region	No of Outbreaks	Suspected deaths	Number of Animals killed	Number of animal Vaccination
TIGRAY	24	179	5	1754
AMHARA	14	169	0	0
OROMIA	16	99	10	186
SNNP	5	22	0	150
BEN. GUMUZ	2	0	0	36
ADDIS ABABA	1	4	0	40
GAMBELA	1	1	1	0
Total outbreak	63	474	16	2166

Table 3: Rabies outbreaks of 2011 by region (APHRD/MOA)

Region	No of Outbreaks	Suspected deaths	Number of Animals killed	Number of animal Vaccination
OROMIA	14	101	17	20
TIGRAY	13	54	4	389
AMHARA	9	41	0	1826
SNNP	3	49	0	0
GAMBELA	1	0	0	0

Total outbreak	40	245	21	2235
Table 1. Rabies outbreaks of	of 2012 by regio			

Table 4: Rabies outbreaks of 2012 by region (APHRD/MOA)

Region	No of Outbreaks	Suspected deaths	Number of Animals killed	Number of animal Vaccination
BEN. GUMUZ	5	83	0	258
OROMIA	14	39	0	4657
TIGRAY	10	37	2	644
AMHARA	4	21	6	340
SNNP	5	15	0	0
ADDIS ABABA	1	2	0	0
Total outbreak	39	197	8	5899



Gaps and challenges on Domestic and wild animal Rabies prevention and control

There are many gaps, which hinder the control and prevention of rabies in domestic and wild animals. The following gaps are considered to be the majors:

- There is no animal disease control regulation in general and that of rabies in particular.
- The animal disease reporting system in the country is not as required by World Animal Health Organization (OIE) and mostly not supported by confirmatory diagnosis.
- The presence of only one rabies diagnostic laboratory in the whole country,
- The vaccination coverage of owned dogs and supply of adequate rabies vaccine is poor.
- Stray dog control activities are not adequately planed and luck regularity, and
- The dalliance of APHDR/MOA rabies control strategy implementation.

Best practice and current intervention method put in place

Currently there are some practices in the country which have to be continued and intensified. The existing practices of post outbreaks stray dog control, Isolation and observation of animals bitten by rabies suspected dog and the suspected dogs in some cities, towns and even in rural kebeles, where animal health services exists is highly contributing to control and prevent animal rabies in the country, so; these activities should be strengthen.

In order to control and prevent animal rabies a sound strategy is required and this strategy is already prepared by APHRD/MOA. In addition to that Addis Ababa animal health services have drafted an animal rabies control and prevention regulation, which will be a valuable tool to control this disease in the city.

Regarding to wild animal especially those endemic and rare canid i.e. Ethiopian wolves, the Ethiopian Wildlife Conservation Program (EWCP) prepared Strategic planning for Ethiopian wolf conservation and starts its implementation with the objectives:

- 1. To reduce dog populations living in or using wolf habitat,
- 2. To reduce the incidence of rabies in 5-12 years to zero (no out breaks)
 - Continuing vaccination of domestic dogs in and around the Ethiopian wolf habitat
 - Trials of oral rabies vaccine SAG2 on wolves and domestic dog, trial in Ethiopian wild wolves confirming success.

The way forward on Domestic/ wild animal Rabies prevention and control

To effectively control and prevent animal rabies in Ethiopia the following measures should be taken:

- Issuance of rabies control regulation
- Applying the existing rabies control and prevention strategy, which includes the following major intervention areas:
 - 1. Provision of safe and effective vaccines
 - 2. Compulsory vaccination of all dogs and cats found in cities and towns
 - 3. Destroy all infected and dangerously contact animals
 - 4. Destroy all stray and uncontrolled dogs and cats
 - 5. Quarantine and movement control
 - 6. Strengthening the diagnostic capabilities
 - 7. Sectors collaboration
 - 8. Public education
- Supporting EWCP to fully implement its Strategic plan for Ethiopian wolf conservation, and
- Supporting EWCP's trial of oral rabies vaccine/SAG2 in Ethiopian wolves and domestic dogs in and around the wolf habitat.

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4.4 Production of Cell Culture based Anti-Rabies Vaccine in Ethiopia Abebe Mengesha (BSc)

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Introduction

Since the first rabies vaccination in 1885 by Louis Pasteur, significant progress has been made in improving the pre- and post-exposure treatment of human rabies. There are several types of vaccines: live attenuated, inactivated (killed), DNA-based, and vector vaccines. For the production of anti-rabies vaccines, a number of attenuated vaccine strains are employed: the Pasteur Virus (PV), Evelyn Rokitniki Abelseth (ERA), Street-Alabama-Dufferin (SAD), 3aG, Fuenzalida S-51 and S-91, Ni-Ce, SRV9, PM, Nishigahara, RC-HL, Kelev, Flury, "Shelkovo-51", "O-73 Uz-VGNKI", "RV-71", "Krasnopresnenskii-85", and the RV-97 strain. The PV is one of the first vaccine strains; it was isolated from a rabid cow in 1882 and attenuated by multiple passages in rabbits. The SAD strain was isolated from a rabid dog in Alabama (USA) in 1935 and adapted for cultivation in the mouse brain and in the baby hamster kidney cell culture. It has two main derivates: ERA and Vnukovo-32. Several variants of the SAD strain exist: SAD-Berne, SAD B19, SAD-P5/88 etc., and also non-virulent mutants SAG-1 and SAG-2. The vaccine strains belonging to the SAD group are widely used throughout the world. One of the most widely used oral anti-rabies vaccines is prepared from the SAD B19 strain, the high immunogenicity and relative safety of this strain has been demonstrated experimentally.

<u>Live attenuated vaccines</u> are still in use in some developing countries for parenteral vaccination of animals and humans. These contain live attenuated rabies virus which has been developed in cell cultures or in live animals such as sheep. In the developed world, live attenuated vaccines are only used for the oral immunization of wild animals. Oral vaccines are widely used and several vaccine strains are used for the production of such vaccines: the SAD B19 and other SAD-strains, SAG1 and SAG2 – apathogenic mutants, Vnukovo-32, and the VRG strain. The vaccine strain RV-97 is used in Russia for producing the oral anti-rabies vaccines "Sinrab" and "ORALRABIVAC". This strain was obtained in the FGI "Federal Centre for Animal Health" (Vladimir, Russia) from strain RB-71. The strain RV-97 is adapted for cultivation in cell culture BHK-21. <u>Inactivated vaccines:</u> Complete inactivated rabies virus particles are highly immunogenic. The vaccines based on this principle are used for the pre- and post-exposure immunization of humans and domestic animals. The inactivated chicken embryo vaccines and vaccines based on virus cultivated in cell cultures are used for veterinary and medical purposes. Modern medical vaccines can be administered by the intradermal route.

<u>DNA vaccines</u> are based on plasmid vectors expressing rabies virus glycoprotein. These vaccines have been tested for their efficiency in several animal species (mice, dogs and nonhuman primates), and it has been found that the DNA vaccine develops VNA levels and offers protection comparable with those obtained with the inactivated vaccines. On the basis of the results of the study conducted in mice, a single administration of the rabies DNA vaccine may be as effective as at least five injections of the cellculture-derived vaccine.

<u>Vector vaccines</u> are based on recombinant viruses, and several viruses have been tested for these purposes. The VRG vaccine was designed on the basis of poxvirus (vaccinia virus) expressing SAD strain glycoprotein and used for oral immunization of wildlife. The Adrab.gp - vaccine is based on the adenovirus expressing the ERA strain glycoprotein and was found capable of inducing an immune response in dogs. The canine herpes virus (CHV) expressing the glycoprotein of rabies virus has also been used successfully as an anti-rabies vaccine. A raccoon poxvirus (RCNV) recombinant vaccine for the immunization against feline panleukopenia and rabies has been developed and tested in cats. A recombinant rabies virus vaccine carrying two identical glycoprotein (G) genes (SPBNGA-GA) has also been constructed.

The rabies virus vaccine strain based on vectors have shown great promise as vaccines against other viral diseases such as human immunodeficiency virus type 1 (HIV-1) infection and hepatitis C, but a low residual pathogenicity remains a concern for their usage.

Plant-derived antigens can also be used for the immunization against rabies. The coat protein of alfalfa mosaic virus has been used as a carrier molecule to express the antigenic peptides from rabies virus. The *in vitro* transcripts of the recombinant virus with sequences encoding the antigenic peptides have been synthesized from DNA constructs and used to inoculate tobacco plants. The plant-produced protein (virus particles) has been purified and used for the immunization of mice, and specific anti-rabies virus-neutralizing antibodies in immunized mice have been found;

spinach has also been used for this purpose. The transgenic maize expressing the G protein of the Vnukovo strain has also been obtained and tested in mice. It was shown that the mice developed virus neutralizing antibodies which were able to provide protection of 100% against the challenge of a vampire bat strain.

The attenuated Fermi vaccine which has been in use since 1944 G.C produce both humoral and cell mediated immunity which could revert to virulent forms and produce undesirable side effects. Besides its virulent form reversion the vaccine has 5% phenol suspension which alters the normal immune system. Therefore production of a safer and effective tissue culture based rabies vaccine is needed.



Currently consumption of Fermi vaccine is 36,000 doses for human use and 12,000 dose for animal purpose.

The objectives of the technology transfer in manufacturing of improved Anti-rabies rabies vaccine as recommended by WHO has three phases: -

- production of appropriate primary or continuous cell-cultures must be adequate for vaccine manufacture
- The use of seed virus and tissue cultures to manufacture rabies vaccine for use in animals must meet national needs.
- The use of seed virus and tissue cultures to manufacture rabies vaccine for use in humans must be based on national needs.

Methods

Virus

The seed virus strains for anti-rabies vaccine production are the ERA and PV. The seed strain ERA is attenuated rabies virus strain derived from SAD strain after 10 passages in chicken embryos and 30 passages in cell culture of fetal pig kidney and newborn pigs. The PV seed strain is an inactivated rabies virus strain is a derivative of the original Pasteur Virus after 10 passages was additionally passaged on rabbit brain (PV-11) and was adapted to fetal bovine kidney cell after 33 passages. Both seed viruses were officially supplied to EHNRI by the Centers for Disease Control and Prevention (CDC, Atlanta, USA), which is a regional reference laboratory and collaboration center for rabies.

The aim of the activity was to produce anti-rabies vaccine from ERA strain provided by CDC. Initially the multiplicity of infection of the viruses had to be optimized therefore both the virus seeds were cultured on BHK-21 and Vero cell line. Cell culture suspension of ERA virus with 10⁷-10⁸ TCID₅₀/ml is used as master seed and stored in 1.0 ml aliquots in -80°C. Working seed with same suspension passaged and stored at -80°C. Master seed can only be used to obtain more aliquots of the working seed virus. Working seed is used to infect cell cultures in roller bottles and tissue culture flasks. ERA strain can be propagated in Vero or BHK-21 cell cultures.

Cell cultivation

Types of cells used are baby hamster kidney cell (BHK) and kidney epithelial cells of African green monkey (vero). BHK is obtained from Pan African Veterinary Vaccine (PANVAC) and Vero cells are obtained from National Veterinary Institute (NVI). Cells are cultivated separately in monolayer in culture flask () or rollers () in media (Commercially prepared Minimum Essential Medium Eagle Auto-ModTM (MEM) powder, formulated at 9.4 grams/liter supplemented with NaHCO₃, FCS, L-glutamine and Penicillin-Streptomycin. Initially cells showed sluggish growth pattern but then continuously and exponentially grown to 95% confluent or highest metabolic activity with the density of 3x105 cells/ml. Cells are cultivated into culture flasks (surface area = 1ml per 25cm²) roller bottles (2500ml). After splitting and collection of BHK-21 or Vero cells, they were centrifuged at about 800 rpm for 8-10 minutes, supernatant discarded and cell pellet reconstituted in half volume of the original cell suspension of MEM. Then a tenfold dilutions were made for counting. A total of 75,000,000 cells were used for one 2500ml roller bottle or 1,200,000 cells for one T75 culture flask. When cells are reaching semi-confluent was prepared for virus cultivation.

Master Seed Virus Preparation

Titration for the original virus was done and 10⁸ TCID/ml for ERA, 10^{7.75} TCID/ml for PV and 10⁷ ID/ml for CVS-11 were obtained.

Titration
10 ⁸
10 ^{7.75}
10 ⁷

Table 1. The result of titration of original ERA, PV and CVS-11 strains obtained from CDC Atlanta.

From the original virus strain 8x tenfold dilution were made and from each dilution titration was conducted to determine multiplicity of infection and incubation period of the virus. Infectivity dose $10^{7.25}$ ID/ml for ERA and $10^{7.75}$ ID/ml for PV were obtained after titration. The best infectivity dose found was 0.001 ID/cell at 96 hours for ERA and 0.01 ID/cell at 72 hours for PV. The titration of the virus, done both on cell culture and mice, showed an optimal incubation period and multiplicity for the virus strains.

Multiplicity	Incubation period							
TCID/vero cell	48hrs	96hrs						
0.1	10 ^{6.75}	105 ^{5.75}	10 ^{6.75}					
0.01	10 ^{6.50}	10 ^{5.50}	10 ^{6.25}					
0.001	10 ^{4.50}	10 ^{5.50}	10 ^{7.25}					

Table 2a. The result of titration of ERA strainobtained from cultivation in Vero cells in flask.

Multiplicity	Incubation period						
TCID/vero cell	48hrs	96hrs					
0.1	10 ^{5.50}		10 ^{6.50}				
0.01	10 ^{5.50}	10 ^{7.75}	10 ^{4.50}				
0.001	10 ^{6.75}	105 ^{5.75}	10 ^{6.75}				

Table 2b. The result of titration of PV strain obtained from cultivation in Vero cells in flask.

Working Seed Virus

After selection of effective multiplicity, the master seed virus once again passaged and considered as working seed virus and called "Seed Virus Passage 1 Working Virus (P1)".

For the same passage titration was conducted and $10^{7.75}$ ID/ml was obtained for ERA P1. Similarly the same procedure was repeated for PV and PV P1 is $10^{7.25}$ ID/ml. P1 for both virus strains are stored at - 80° C as working seed bank in 1.0 ml aliquots. From P1 again passaged and obtained P2 virus suspension for vaccine production which contains $10^{6.5}$ ID/ml for ERA P2 and $10^{7.0}$ ID/ml for PV P2.

					1		1	2	З	4	5	6	7	8	9	10	11	12
Virus	Inoculat	Dead	Survive	Percent		А	-	-	-	-	-	-	-	-	-	-	-	-
dilution	ed mice	mice	d mice	mortalit		B (-8)	-	-	-	-	-	-	-	-	-	-	-	-
				У		C (-7)	-	-	-	-	-	-	-	-	-	-	-	-
1 0-1	6	6	0	100		D (-6)	-	-	-	-	-	-	-	-	-	-	-	-
1 0 ⁻²	6	6	0	100		E (-5)	+	+	-	-	-	+	-	+	-	+	+	-
1 0 ⁻³	6	6	0	100		F (-4)	+	+	+	+	+	+	+	+	+	+	+	+
10-4	6	6	0	100		G (-3)	+	+	+	+	+	+	+	+	+	+	+	+
10-5	6	5	1	83.3	1	H (-2)	+	+	+	+	+	+	+	+	+	+	+	+
10 ⁻⁶	6	3	3	50	1		Т€	est	viru	JS	_				_			
1 0 ⁻⁷	6				1		1				Te	est v	irus	52	Te	est vii	rus 3	

Virus Cultivation

Cells of 75×10^6 were infected with $10^{6.5}$ ID/ml of ERA P2 and incubated for 96 hrs at 37^0 C in 5% CO₂ incubator. Since the required pH is 7.2 to 7.4, a daily visual pH check up is inevitable and can also be maintained by 7.5% sodium bicarbonate solution. The harvest schedule for the ERA and PV –strains are 96hrs and 72hrs, respectively. When virus-cell suspensions are reaching semi-confluent the virus harvested, then frozen and thawed, in order to destroy cells to release virus. The thawed viral suspensions were clarified using Sorvall centrifuge (Sorvall centrifuge with rotor 6000), at 2500 rpm for 15 minutes at 4^0 C, to remove cells debris. The clarified supernatant viral suspension was transferred into sterile test tube or bottle, viral inactivation conducted.



Inactivation

Inactivation was performed using formalin and phenol. The preferable inactivant is β -propiolactone but for the time being it is not available. Formalin inactivation was done using a concentration of vol/vol 1:5000 incubated at 37^oC for 48 h with shaking twice a day. But in case of phenol inactivation process was different which was 3.75ml of 10% in PBS to 46.25 ml of viral suspension was mixed and incubated for 48hrs at room temperature with 200 rpm. Formalin inactivation against phenol inactivation was compared and the result accordingly showed that phenol could not completely inactivate the virus therefore all the mice were dead whereas formalin inactivation was complete and became 8.32 IU/ml for ERA and 2.5 IU/ml for PV respectively.

Safety and Potency test

After inactivation safety and potency tests was determined. Safety test determines the presence of residual virulent virus in the vaccine whereas potency determines the degree of protection conferred by the vaccine in immunized mice challenged with virus. A safety tests for both inactivated vaccines were performed and all mice survived for the formalin-inactivated vaccine whereas all the mice were dead in case of phenol inactivation. Safety test regarding standard bacteriological test for contamination was conducted and the vaccine was free of any bacterial contamination.

According to National Institutes of Health (NIH) a vaccine passes the test if the estimated potency is not less than 2.5 IU per dose. The test was conducted by vaccinating four groups of mice twice in 7 days difference with dilutions of reference vaccine and the vaccine under test. Seven days after the second immunization mice was challenged with challenge virus strain and observed for 14 days and the median effective dose of the reference and the test vaccine determined.

Standard CVS strain from CDC was used for challenging. From the master CVS, working CVS was prepared by passaging once on mice brain and performed titration to determine the LD_{50} of the challenge virus. The brains of these mice were collected and prepared of working CVS-11 and stored at - 80° C. The lethal dose of the working CVS is 10^{5} LD₅₀.

For immunization, both test vaccine and reference vaccine were diluted and immunized 3 groups of mice each group consisted 16 mice. For the test vaccine mice were immunized intraperitoneally with the volume of 0.5ml. Similarly we had 2 control groups; the first control group was used against the reference vaccine immunization and the second control group was used against CVS challenging.

Immunization will be repeated after 7 days. All mice were challenged after 14 days of the first immunization with diluted CVS and challenged intrcerebrally with 0.03ml. Daily observation was made for 14 days and the number of dead mice after 5th day recorded, any death before the 5th day is considered as non-specific death.

Inactivant	Group name	No. mice	Titre	1 st Imm.	2 nd Imm.	Challgd	Dead	Live	Non-SD
	Gr-7	16	1:25	0	7	10	3	13	-
	Gr-8	16	1:125	0	7	10	4	12	-
	Gr-9	16	1:625	0	7	10	1	15	-
	Gr-10	16	1:3125	0	7	10	9	7	-
	Gr-11	16	1:10	0	7	10	6	10	-
Control	Gr-12	16	1:50	0	7	10	6	10	-
vaccine	Gr-13	16	1:250	0	7	10	12	4	-
	Gr-14	16	1:1250	0	7	10	0	16	-
	Gr-15	10	10 ⁻¹ ,	-	-		6	4	-
CVS	Gr-16	10	10 ⁻²	-	-		3	7	1
	Gr-17	10	10 ⁻³	-	-		0	10	-

Table 3. The result potency of formalin inactivation.

A volumetric method of calculation of potency, compares the 50% end-point dilution (vaccine dilution protecting 50% of mice) of the vaccine under test with that of the standard (commercial vaccine diluted to a final potency of 1IU/ml in our case). The relative potency (RP) of the vaccine under test is determined by the formula:

Where:

TV = test vaccine

RV = reference vaccine

Dose = volume of a single vaccinal dose, as stated by the producer

ED₅₀ – the 50% effective dose, which is calculated using Spearman-Kärber formula.

Where;

 $x^0 = \log_{10}$ of the lowest dilution with all wells positive

 $d = log_{10}$ of the dilution step, one in this case

ni = number of replicates, six in this case

ri = number of positive wells

Formalin ED₅₀ Test vaccine

= 0-0.35 + 0.7 x 3.8125

= 2.31875 ≈ 2.32

Control vaccine ED₅₀

= 1.4

If it is assumed that a single dose of vaccine is 1ml then,

 $= 10^{0.92}$

= 8.32 IU/ml for ERA vaccinal strain and 2.56IU/ml for PV vaccinal strain

Preclinical trial

Pre-clinical studies use tissue-culture or cell-culture systems and animal testing to assess the safety of the candidate vaccine and its immunogenicity, or ability to provoke an immune response. Animal subjects may include mice, dogs and monkeys. These studies give researchers an idea of the cellular responses they might expect in humans. They may also suggest a safe starting dose for the next phase of research as well as a safe method of administering the vaccine.

Researchers may adapt the candidate vaccine during the pre-clinical state to try to make it more effective. They may also do challenge studies with the animals, meaning that they vaccinate the animals and then try to infect them with the target pathogen. Challenge studies are never conducted in humans.

Duration: 120-160 days excluding 15 quarantine days.

Animals: 24 Dogs used for preclinical test of both ERA and PV vaccinal and mice for NIH potency test prior to preclinical test.

Positive and negative serum: OIE or WHO standard positive serum were used to compare immunogenicity of test serum obtained from dogs immunized with candidate vaccine. Serum from dog which is not immunized before and do not have any antibody titer will be used as negative serum.

Number of experimental animals: 2 groups (vaccinated and control), 6 dogs in each group for both ERA and PV virus strain vaccine. Experimental dogs are kept under observation for 2 weeks, each vaccine have control group of 6 dogs. All seek animals and animals with increased body temperature removed from the experiment.

Blood sampling: Blood is sampled on days 0 (before vaccination and testing for the absence of rabies virus neutralizing antibodies), 7, 15, 21, 30, 60, 90, and 120. Brain is sampled for rabies diagnosis using FAT method for all the animals dead during the experiment and after finishing the experiment (on the 120th day). The rapid rabies fluorescent focus inhibition test (RFFIT) or





test (FAVN) is used in comparing the immunogenicity of ERA and PV strain vaccines. No substitute test reagents or substitute tests shall be accepted.



Any dogs with detectable antibody at day 0 will be removed from the experiment and all test animals should be negative at day 0.

Vaccine and vaccination: experimental vaccine suspension prepared according to "Laboratory manual for vaccine production", formalin-inactivated, containing before inactivation at least $10^{6.0}$ TCID₅₀ used. Completeness of inactivation of vaccine checked on mice and only completely inactivated vaccine is used. Potency of the vaccine determined on mice using NIH test before immunization of the dogs. Experimental dogs were vaccinated subcutaneously in shoulder (scapular) area once with the volume of 2.0 ml or 2 IU determined in NIH test. Each group contains 6 dogs. One group unvaccinated, control groups are kept separately until challenging day.

Determination of virus neutralizing antibody

This test is based on the detection of the specific (neutralizing antibodies, nab) and unspecific neutralizing activity of sera against the rabies virus. The antibody titer is determined by dilution of the sera in logarithmic steps and comparison with a positive or negative reference serum. The basic principle is the virus-specific inhibition of a serum to be tested in cell culture by the identification of infected cells using FITC marked anti-rabies hyperimmune serum.

$10^{T \text{ test}} \div 10^{T \text{ ref.}} \times 0.5$

T test. – logarithmic titers of tested serum *T ref.* – logarithmic titers of reference serum
0.5 – activity of reference serum, IU/mI.

Based on the result obtained, more than ten times WHO minimum recommendation (0.5IU/ml) antibody titer is obtained for all dogs immunized. Based on the result, this vaccine can be used for the immunization of animal and additional purification and clinical trial is required to use for human immunization.

Inoculation: Dogs were inoculated intramuscularly in mandibular area from both sides. The dose given each dog shall not exceed 200,000 mouse intracranial 50 percent lethal doses (MLD50), or a demonstrably equivalent dose as determined by an alternate method of titration. All animals, vaccinates and controls, challenged at the same time. A separate needle and a separate syringe used for each dog.

Challenged dogs, retained in isolation, shall be observed for a minimum of 40 days prior to final bleeding, euthanasia, and rabies testing of their brains. All the dogs shall be euthanized after the experiment finished. All dogs dead after inoculation or killed after finishing the experiment will be tested in FAT/MIT. This trial is not completed and under way to finalize this trial.

Currently VDPD produce more than 100,000 dose cell culture based anti-rabies vaccine which can be used for animal immunization. The test is ongoing for clinical trial of the vaccine to be used for the immunization of humans. The vaccine is produced in the same process but for human consumption, different special purification system is required.

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4.5 National Rabies Baseline Survey Preliminary Report: Household Assessment

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Background

Rabies is an important disease that has been recognized for many centuries in Ethiopia (1). The disease is endemic throughout the country and results in a significant loss of human life every year (2). There has never been any nationwide epidemiological survey undertaken in the country except the survey conducted in the capital city Addis Ababa, in which a total of 464 human rabies fatal cases were reported (3).

Previously estimation of rabies mortality have been based on the projection utilizing the data base from the Ethiopian health and Nutrition Research Institute (EHNRI) and survey findings from Addis Ababa applied to the total population of the country assuming that there is a uniform distribution of risk factors throughout the country (3,4 & 5). However, different studies revealed that the burden of rabies is not uniformly distributed across sectors of the society and is also influenced by socioeconomic and other related factors (6, 7& 8).

Studies showed that better understanding of the basic determinants attributed to the occurrence of rabies have been used to estimate human rabies deaths (9) and to design appropriate intervention measures. However, the burden of the disease is underestimated and not well documented in most developing countries including Ethiopia. Thus, up to date information on rabies and rabies related conditions are scanty, unreliable and controversial due to poor surveillance/reporting system in all sectors. As a result of this huge information gap, designing and applying appropriate prevention and control measures at national as well as regional levels has still proved to be difficult. Therefore, taking into consideration the gaps indicated above and in order to obtain accurate information to address the problem in coordinated manner, conducting this nationwide epidemiological survey on rabies has great significance to fully understand the disease dynamics in the country.



Objective

General:

• To generate a comprehensive data on rabies and its related aspects in human and animals population in Ethiopia

Specific:

- To estimate the annual clinical incidence of human rabies exposures and fatal human rabies cases
- To identify the commonly responsible animal for rabies exposure and fatalities in human
- Estimate the overall dog population and their vaccination coverage

Methodology

Study Design and area

This national rabies baseline survey is a population based cross sectional study design using a multistage cluster sampling method with stratification by region and by urban/rural strata. The survey was conducted in 9 regions (Tigray, Amhara, Oromia, Southern Nations, Nationalities and Peoples (SNNP), Somali, Gambella, Benishangul-Gumz, Harari and Afar) and 2 special city administrations (Addis Ababa and Dire Dawa) during May to June 2012.

Sample size estimation

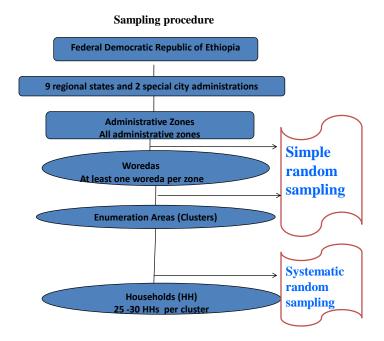
A total sample size of 5280 households were calculated using Epi Info version 6 based on the following assumptions; a 2007 census total estimated population of 73,918,505, an expected prevalence of Human rabies 50%, a 95% confidence interval, including a design effect of 2 and a 15% increase for non-response rate. Subsequently, the required samples size was calculated to be 20644 (~20700) individuals. The average household size in Ethiopia is estimated to be 4.7 persons (10). Therefore, to obtain 20700 persons, approximately 4404 households were needed. According to WHO (11) World Health Survey Guideline between 10 and 30 households are recommended in a single cluster for household surveys. Therefore, for this study, a cluster size of 25 households were used that give country total of 176 clusters. In order to increase the precision level, additional 5 households were included from each cluster that gave a total of 5280 households for the country.

Sample allocation and Sampling strategy

The total sample size calculated for the country was distributed for the regions based on their population size; sample allocation using probability proportional to size (PPS) technique and power allocation calculation. All zones in the country were included in the survey in order to avoid too much clustering of the survey areas and to increase the national geographic coverage. The number of clusters in each region was distributed to zones proportional to the size of their population. At least one district was selected from each zone. A simple random sampling technique was used to select district in order to give equal chance for every district in the zone to be selected for the survey. One Enumeration area (EA), considered as cluster, was selected randomly from each district the central statistics authority (CSA) and all the necessary information about the selected EAs were also obtained from CSA (10).

Finally, the households registered in the selected EAs were enumerated and listed before the actual data collection was initiated. The households were selected from EAs using systematic random sampling techniques (Figure 1). All the selected households were visited separately and face to face interviews conducted with the head /spouse/ adult respondent's \geq 18 years of age using structured and pre tested questionnaires.

Figure 1: Sampling procedure





Data collection procedure

Nine teams were assigned for field data collection from the centre (EHNRI). Each team was composed of one coordinator, one supervisor and two-three data collectors. Besides, one health professional, one veterinarian, one-three translators and one guider were recruited and trained from each selected district respective offices and the community to facilitate the data collection process. Trained data collectors gathered the information by moving house to house under close supervision of the field supervisors, coordinators and local health/vet professionals. The informants were asked to report any human rabies deaths and exposure cases that occurred during the past five year's period (from 2000-2004 E.C.) among the household members. The exposure status and fatal human rabies cases were categorized based on WHO case definitions (12). The household questionnaire survey also included questions related to dog population and their management systems in the selected households and enumeration areas as a whole.

Data Quality assurance and control

The questionnaire was first prepared in English and later translated in to Amharic. It was pretested in peri -urban area setting and the questionnaire was modified based on the findings. Field coordinators, supervisors and data collectors were also trained for three days on the survey process and the questionnaires including practical field –testing of the data collection instrument. The supervisors and coordinators followed the data collection procedure throughout the study period. All the questionnaires gathered from each household were checked and approved by the supervisors for completeness and consistency. Besides, 30 % of the questionnaires collected from every EAs were checked randomly at the household level. The data entry template was prepared and tested by EHNRI data manager. The data clerks were briefed about the template and the variables. The whole course of data entry and cleaning process was closely supervised by the data manager and the research team members.

Data analysis

After the data collection was completed, the data from the questionnaires were entered into CSPro version 4 and transferred to SPSS version 16.0 for cleaning and analysis. The data analysis was done at individual sampling unit level (Simple sample Analysis) and the annual incidence rates were estimated based on the mid- year population using 2007 census for projection.

The stray dog population density was calculated by dividing the average stray dog population in EAs by average area coverage of the selected enumeration areas.

Ethical considerations

The survey (Project number: *SERO-86-2-2004*) was approved by the scientific and ethics review office of the EHNRI. Permission was also granted from all regional health bureaus and local administrative of all zones and districts included in the study. Each participant was informed about the purpose of the study and informed consent was obtained from each respondent.

Results and Discussion

Characteristics of Households and Respondents

In this study, a total of 5280 households were identified and listed. Incomplete questionnaires and households in which no one was present on the day of interview (308) were excluded from the analysis. Only questionnaires collected from 4972 households were considered for analysis. Of these 19.8 % households were in urban and 80.2% were in rural areas and the proportion of men and women respondents in the sampled population were 44.0% and 56.0%, respectively. Of all respondents, about half had undergone formal education and lead their life by agricultural practices. Regarding religion, most of the respondents (65.0%) were Christian followed by Muslim (33.1%) and others accounted to 1.9%.

Human Rabies exposure and fatal rabies cases

During the survey, a total of 354 human rabies exposures and 45 fatal human rabies cases were reported by the selected households. The majority of human rabies victims (86.7%) and rabies exposure cases (79.9%) were from rural areas. Rabies exposure cases were reported from all regions while fatal human rabies cases were recorded only in 8 regions and one city administration. Nevertheless, the highest exposure and fatal cases were observed in Amhara and Oromia regional states, respectively (Figure 2 & 3). None of the human rabies deaths were laboratory confirmed (Probable cases) and only few exposure cases (2.6%) were classified as confirmed exposure cases.

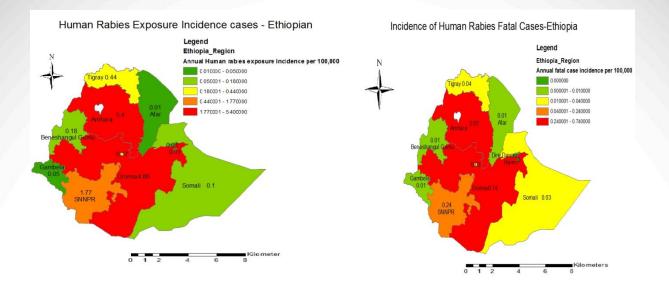


Figure 2: Incidence of human rabies exposure cases by region

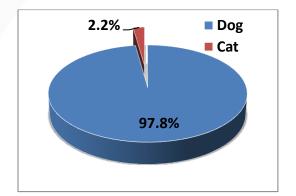
Figure 3: Incidence of Human rabies deaths by region

The overall national annual incidence rate of exposures and rabies deaths were 12 exposure cases/ 100,000 population and 1.6 rabies deaths /100,000 populations, respectively. Almost similar estimation, more than 1000 deaths/year, was reported by the Ministry of Health in 1977(14). This is the second highest incidence rate of human rabies death in the world next to India reported as 1.7 deaths per 100,000 population from community based survey (13).However, both the incidence of exposure and human death in this study are by far lower as compared to the previously reported incidences (74.4 exposure cases per 100,000 population and 18.6 deaths per 100,000 population) in Ethiopia (3). Majority of fatal human rabies (60.0%) and exposure cases (52.8%) were reported in children under the age of 15 years. Similarly, WHO has also reported 5 times higher incidence rate of rabies in children aged <15 years than adults (15). This is mainly associated with their close contact with dogs, unable to identify & escape from rabid animals.

Approximately 50% of human rabies victims had resorted to traditional treatment following animal bite. In line with this finding high preference of people from inaccessible areas to seek medical care from traditional healers instead of traveling long distance to get post exposure vaccine were reported in Ethiopia (1, 4 &5). This is due to the deep rooted belief in traditional medicine for rabies treatment among the rural community, inaccessibility of alternative modern post exposure treatment in the nearby areas and lack of knowledge on the availability of modern medical treatment.



The animal mainly responsible for human rabies deaths (97.8%) and exposure cases (90.4%) was dog (Figure 4 & 5). Unlike other canine rabies endemic countries (9 &13) that reported high proportion of human rabies deaths from the bite of stray dogs. The findings of this study showed that stray and owned dogs were equally responsible for human rabies deaths in Ethiopia. This can be associated to the overall low vaccination coverage and poor management of owned dogs.



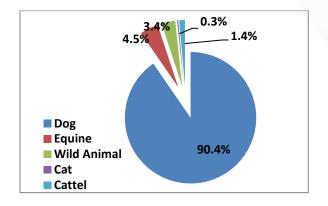


Figure 4: Responsible animals for Human Rabies Deaths in Ethiopia Figure 5: Responsible animals for Rabies Exposure Cases in Ethiopia

Dog population and their management

In this sample survey 21.0% of the households indicated as owning one or more dogs. The combined households dog ownership proportions of all urban areas (24.0%) were higher than the rural areas (21.0%).Only 19% of the dogs in the sampled areas were vaccinated and the vaccination coverage of dogs against rabies were relatively higher in urban than rural areas (Figure 6) at the time of the survey. Moreover, the vaccination coverage reported both in urban and rural areas were below the WHO recommended coverage (>=75%) to control canine rabies certain area.

Among respondents those who did not vaccinate their dogs against rabies, 46% and 41% of household informants gave lack of knowledge on the vaccine availability and lack of vaccination service in the nearby as the main reasons, respectively (Table 1).

The stray dog population densities were 11 / Km2 and 0.12/Km2 for the urban and rural areas respectively. This means that for the areas surveyed, the population density of stray dogs in the urban area is almost 100 times more than that of rural areas. In agreement with this finding, the presence of higher population density of stay dogs in urban areas was reported in other developing countries where sustainable stray dog population control measures are lacking (16).

Figure 6: Dog population and vaccination coverage by place

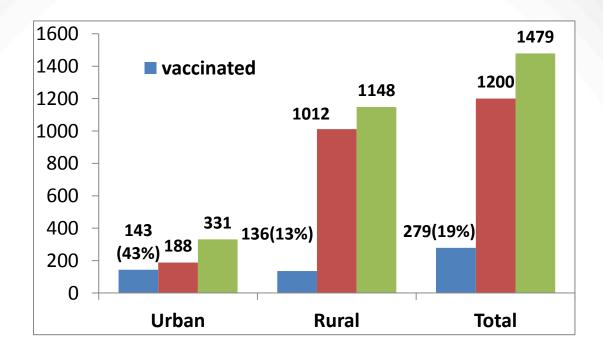


Table 1: Reasons for low dog anti-rabies vaccination coverage by place

Reasons why the dogs are not get vaccinated			
	Urban	Rural	Total
I don't know about the availability of vaccine	32	366	398 (46%)
No vaccination service nearby	59	296	355(41%)
Due to the expensiveness of the vaccine	10	15	25 (3%)
since it is leashed no need to vaccinate	32	57	89 (10%)
Total	133	734	867(100%)

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Limitations of the study

- Only Bite and scratches were considered to assess the exposure status (exposure due to contact with saliva of rabid human and rabid animals were not included).
- None of the fatal cases reported in this study were laboratory confirmed
- Paralytic forms of human rabies might have been missed
- Memory recall intervals
- Stray dog population estimation was based on a single day count/observation and community estimate
- Vaccination status of dogs were taken as informed by household informants
- In communities that are not aware of rabies and did not report any death were considered as zero report.

Conclusion

Rabies is a major public health concern in all parts of the country although the magnitude of the problem varies from region to region. Besides, this study showed that the magnitude of the problem is much higher in rural areas where 85% of the population lives. School children are at a higher risk of being exposed to rabies and death. Traditional medicine for the treatment of rabies and low health seeking behavior for animal bite among the communities have contributed for the occurrence of higher rabies mortality rates in rural areas. Dogs (owned and stray) are the major animals responsible for the spread and transmission of rabies to human in Ethiopia. However, the level of canine(dog) rabies control and prevention intervention measures like (dog mass vaccination and stray dog population control) are not sufficient enough to put the disease under control in the country.

Recommendations

Therefore, to safeguard the public from rabies:

- Primary intervention measures should focus on controlling the disease in dogs
- Laboratory diagnostic systems should be established at least at regional level
- Educating the public about the dangers of rabies, its effective control measures and scientifically proven post prophylaxis treatment
- Strengthen responsible pet ownership system
- Incorporation of rabies education into elementary school curricula
- Make PEP and dog vaccination services easily accessible in areas that are nearby to the community

Acknowledgements

- EHNRI
- Regional Health Bureaus
- Regional Agricultural Bureaus/Livestock agencies
- District health and agricultural offices
- Study participants (Households)
- Central statistics Agency
- Ethiopian Wildlife Development and conservation Authority

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4.6 National Rabies Baseline Survey Preliminary Report on Facilities Based: Public Health, Veterinary and Wildlife

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Introduction

Rabies is an acute viral disease which causes encephalomyelitis in virtually all the warm blooded animals including man. Rabies virus belongs to the family Rhabdoviridae and genus Lyssavirus (Lyssa: Greek: rabies). The causative agent is found in domestic and wild animals, and is transmitted to other animals and to humans through close contacts with their saliva (i.e. bites, scratches, licks on broken skin and mucous membranes). In most of the developing countries, dogs are the principle reservoirs of rabies (canine rabies) whereas sylvatic rabies involving animals such as foxes, racoons and coyotes are principle reservoirs of this disease in developed countries.

Rabies continues to persist as a major public health problem. It is perhaps the most gruesome and dreadful of all communicable diseases afflicting human beings (7). WHO pointed out that more than 99% of all human rabies deaths occur in the developing world, and that the disease has not been brought under control throughout most of the affected countries (8). It is widely recognized that the number of deaths officially reported in most developing countries greatly underestimates the true incidence of disease, with several factors contributing to widespread underreporting. It leads to lack of attention by national authorities, and by the international organizations concerned. Disparities in the affordability and accessibility of post-exposure prophylaxis, levels of rabies awareness and risks of exposure to rabid dogs result in a skewed distribution of the disease burden across society, with the major impact falling on members, particularly children, of poor rural communities. Thus, it is necessary to conduct new assessments of the rabies burden in country to promote the development of alternative technologies and other interventions, as part of the new thrust for rabies control in countries.

Based on the Ethiopian Health and Nutrition Research Institute (EHNRI), Zoonoses Research Team report, from 35 to 58 human fatal rabies cases are recorded every year from Addis Ababa city and its surrounding towns (1, 2, 9).

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This is only an indication of the human rabies death in these areas i.e. it does not represent the national level figure as a whole. Therefore, in order to obtain accurate information in Ethiopian context conducting a nationwide rabies base line survey on public, veterinary and wildlife facilities have a great significant impact in terms of better intervention technique as well as designing strategies on prevention and control of rabies at national level.

Objective

General objective:

 To generate a comprehensive data on rabies and its related aspects in human and animal populations in Ethiopia

Specific objectives:

- To estimate the incidence of animal and human rabies cases and deaths
- To identify the distribution of rabies among different animal species
- To measure the level of vaccination coverage
- To assess the challenges on rabies prevention and control activity

Material and Methods

Study Design

The facility survey was designed on Population based cross sectional study using a multi- stage cluster sampling with stratification by region

Study area

The survey was conducted during the month May to June 2012 in all regions and city administrations of the country that was included randomly selected facilities survey i.e. Public, Veterinary and Wildlife sectors.

Sample size estimation for facility Survey

The sample size estimation for facility survey was calculated based on household survey i.e. 176 clusters.

The sample size is calculated using Epi Info version 6 based on the following assumptions:-

- From the Total population of 73,918,505, 50% expected prevalence of Human rabies,95% confidence interval ,0.01 degree of precision, Design effect of 2 and a 15% non- response
- Over all sample size of 20644 (~20700) is obtained from above, and
- To calculate the total number of HHs required for this survey:-

<u>Total sample size</u> = <u>20700</u> = 4404 Households

Average household size 4.7

• For this survey 25 Households/cluster is enough, so that the total number of clusters is obtained from calculation of 4404/25= 176.

Thus, by using Non probability sampling technique from randomly selected districts for Household survey 176 Health and Veterinary facilities had been used at district level. However, Wildlife facility survey was assessed based on the animal species they conserve, because the animal(s) play an important role in the epidemiology of rabies, so that among 15 national parks registered by Ethiopian wildlife conservation Authority, 14 were planned to conduct the study.

Data collection Technique

The data was collected using structured and pre-tested questionnaires. During data collection the following three Techniques were used:

- Health facility:- *Record review*
- Veterinary service facility:-*Record review & key informant interview*
- Wildlife facility based surveys:-*Record review & key informant interview*

Data management and Analysis

After the data collection was completed based on the schedule, all facilities data was entered into Epiinfo 2008 version 3.5.1 and Transferred to SPSS version 12.0 for further analysis. The expected data from each facility were:

- Health Facility-
 - Human Rabies
 - Exposure cases
 - Annual Rabies Vaccine used
- Veterinary Facility-
 - No. of Animal Rabies cases
 - Vaccination coverage
 - Dog population management
 - Challenges
- Wildlife Facility
 - No. of Animal Rabies cases
 - Rabies prevention& control
 - Challenges

Results

Preliminary results (Major Findings) on Public Health Facility Survey

For the survey purpose it was expected to conduct the survey from 176 Health facilities, in this survey only 135(79%) of them responded for questionnaire (figure 1).

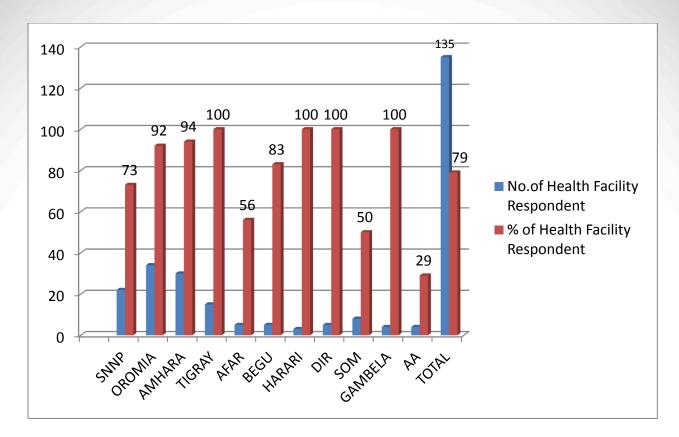


Figure 1. Percent of Health Facility Respondent per Region

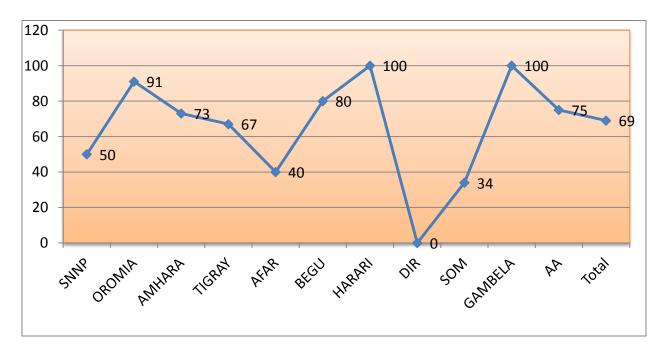


Figure 2.Percent of respondent: Rabies as a Public Health Problem

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As it was showed in the above Figure 2, the percent of respondent that said rabies is a public Health problem is 69% as total, but Harari and Gambella regions said the problem is100%. The rest regions like Oromia, Benshangul, Amhara, Addis Ababa and Tigray said that the problem is 91,80,73,69 and 67 percent, respectively.

Eventhough, The rabies is a major Health problem within the community as indicated in fig.2, the data record per region showed that only 46% the total of surveyed region (figure 3).

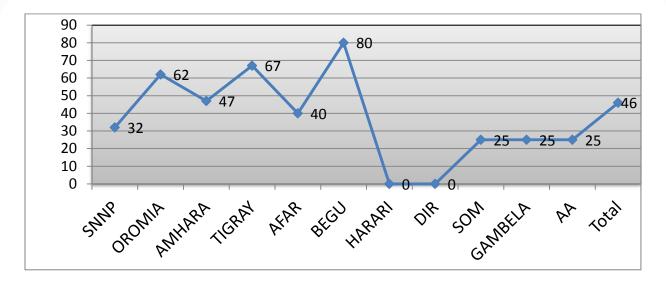


Figure 3. Rabies data record per Region

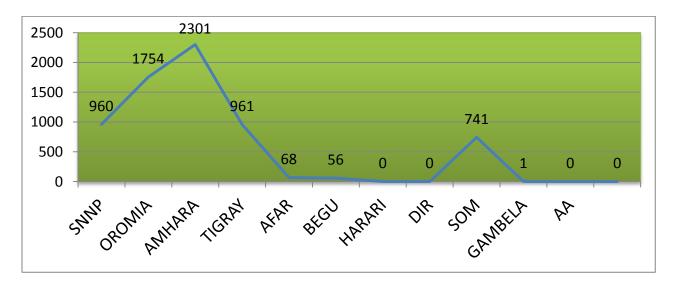


Figure 4. Human Exposure/Bite (1999-2004 E.C)

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The retrospective data record from the year 1999 to 2004 E.C indicates that there was Human exposure/bite by suspected rabid animal in total number of 2301,1754,961,960 and 741 in Amhara, Oromia,Tigray, SNNP and Somali regions, respectively(figure 4).

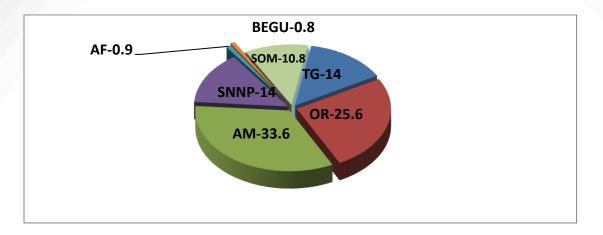


Figure 5. Human Rabies Death (1999-2004E.C)

The last five years data records in fig. 5 from 1999 to 2004 E.C. showed that High percentage of Human Rabies Death in Amhara and Oromia, i.e. 33.6 and 25.6, respectively. Region like Tigray and SNNP have indicated 14% each.

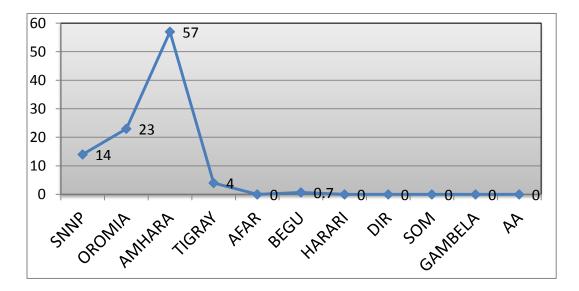


Figure 6. Anti-Rabies Vaccination Coverage (1999-2004 E.C.)



As it is stated in the above Fig.6 regarding Anti-Rabies Vaccination Coverage in Human from 1999 to 2004 E.C. from the total percentage, 57 of them was utilized by Amhara region, 23 by Oromia, 14 by SNNP and 4 by Tigray Region.

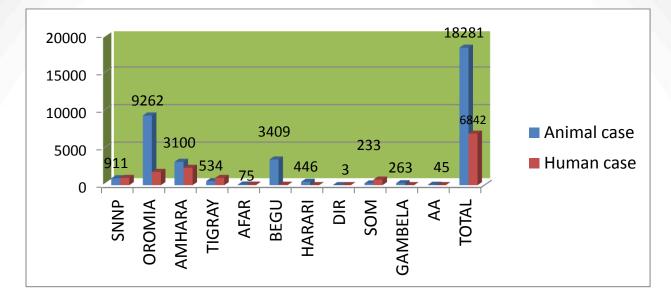
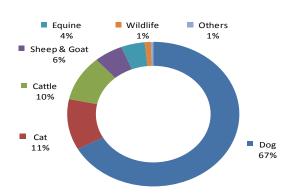


Figure 7. Comparison of Animal & Human Rabies cases (1999-2004E.C.)

As it is known in our country specially, in rural area most of the people handle animals for their economic purpose. The Comparison of Animal & Human Rabies cases in Fig.7 showed that where there is high animal cases always human cases, due to day to day interaction in their life and also indicates that how important animals are the main source for the rabies cases in humans.



Preliminary results (Major Findings) on Veterinary Facility Survey

Figure 8. Distribution of Rabies case according to animal species



The data record of randomly selected veterinary facilities from 1999 to 2004 E.C on the Distribution of Rabies case in different animal species indicates that 67% of Dogs, 11% of Cats and 10% of Cattles are the main sources of rabies as a whole(figure 8).

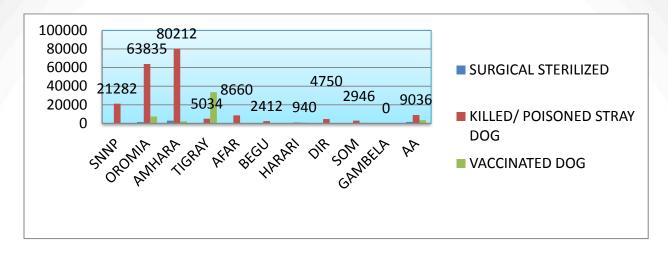


Figure 9.Dog Management record from 1999 to 2004E.C.

One of the major factors that affect rabies distribution is improper dog management. Currently, in our country the management of dog is conducted by using vaccination of dog, killing/destroying of stray dog and surgical sterilization technique. Figure .9 describes that, among the above stated techniques Amhara, Oromia and SNNP regions are used killing/destroying of stray dog, where as in Tigray region vaccination of dog was highly utilized. Regarding surgical sterilization technique only Amhara, Addis Ababa and Oromia were used in a very low number.

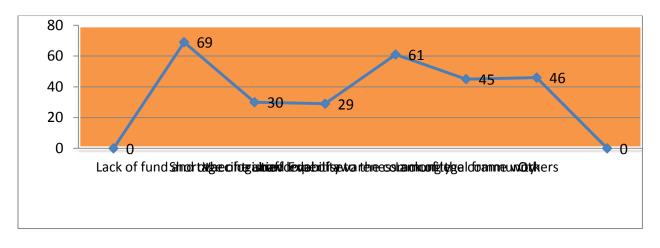


Figure 10. Challenges on Vaccination of Dogs

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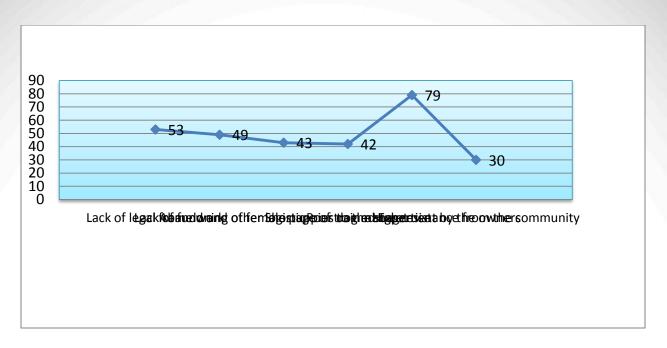


Figure 11. Challenges on Management of Dogs

During this survey, it was observed different challenges on vaccination and management of dog as indicated in the Figures 10 and 11 most of the respondents said that they have a lack of fund, low level of awareness on rabies, lack of legal frame work, and poor dog management in all regions.

Preliminary results	(Major Findings) o	on wildlife Facility Survey
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Factors	Nation	al (10)	Regio	nal(4)
	Yes	No	Yes	No
Protected from Human/Animal contact	1	9	1	3
Dwellers inside Facility	7	3	3	1
Keeps Dogs	6	4	2	2
Seen Rabid Animal	4	6	2	2

Table 1. Wildlife Facility Data per National & Regional Park

Wild animals should be protected from different diseases, because they are necessary for nature conservation, Tourism and economic growth of one country. However, as described in Table 1 both the national as well as regional parks not protected from human and animal contact, Dwellers live and keeps dog inside the facility and also there had been seen rabid animals with in the compound.

Observations

- Lack of Legislation:
 - ✓ Dog management
 - ✓ Compulsory vaccination
 - ✓ Control of stray dogs
- Weak intersectoral coordination among Veterinary service, Human Health, Municipality and others
- Inadequate data and information
- No specific reporting system on rabies and/or Under reporting
- Inadequate Diagnostic facilities No laboratory confirmatory test
- Inadequate Awareness and No fund allocation

Conclusion

The survey result showed that, rabies is a Public as well as Animal health problem as a whole, and also most of the regions have poor data handling, underreporting systems, and lack legal frame work. The distribution of rabies in human and animal and Anti-rabies vaccination coverage are more dominant in Amhara, Oromia, SNNP and Tigray regions, however, due to cultural and religion influence it is less in Afar, Somali and Harari regions. The dog management practice is very poor in almost all regions, and it needs emphasis in rabies prevention in human. Regarding the wildlife sectors, the national and regional parks are not protected from human and animals, Dwellers are living and keeps dogs inside, due to all these problems currently both parks are endanger, so that it needs all rounded attention by all stakeholders in terms of rabies prevention and control in wildlife as well as in country level.

Recommendations

- Legislation: Registration/Licensing and vaccination of dogs.
- Development and implementation of comprehensive and sustainable national rabies prevention and control strategy
- Establish/Strength State-level coordination committees
- Establish surveillance and diagnostic laboratory on human as well as animal side
- Improve understanding of rabies through advocacy, awareness, education and operational research
- Provide coordinated support for anti-rabies drive with the involvement of community, civil society, government and non-government sectors and international partners
- Strength multisectorial/intersectorial Collaboration(in Report, Data & Information exchange)
- Promote dog population management and mass dog vaccination
- Active contribution of Veterinary Services to the goal of prevention and control of human rabies at the animal source

Acknowledgments

- EHNRI
- Regional Health Bureaus
- Regional Agricultural Bureaus
- District Health and agriculture offices
- Ethiopian Wildlife Development and conservation Authority

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4.7 Rabies Public Education Booklet

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Summary

Health education is any combination of learning experiences designed to help individuals and communities to improve their health, by increasing their knowledge or influencing their attitudes. The Ethiopian Health and Nutrition Research Institute (EHNRI) has been disseminating information on rabies to the public through brochures, magazines, television and radio programs. The EHNRI is now developed an educational booklet on Rabies with the objective of increasing public awareness on the diseases and reaching the public in large. The booklet was pretested in selected target groups comprising 31 participants from school children, health professional, veterinarian and members of media organizations aiming at examining how effective the booklet serves its purpose. The pre-testing was conducted using focus group discussions and individual interview. The parameters measured were understandability, convincingness, social acceptability, accurateness and attractiveness. The minimum of 81% for the convincingness of the booklet was observed in school children and a maximum of 95% for accuracy was observed in journalists. In general, the educational booklet was given satisfactory level of understandability, convincingness, acceptability, accuracy, usefulness and attractiveness by the participants. The booklet was officially launched at the national rabies workshop on October 8 and 9, 2012 held at Adama town, Ethiopia. The booklet will be disseminated to schools, health and veterinary facilities in Addis Ababa primarily and will reach to regions of the country later.

Key words: Rabies education booklet, pretesting

Introduction



Health education has been variously defined by Green (1980)as any combination of learning experiences designed to facilitate voluntary adaptations of behavior conducive to health; by Simonds (1976) as a strategy aimed at bringing about behavioral changes in individuals, groups and larger populations, from behaviors detrimental to health to behaviors conducive to present and future

health.

Rabies education practice

The Ethiopian Health and Nutrition Research Institute has been disseminating information on rabies to the public through brochures, magazines, television and radio programs. There were limitations in using broachers and magazines that there were page restriction and hence all the required information was not given at the satisfactory level. By way of television and radio programs because they have only one time contact and it lacks repeatability only those people at home had access to gain the information.

Rabies education booklet

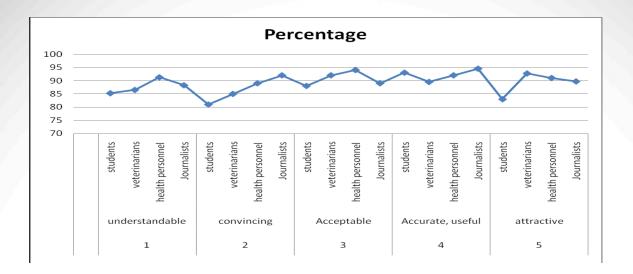
The EHNRI is now developed an educational booklet on Rabies to reach the public in large. The educational booklet is intended to convey information on rabies to the public for the understanding of the diseases and there by teaching important preventive measures like avoiding dog bite incidences, first aid treatments, post exposure treatments, dog ownership and managements and many other. The booklet is targeting mainly children at school and public visiting health and veterinary facilities.

Rabies booklet pretesting

The booklet was pretested in selected target groups comprising school children, health professional, veterinarian and members of media organizations with the objective of examining how effective the booklet serves its purpose and it was conducted by the rabies laboratory experts and Public Relation Office of the EHNRI. The pre-test was conducted by rabies laboratory staffs and the Public Relations Office of EHNRI from March 10 and 11, 2012. The pre-testing was conducted using focus groups and individual interview for an in-depth discussion of the booklet relevance and effectiveness. The conducted focus group discussion allows the solicitation of opinions from 31 people in a short time. The parameters measured were understandability, Convincingness, Social acceptability, Accurateness and Attractiveness.

Results

Health professional respondents believed that the educational booklet is 91% understandable to health professionals, 88 % understandable for journalists and 86 % for veterinarians and 85% to school children. The booklet is 92 % convincing to journalists, 89 % and 85 % to health professionals and veterinarians respectively and 81% to school children. Social acceptability of the booklet is 94 % to health professionals, 92 % for veterinarians, and is 89 % and 88 % for journalists and school children respectively. Accuracy and usefulness level of the booklet is 95% for Journalist, 93% and 92 % for School children's and the health personnel respectively. Veterinarians believed that accuracy level of the booklets is 89 %. Attractiveness of the booklet is 93 % for veterinarians 91 %, 90 % and 83 % for health personnel, journalists and school children.



Conclusion

Generally, the rabies educational booklet is given a satisfactory level to its understandability, convincingness, acceptability, accuracy, usefulness and attractiveness. The participants have also provided valuable information and were incorporated in the booklet. The booklet was officially launched at the national rabies workshop on October 8 and 9, 2012 held at Adama town, Ethiopia. The booklet will be disseminated to schools, health and veterinary facilities in Addis Ababa primarily and will reach to regions of the country later.

Future initiatives

- In the short term, broad media coverage through television and radio program to publicize the booklet.
- In the middle term, the booklet will be translated in to different local language to reach public in regions of Ethiopia and dissemination and public teaching will be effective through health extension workers in respective regions. A documentary film (VCD/DVD) focusing on important practices like a) animal bite avoiding, b) recognizing rabies episode in animals c) bite wound management. d) Hospital care for human rabies etc.

 In the long term, the EHNRI will be highly attracting, encouraging, assisting and give technical advice to any organization committed to work in community rabies education and give humanitarian support in preventing human rabies.

Acknowledgments

All authors participated in the booklet writing and editing, the public relation office and General Director of the EHNRI, all participants during the booklet pretesting session are all highly acknowledged for their active and kind contributions.

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4.8 Current Rabies Diagnosis and Future Prospect in Ethiopia: One health approach

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Introduction

The history of Rabies diagnosis and anti-rabies vaccine production in Ethiopia is as per the agreement reached between the Imperial Ethiopian Government and the Institute de Pasteur of Paris in 1944 G.C. Since then Ethiopian Health and Nutrition Research Institute (EHNRI) is the sole institution to provide rabies diagnosis in Ethiopia.

Currently, the incidence of dog bite and rabies is increasing significantly due to increase in dog population, lack of dog movement control and irregular vaccination program in Ethiopia (Asefa et al., 2010). There are also a number of rabies cases that have not been diagnosed in the country, due to lack of established surveillance systems and absence of diagnostic facilities in different geographical locations of Ethiopia. Regarding, the public health concern of rabies in Ethiopia, the annual reports from Ethiopian Health and Nutrition Research Institute (EHNRI) shows figures between 1500-2000 post exposure treatments and between 35-50 human deaths each year in and around Addis Ababa. Thus, lack of accurate, simple and less costly diagnostic tests for field use has caused most of the rabies cases undiagnosed. This has become a major threat for understanding of the epidemiological patterns and scaling the magnitude of public health impacts of the disease.

Rabies Animal –Human interface: One health approach

A new model that replaces highly expensive equipment required for diagnosis, with a less expensive test (DRIT) to conduct epidemiological research required to better elucidate the epidemiology of rabies both in humans and animals; The rabies transmission dynamics among domestic wild and human interface within the same ecosystem is briefly mentioned to identify the inter species rabies virus genotypes circulating in Ethiopia.

Decentralization of rabies diagnosis both for humans and animals

Decentralization of one centered laboratory can improve access to better diagnosis capacity and facility with reliable testing of suspected humans and animal rabies cases in the country. It will also create an opportunity to conduct training sessions for health professionals in 'one health' environment in both regional public health and veterinary laboratories for humans and animal rabies diagnosis.

In this regard, efforts are under way to establish rabies diagnosis facilities at regional public health and veterinary laboratories to scale-up rabies rapid diagnostic tests mainly the newly introduced diagnostic test kit direct rapid immunohistochemical test (dIRT). The Ethiopian Health and Nutrition Research Institute (EHNRI) in collaboration with international partners like CDC will scale-up rabies laboratory activities to regions to carry out enhanced laboratory based surveillance and establish monthly reporting system at grass root level. With the principle of one health approach establishing local training centers at regional universities and linking up with laboratory net work at public and veterinary health facilities would also enhance the capacity of regional laboratories to collect specimens and perform some laboratory works for further investigations of the disease using molecular techniques at national referral laboratories both for human and animal subject.

With this background information the objective of this paper is to sensitize the national rabies prevention and control workshop participants the current status of rabies diagnosis with its, challenges and future directions in Ethiopia. This paper also tries to highlight animal rabies diagnosis facilities and techniques routinely exercised at EHNRI, and challenges encountered to scale-up the technique Fluorescent Anti-body Test(FAT) across the country, attempts made so far in rabies diagnosis and the way forward are briefly presented.

Rabies diagnostics at Ethiopian Health and Nutrition Research Institute (EHNRI)

Animal rabies diagnosis

At present, routine animal rabies diagnosis is carried out in two settings:

1. Clinical Examination on live animals (based on clinical signs of rabies)

Clinical rabies diagnosis is performed on live animals being suspected and affected by rabies and are kept under quarantine for 10 days either at EHNRI and/or at home depending up on the risk of clinical observation at arrival. The following observation parameters are routinely exercised at EHNRI:

- Dropping jaw
- Abnormal sound in barking
- Dry dropping tongue
- Licking its own urine
- Abnormal licking of water
- Regurgitation

- Altered behavior
- Biting and eating abnormal objects
- Aggression
- Biting with no provocation
- Running without apparent reasons

- Stiffness up on running or walking
- Restlessness
- Appearing sleepy
- Imbalance of gait
- Dog sitting position

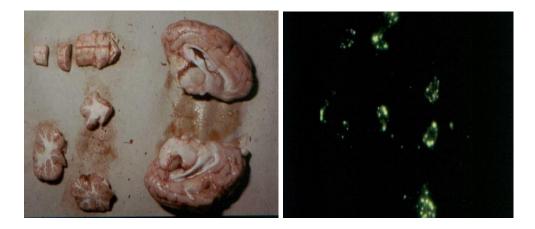
The diagnostic facilities are located at the campus of Ethiopian Health and Nutrition Research Institute (EHNRI) only. The 10 days animal quarantine facility has the capacity to hold 20 animals (one per cage) with adequate feeding and watering materials. Two types of animal quarantine are practiced one at the institute's facility and the second at home. If the animal does not manifest clinical signs of rabies during clinical examination at our facility then the owner is advised to perform some procedures like not to let outside the cage and tie the animal for 10 consecutive observation days under adequate supply of feed and water, and then telephone call and reporting to EHNRI in case of abnormal behavioral change, and also if the animal disappears and/or dies out. The quarantine facility at EHNRI has also post mortem diagnosis unit for removal of the brain and preparation of specimen for examination. This quarantine facility is also capacitated with all required materials like post-mortem kit, deep freezers, disposal materials and incinerator. The working space for specimen storage is also available separately. The Rabies diagnosis on live animal facility is also structured in front of the main building of rabies laboratory to perform clinical observation of suspected live animals. This unit is also equipped with case registration books, animal restraining equipments, feeding and watering materials and cages.

2. Laboratory confirmation work:

Fluorescent Anti-body test (FAT) which detects viral antigen and also the laboratory confirmatory diagnostic method for rabies infection.

In Ethiopia Rabies diagnosis is performed on live animals and brain samples of animals that are submitted to the Ethiopian Health and Nutrition Research Institute's laboratory live, suspected of being affected by rabies and are kept in quarantine and finally die or brain of animals submitted by health care seekers/customers after being killed or died.

In addition, **Mouse Inoculation Test (MIT)** is also used in our laboratory in case of FAT test result failure due to sampling and human error for further confirmation. Then the inoculation of rabies suspected tissue suspensions in to a mouse brain, amplifies live virus present and manifest sign of rabies in the inoculated mice.



FAT: is Gold-standard[®] test

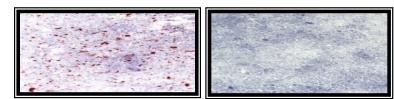
- Antigen detection
- Brain smear
- Brain tissue samples are aggregates of Hippocampus, medulla, cerebellum
- FAT positive looks apple green staining
- Fresh tissue should be examined, whenever possible
- FAT is more sensitive than direct microscopic examination(DME)
- FAT is more specific than DME
- 99% agreement between FAT and MIT
- Sensitivity 90 100%
- Sensitivity reduced in autolysed sample

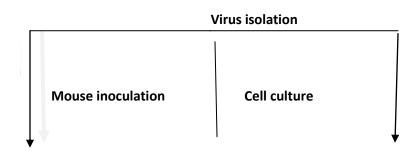
Rapid rabies diagnostic test kits for field use:

Direct Immunohistochemistry test (dRIT)

- Newly introduced Rapid Diagnostic Test at EHNRI
- Detects antigen
- Sensitivity and specificity equivalent to the DFA / FAT (100%)
- Frozen and glycerol-preserved brain samples
- No specialized equipment or infrastructure required
- Ideal for use in developing countries, especially under field conditions

DRIT Positive (source CDC) DIRIT Negative (Source CDC)







Animal rabies diagnosis challenges to scale-up FAT

- FAT requires a fluorescent Microscope/expensive
- No logistical support (sustainable laboratory supply)
- Lack of infrastructure

Attempts made so far in animal Rabies Diagnosis

So far attempts were made to strengthen rabies diagnosis capacity in the following ways:

- In developing rabies Laboratory working manual (for dRIT and FAT)
- Training of staff on dRIT at EHNRI level
- Preliminary evaluation of dRIT is done
- Veterinary and medical laboratory technicians from Addis Ababa were trained on dRIT
- The following Veterinary and Public health laboratories are assessed and selected for scaling-up of rapid rabies diagnosis tests for the purpose of field use:
- Tigray Regional State:
- Mekele Regional Public health and Veterinary laboratories
- Amhara Regional State:
- Bahirdar Regional Veterinary and Public health laboratories
- Kombolcha Veterinary laboratory
- Dessie Public health laboratory

• Oromiya Regional State:

- Asela Veterinary laboratory
- Adama Regional Public health laboratory
- SNNP Regional State:
- Wolaita sodo veterinary laboratory
- Hawassa Regional Public health laboratory

Human rabies diagnosis

Most human fatal cases were observed after the on-set of the clinical sign and in case of Ethiopia victims were seeking our medical care after exhaustive traditional healers and holy water treatment. By the time rabid human cases were arrived there is no facility to admit the patient the only thing we can do is observing the clinical manifestations like hydrophobia, aerophobia and many others and register in case fatality record book. After death occurred all exposed individuals with contaminated saliva from the rabid person are advised to take Post Exposure Prophylaxis (PEP).

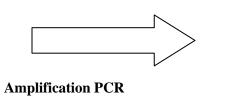
Laboratory confirmation of human rabies in South East African countries

- Experiences of South East African countries show intravitam (ante-mortem) diagnosis of human rabies through hospitalization and supportive treatment of human encephalitis with clinical cases of rabies
- Tests are performed on samples of saliva, serum, spinal fluid, and skin biopsies of hair follicles at the nape of the neck
- Saliva can be tested by virus isolation or reverse transcription followed by polymerase chain reaction (RT-PCR)
- Serum and spinal fluid are tested for antibodies to rabies virus
- Skin biopsy specimens are examined for rabies antigen in the coetaneous nerves at the base of hair follicles.

Molecular technique



PCR Process



NA Extraction

Detection/Sequencing

Figure 1. Rabies RT/PCR with subsequent nucleotide sequencing

The principle of RT/PCR with subsequent nucleotide sequencing

The reverse transcriptase polymerase chain reaction (RT-PCR) with subsequent nucleotide sequencing permits the diagnosis of rabies, typing, and molecular epidemiological studies. Since the rabies genome is RNA, the amplification procedure consists of the reverse transcription of the target RNA strain into complimentary DNA (cDNA), followed by the amplification of the cDNA by PCR (Tordo et al., 1995, 1996).

The RT-PCR procedure consists of the following steps: total RNA extraction, cDNA synthesis with random or specific primers, amplification of the cDNA with specific primers, and visualization of the results with horizontal electrophoresis in agarose gel containing ethidium bromide observed under UV light (Heaton et al., 1999).

A rapid RT/PCR technique was developed for the detection of the classical rabies virus (genotype 1) and the rabies related EBLVs (genotypes 5 and 6), and also to distinguish between the six established rabies and rabies-related virus genotypes (Black et al., 2000, 2002). The PCR can also be applied to detect the rabies virus genome in formalin-fixed paraffin-embedded brain tissue (Kulonen et al., 1999) and for the intravitam diagnosis of rabies in humans by testing saliva and cerebrospinal fluid (Crepin et al., 1998). The Real-time PCR is a quantitative technique which allows the detection of an increase in the amount of DNA (cDNA) during amplification. It is currently used for the ante- and post-mortem diagnosis of rabies and the discrimination of the Lyssavirus genotypes (Wakeley et al., 2005; Nagaraj et al., 2006; Saengseesom et al., 2007).

Human Rabies Diagnosis Challenges

Rabies in humans is fatal and presents a horrifying dinical picture. Human rabies can manifest in either Encephalitic (furious) or paralytic (dumb) forms. The brainstem is preferentially involved in both dinical forms, though there are no Clinical signs of Brainstem dysfunction. Differences in tropism at the inoculation site or the CNS, in the route of spread, or in the triggering of immune cascades in the brainstem may account for clinical variation (Perrin et al., 1986). Rabies still poses diagnostic problems, particularly the paralytic form, which closely resembles Guillain-Barré syndrome, or when a patient is comatose signs may be lacking and cardinal. Molecular methods allow reliable detection of rabies-virus RNA in biological fluids or tissue before death. The current human rabies diagnosis in Ethiopia is human clinical observation and Post Exposure prophylaxis (PEP) without laboratory confirmation which needs strong commitment of stake-holders with the principle of one health for better public health delivery and veterinary services.

In this regard, the future direction is set for hospitalization of humans' encephalitis with clinical cases of rabies and supportive treatment in isolated space till death occurs across all health facilities in the country. Training of Trainees (TOT) for public health professional on PEP algorithm decision and rabies case management is required with training manuals. The issue of traditional medicine should be investigated scientifically and if disproved awareness creation work must be designed among traditional healers in particular and the community at large.

The Way Forward in Animal Rabies Diagnosis

- Continue with scaling up of rapid rabies diagnosis tests (dRIT and others)
- o Strengthening of Resource mobilization for the scaling up of rabies diagnosis
- TOT on rabies diagnostic techniques and technologies
- PEP algorithm across the country
- Work towards the establishment of PCR-based laboratory confirmation of animal rabies diagnosis at EHNRI
- Laboratory based animal rabies surveillance using rapid diagnostic tests at region level

The Way Forward in human Rabies Diagnosis

- Work towards the establishment of PCR-based laboratory confirmation of human rabies diagnosis in public health system
- Piloting intravitam diagnosis of human rabies diagnosis through
- Hospitalization, and supportive treatment
- o Determination of rabies virus-specific antibodies from serum & CSF
- Rapid fluorescent focus inhibition test (RFFIT)
- Fluorescent Antibody Virus Neutralisation test (FAVN)

Conclusion

- The intravitam diagnosis of human rabies should start somewhere and being implemented across all health facilities in the country.
- Memorandum of understanding between EHNRI and regional veterinary and public health laboratories should be based on:
- Laboratory based animal and human rabies surveillance.
- Establishment of reporting system.
- Rabies suspected specimen shipment for further investigation and laboratory referral linkage between EHNRI National Rabies Reference Laboratory and Regional Public health and Veterinary laboratories.

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4.9 Rabies Legislation; best practice from Addis Ababa City Administration

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Introduction

Rabies is primarily a disease of dogs in Ethiopia. Since August 1903, Rabies is an important disease and a public health problem in Addis Ababa. Currently, many people receive Post exposure treatment in the city every year due to very high man – dog contact. The retrospective information recorded here at Ethiopian Health and Nutrition Research Institute (EHNRI) during the period 1990-2000 indicates canine rabies in Addis Ababa is well established with no decline in the annual number of confirmed rabid cases.

Rabies in Addis Ababa

Dog's population in Addis Ababa is estimated 230,000 - 300,000. Out of which 30% are owned and 70% are ownerless (stray dogs). Rabies in Addis Ababa is primarily a disease of domestic animals, particularly dogs. According to EHNRI reports, Dogs are responsible in maintaining & dissemination of rabies, in the city.

Year E.C	Dogs vaccinated	killed	Spay & neuter
1995/1996		19945	-
1996/1997		6985	-
1997/1998	5716	8214	-
1998/1999	8982	15963	-
1999/2000	4567	11488	-
2000/2001	4468	6684	-
2001/2002	6809	9544	736
2002/2003	4048	13807	-
2003/2004	8194	14692	-

Table 1. Activities done from 1995 – 2004 E.C Sou

Source: Addis Ababa urban Agriculture

Gaps and challenges on rabies

- There is no legislation for registering, licensing and vaccinating dogs,
- No dog population data,
- Weak intersectoral coordination,
- No control strategy,
- Inadequate diagnostic facilities (only 1 laboratory),
- Lack of attention & resource allocation
- Neglected disease,
- Lack of public education,
- Poor dog management(breeding, feeding & waste)

Best practices and intervention methods

1. ABC (animal Birth Control) in 2001/2002 E.C

- Spaying & neutering (it was sponsored by 3 NGOs (Best Friends Animal Society, human society international and Amsale Gusesse Memorial Foundation)
- **1200** planed & **736** achieved, within 9 months.
- Training for **37** Veterinarians and paraprofessionals,
- Support surgical equipments & medicaments,
- Got one media coverage,

Gaps:

- It was started as a pilot program,
- High cost for the service plus service charge,
- No continual/regular vaccination of dogs,
- Unacceptability by the public and officials,

2. Addis Ababa city administration rabies prevention and control regulation No...... /..... (It is on process).

The way forward:

- Rules & regulations should be in place for regular vaccination, registration etc,
- Dog census should be done,
- Strengthen the intersectoral collaboration,
- Establish a sustainable rabies control program or strategy,
- Strengthening resource mobilization & give priority,
- Public education and awareness
- Produce and distribute rabies vaccine for local use
- Control rabies in urban by:
 - 1. Rabies monitoring;
 - 2. Mass vaccination;
 - 3. Control of the population density. (ABC, Destroy ownerless dogs...)

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4.10 Workshop schedule Day One Topics Responsible										
Oct.18,2012										
8:30am-9:00am	Registration	Organizers								
9:00am-9:05am	Master ceremony	Ato Abel Yeshaneh, PR, EHNRI								
9:05am-9:10am	Well Come Address	Dr. Almaz Abebe, INDRD, EHNRI								
9:10am-9:20am	Opening Remark	Dr. Amha Kebede, Director General, EHNRI								
9:20am-9:30:am	Workshop Overview	Dr. Almaz Abebe, INDRD, EHNRI								
9:30am-10:00am	Tea Break	Organizers								
	Chairpersons: Dr. Shiferaw Jenberie ,NVI & Ato Kelbessa Urga, Director, VDPD, EHNRI									
10:00am-10:20am	An overview of Rabies	Dr. Eshetu Yimer, EVA								
10:20am-10:40am	Human Rabies Surveillance in Ethiopia	Ato Haftom Taame, Member PHEM, EHNRI								
10:40am-11:00am	Animal rabies in Ethiopia	Dr. Melaku Assefa, FMOA								
11:00am-11:20am	Anti-Rabies Vaccine production in Ethiopia	Ato. Abebe Mengesha, VDPD, EHNRI								
11;20am-12:30am	Discussion									
12:30am-2:00pm	Lunch break	Organizers								
	Chair persons:Dr. Eshetu Lemma, EHNRI & Dr. Kifle Woldemichael, Jimma University									
2:00pm-2:30pm	National Rabies Baseline survey Preliminary Report: Household assessment	Dr. Abraham Ali,INDRD,EHNRI								
2:30pm-3:00pm	National Rabies Baseline survey Preliminary Report : Facility Based assessment	Dr. Hailu Mamo, INDRD,EHNRI								
3:00pm-3:30pm	Tea break	Organizers								
3:30pm-4:00pm	Discussion									
4:00pm-4:20pm	Rabies Public education	Dr. Fasil Mengistu, INDRD,EHNRI								
4:20pm-5:00pm	Material inauguration	Ato Fekadu Beshah,PR,TU/EHNRI								

Day Two	Topics	Responsible
Oct.19,2012		
8:30am-9:00am	Registration	Organizers
	Chair persons: Ato. Gonfa Ayana.A/Director RLCBD,EHNRI., Dr. Eshetu Yimer,EVA	
9:00am-9:20am	Current Rabies diagnosis in Ethiopia and future prospective	Dr. Asefa Deresa, INDRD, EHNRI
9:20am-9:40am	Rabies legislation: Best Practice from Addis Ababa	Dr. Zerihun Abegaz, AACUAB
9:40am-10:10am	Tea break	Organizers
10:10am-12:30am	Discussion	
12:30am-2:00pm	Lunch break	Organizers
	Chair persons: Dr.Amha Kebede, General Director, EHNRI & Proffessor Getachew	
	Abebe,Livestock Manager,FAO- Ethiopia	
2:00pm-2:10pm	Major Challenges and gaps in rabies prevention and control in Ethiopia	Dr. Almaz Abebe,INDRD,Director,EHNRI
2:10pm-3:10pm	Panel Discussion	Dr. Dadi Jimma.,D/Director,EHNRI, Dr. Birhanu Admasu,
		EVA ,& Dr. Melaku Asefa, FMOA-APHID, Dr. Kifle
		Woldemichael, Jimma University
3:10pm-3:30pm	Tea break	Organizers
3:30pm-4:30pm	General Discussion	Dr. Dadi Jimma.,D/Director,EHNRI, Dr. Birhanu Admasu,
		EVA ,& Dr. Melaku Asefa, FMOA-APHID, Dr. Kifle
		Woldemichael, Jimma University
4:30pm-4:40pm	The Way Forward	Dr.Abraham Ali/Dr.Hailu Mamo
4:40pm-4:50pm	Closing Remark	Dr.Amha Kebede, General Director, EHNRI,
6:00pm	Reception	Organizers

Question	Strongly Agree Agree				Neutral		Disagree		Strongly Disagree		Total
	#	%	#	%	#	%	#	%	#	%	
The workshop met its stated aims and objectives	30	83.3	6	16.7		0		0		0	36
The workshop was scheduled at a suitable time	19	52.8	10	27.8	3	8.3	2	5.6	2	5.6	36
The workshop location was appropriate and satisfactory	25	69.4	8	22.2	1	2.8	2	5.6		0	36
The arrangements were appropriate and satisfactory	27	75.0	5	13.9	2	5.6	2	5.6		0	36
The workshop material was presented in a clear and organized manner	18	50.0	12	33.3	4	11.1	1	2.8	1	2.8	36
The workshop was paced appropriately	29	80.6	4	11.1		0	2	5.6	1	2.8	36
The presenters were well prepared	28	77.8	6	16.7		0	1	2.8	1	2.8	36
The presenters responded to questions in an informative, appropriate and satisfactory manner	30	83.3	4	11.1		0	1	2.8	1	2.8	36
The time allocated to presentations was appropriate and satisfactory	21	58.3	12	33.3		0	1	2.8	1	2.8	36
Over all, the sessions were informative and valuable	22	61.1	11	30.6		0	2	5.6	1	2.8	36
What Aspect of the conference if any would you change in future? Why		sharing before the workshop			Time allocated for Change the workshop discussion-make it longer		rkshop site to regions				
Would you recommend this or similar conference to a colleague?	Yes=36										
Which session elements of the conference did you find most useful? Why?	Survey finding=25	Global Burden=17	National burden=17	Vaccine production=20	Lab. Diagnosis=1 7	Education material=1 6	Legal (AA experience)=16	Discussion= 21	Mgt participat ion=1	No response= 4	
Do you have any suggestions for future workshops, conference/events that you would like us to organize?	Workshop for higher officials		Final survey res	ult	Prepare work other zoonoti		Hard copies of p	resentations	Involve oth stakeholde	-	
What new skills have you learnt from the conference that you think you will be able to put in to practice?	One health app	oroach									
Other Comments	The work shop successful	was very	Keep up the co	laborative work							

4.11 Workshop Evaluation Report

As it is indicated in the above table, from all participants only 36 were willing to participate and fill the questionnaire. More than 80 %(30) agreed that the workshop had achieved its objectives and believed that the presenters responded to questions raised in an informative, appropriate and satisfactory manner. About 29(80.6%) of the respondents accepted that the workshop was paced appropriately and 28(77.8%) said the presenters were well prepared. However only 19(52.8%) of the respondents agreed that the workshop materials were presented in a clear and organized manner and 18(50%) said the workshop was conducted at an appropriate time.

All of the 36 respondents commented that similar conferences should be delivered to others working in the area and advised EHNRI to strengthened collaborative works.

Twenty five of the respondents said the survey result presentations (community and facility based) were more useful than of all other presentations. This is because the work was the first of its type in Ethiopian and probably in Africa. Some of the respondents appreciated the full participation of the EHNR management till the end.

The respondants recommended on issues that should be improved in the future. Some of the things are sharing of documents before the workshop, including regional experiences in the programme, allocating adequate time for discussion and change the venue of the workshop to other places.

In general all the participants agreed that the workshop was very successful and met its objectives. Collaborative works should be encouraged to achieve the one health goal.

4.11 Workshop Working Document for the National Rabies Prevention and Control in Ethiopia

Workshop Rationale

Rabies is major public-health problem in most parts of the developing world where dogs play principal role as reservoir and transmitter of disease to humans. Dog bites and scratches are poorly understood and represent the most important public health issue related to dogs and cats because of the risk of rabies transmission as these attacks result in millions of injuries and thousands of deaths all over the world.

In Ethiopia, rabies is an important disease which has been known for many centuries. In Addis Ababa where dogs are the commonest domestic animal, the total dog population and the number of stray dogs are estimated to be 250,000 and 120,000 respectively. Dogs are the primary animal species implicated in bite injuries ranging from 63-80%. Averages of 1500 humans are bitten by dogs each year and 20,000 to 30,000 doses of anti-rabies vaccine are used to prevent death in human. In relation to this, a number of strategies are suggested for canine rabies control that includes placing rules and regulations which enforces dog ownership and vaccination, controlling dog movement and reducing stray dog population. However, those efforts don't seem to satisfy all the urgent issues of rabies to its satisfactory level.

One of the reason the disease situations is getting very worse from time to time is that, it is only the Ethiopian Health and Nutrition Research Institute, EHNRI which is the only institutes in the country which is known in rabies diagnosis, human exposure counseling and anti-rabies vaccine production for animals and human.

Therefore, it needs to receive serious attention and much of cooperation and collaboration between GOVs, NGOs, Research institutes, universities, professional associations, national and international donors, and individuals in order to maximize the effort towards the disease prevention and control.

As part of this effort, the EHNRI has taken the initiative to transfer the existing knowledge and resources for the establishment of rabies diagnosis of animals and human beings to the regional level in collaboration with its partners. However, as zoonotic diseases have animal-human interface, this effort alone cannot bring the required results. It is mainly because of less collaboration among relevant stakeholders in prevention and control of rabies and lack of multi-sectoral collaboration and divisions of tasks.

For that reason, the Institute planned to conduct the workshop on the agendas of rabies prevention and control in Ethiopia which is aimed at bringing together all concerned stakeholders to address the critical issues of the matter and coordinate the prevention and control effort.

In order to maximize the impact, the workshop is also accompanied by the commemoration of the world Rabies Day and the dissemination of Preliminary Report National Rabies Baseline survey

Objectives of the workshop

The workshop has the following objectives:

- To establish the network among concerned stakeholders and create holistic approach for rabies prevention and control in Ethiopia
- To promote the contributions of the EHNRI towards rabies prevention and control
- To disseminate the Preliminary national baseline survey major findings
- To sensitize regional laboratories on establishment of laboratory rabies diagnosis service at regional level
- To commemorate the world Rabies Day and to create public awareness on rabies
- To officially launch the publishing of the rabies educational material

Task Implementation Plan

In order to conduct the workshop in a planned manner, the implementation plan has been categorized in the following three steps.

Pre-workshop tasks

No.	Type of Activity	Responsible body	Current States	Remark
1	Identify issues and set workshop objectives	Zoontic diseases Research Team (Dr. Hailu Mamo)	✓	
2	Determine number and title of presentations	Zoontic diseases Research Team (Dr. Hailu Mamo)	✓	
3	Identify type and numbers of Participation	Zoontic diseases Research Team (Dr. Hailu Mamo)	~	Suggested participants are indicated in annex
4	Determine date and Venue	Zoontic diseases Research Team (Dr. Hailu Mamo)	~	Adama, October 18-19 , 2012
5	Write and disseminate letter of invitation	Ato Fekadu Beshah		, 2012
6	Form the workshop organizing Committee	Dr. Hailu Mamo	×	List of members of the organizing committee is indicated in annex
7	Prepare draft budget	Dr. Abraham Ali	✓	
8	Determine type of materials, stationeries and material holders to be disseminated to participants of the workshop	The organizing committee (Ato Dereje Nigussie, Ato Kassahun Amenu and Ato Fekadu Beshah	✓	
9	Prepare workshop schedule	Dr. Hailu Mamo	~	Draft workshop schedule is indicated in annex
10	Prepare workshop evaluation form	Ato Fekadu Beshah	~	Draft workshop evaluation form is indicated in annex
11	Select presentation topics and assign presenters and panelists	Dr. Abraham Ali/ Dr. Hailu Mamo	~	
12	 Design workshop promotion Communication Approach and attract media organizations with creative writing to make them cover the event Design and post banners at the compound of the Institute and the event Write and publish news story on the website of the institute Write speeches to be delivered by Officials Prepare press release Select and Identify promotional materials that are going to be 	PR Office/ Ato Fekadu Beshah/Ato Abel Yeshaneh	✓	

During Workshop Tasks

No.	Type of Activity	Responsible body	Current States	Remark
1	Opening session		\checkmark	
	 Registration and material dissemination Assign Master of the ceremony Opening Speeches and keynote addresses Make sure the presence of the selected Rapporteurs 	Ato Alemayehu Godana Ato Fekadu Beshah/ Ato Abel Yeshaneh Dr. Amha Kebede		
	 Ensure the availability of refreshments and lunch service 	Ato Kassahu Amenu/Ato Alemayehu Godana		
2	 Communication Ensure the presence of the representatives of media organizations Disseminate Speeches and press releases to journalists Provide briefing and suggest story ideas to journalists Identify and assign authorized individuals for the media interview Take photos and video record 	PR Office Ato Fekadu Beshah /Ato Abel Yeshaneh/ Wzo.Tsehaynesh	✓	
3	Closing Session • Facilitate formal closing session • Disseminate and collect workshop evaluation form	A person delegated from MoA Ato Haftom Taame & Ato Dereje Nigissie	✓ 	

After the Workshop Tasks

No.	Type of Activity	Responsible body	Current States	Remark
1.	Prepare and submit the workshop proceeding	Dr. Hailu Mamo, Dr. Abraham Ali, Ato Fekadu Beshah, Ato Haftom Taame, and Ato Dereje Nigussie	~	
2	Communication • Monitor the media coverage and report to the institution community • Write updated news stories to be published in the website and by 15 days news letter	PR Office/ Ato Fekadu Beshah/Wzo.Tsehaynesh		

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List of Workshop Participants

Depending on the objectives of the workshop the following participant organizations are selected:

- I. Stakeholders
 - Federal MOA-APHID-2
 - NAHDIC-1
 - MOH-1
 - National Veterinary Institute-1
 - Ethiopian wildlife Development and conservation authority-2
 - Regional Health bureau- 11
 - Regional Agricultural bureau-11
 - Regional Public Health Laboratory (Amhara-1, Oromiya-1, Tigray-1, SNNPR-1)
 - Regional Veterinary Diagnostic Laboratory (Amhara-1, Oromiya-1, Tigray-1, SNNPR-1)
 - FDRE House of Representative Social Affairs Committee-2
 - MoE-1
- II. Partners
 - Professional associations (EVA-4 , EPHLPA-1, EPHA-1)
 - WHO-1
 - FAO-1
 - CDC-1
- III. Staff/Host
 - EHNRI Management group-17
 - EHNRI Staff-26
- IV. Media Organizations
 - Representatives of Electronic and Print Media- 10

List of members of the Workshop organizing committee, EHNRI

Dr. Hailu Mamo -----Chairman, INDRD
 Dr. Abraham Ali ------Chairman, INDRD
 Dereje Nigussie-----Member, INDRD
 Dereje Nigussie-----Member, VDPD
 Alemayehu Godana-----Member, VDPD
 Haftom Taame------Member, PHEM
 Kassahun Amenu------Member, SERO
 Abel Yeshaneh ------Member, PR
 Fekadu Beshah------Secretary/Member, PR/TUTAPE

Workshop Evaluation Form



The Ethiopian Health and Nutrition Research Institute Rabies Prevention and control Workshop Evaluation Form

EVALUATION

Thank you for attending the Rabies Prevention and control workshop that is intended to establish the network among concerned stakeholders and create holistic approach for rabies prevention and control in Ethiopia, commemorate the world Rabies Day and disseminate the Preliminary Report National Rabies Baseline survey. We hope that you found the conference interesting and that we met your expectations. We would be very grateful if you could take a moment to complete the workshop evaluation form. Your comments will assist us in improving future conference that we will organize.

Please complete the form and return to the workshop facilitators.

CONFERENCE CONTENT AND ORGANIZATION												
SCALE: 1- STRONGLY AGR	EE;	2 — AGI	REE;	3 – NI	EUTRAL;	4 – DISAGREE;	5 – STRONGLY DISAGREE					
DI		vrovid	o foor	hack	in the	comments box						
						comments box						
	1	2	3	4	5		Comments					
1. The workshop met its stated aims and objectives.												
2. The workshop was scheduled at a suitable time.												
3. The workshop location was appropriate and satisfactory.												
4. The workshop facilities were appropriate and satisfactory.												
5. The sit arrangements were appropriate and satisfactory.												
The workshop material was presented in a clear and organized manner.												
7. The workshop was paced appropriately.												
8. The presenters were well prepared.												
 The presenters responded to questions in an informative, appropriate and satisfactory manner. 												

10. The time allocated to presentations was appropriate and satisfactory.								
11. Overall, the sessions were informative and valuable.								
8. What aspects of the conference, if any, would you change in future? Why?								
10. Which session/elements of the conference did you find most useful? Why?								
9. Would you recommend this or a similar conference to a colleague?	Yes		No					
10. Do you have any suggestions for future workshop, conference/events that you would like us to organize?								
11. What new skills have you learnt from the conference that you think you will be able to put into practice?								
11. Other comments								
Thank you for completing the questionnaire								

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4.12 Certificates, awarded for Members of the workshop organizing Committee, presenters and panelists



4.13 List and addresses of Workshop Participants

		DECION			TEI	58441
S.N.	FULL NAME	REGION	ORGANIZATION	POSITION/DUTY	TEL.	EMAIL
1.	Oumer Shefi	Addis Ababa	FMOH	Officer	0912048181	umerjoji@gmail.com
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						m
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62	Abel Yeshaneh	Addis Ababa	EHNRI			

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66	Berhanu Admassu	Addis Ababa	EVA	President	0911243302	
67	Getachew Abebe	Addis Ababa	FAO	Livestock prog.mag	0911407260	
68	Hussien Mohammed	Somali	RHB	RL owner	0911547199	
69	Dessalengn Sifer	Addis Ababa	EHNRI	Researcher		
70	Afework Kabtehyimer	Addis Ababa	EHNRI	Technician		
71	Fekadu Beshah	Addis Ababa	EHNRI	Advisor	0911646866	
72	Nurya Edris	Addis Ababa	FMoH	Officer	0911164615	nedrisd@gmail.com
73	Mesfein Aklilu	Addis Ababa	EHNRI	Technician	0911185307	mesfinaklilu@yahoo.com

4.14 Media Coverage

In order to promote the Workshop's events and the Institute's effort towards Rabies prevention and control the PR Office of the Institute has developed communications strategy that insures maximum coverage of the workshop.

Depending on that, the Ethiopian Television and Radio Agency and Ethiopian News Agency have been invited to the event. Following the assignment of reporters, the Ethiopian Television has covered the workshop on its lunch time and evening news broadcast. Similarly, the Ethiopian News Agency has also given coverage to the workshop on its website.

The workshop has also received coverage on the Institute's media. The Institute's website, <u>www.ehnri.gov.et</u> has given a coverage that focuses on the objective of the workshop. At the same time, the workshop is also covered by the Institute's by 15 newsletter, Zena EHNRI

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The Ethiopian Health and Nutrition Research Institute

