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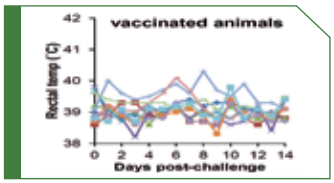

4 Overview of FAO's coordination of peste des petits ruminants control activities

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WWW.FAO.ORG/AG/EMPRES.HTML

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GUEST EDITORIAL

Towards the eradication of peste des petits ruminants / sheep and goat plague

Maria Helena Semedo
Deputy Director-General, FAO

At the International Conference from 31 March to 2 April 2015, the Food and Agriculture Organization of the United Nations (FAO) and the World Organisation for Animal Health (OIE) launched the global campaign to eradicate a devastating livestock disease: peste des petits ruminants. FAO and OIE will rally the international community against this common threat, the eradication of which will vastly improve the lives of hundreds of millions of people. For this reason, I consider it a great honour to be guest editor of this special issue of *EMPRES Animal Health 360*, which affords me the opportunity to describe the momentous PPR initiative shortly after its launch.

Having lived and worked in Ghana and the Niger I know how important small ruminants are for the livelihoods of smallholders, particularly for women. Goats and sheep are a kind of "insurance" in times of crises and disasters. But they are also key in providing milk and meat, both important products to ensure better nutrition of families and especially their children and expectant mothers. Goats and sheep also contribute to local and global growth and development, especially agribusinesses, job creation and, to a certain extent, political stability.

Thus, with some 330 million people depending on small ruminants for food security, nutrition and income, PPR effectively destroys lives and entrenches the most vulnerable of our communities deeper into poverty. The disease reduces sheep and goat numbers dramatically – it is highly virulent and kills upwards of 90 percent of a herd once introduced. Plus, PPR is present in 76 countries and threatens to keep spreading.

PPR is also a major impediment to overall development. Demand for sheep meat is on the rise, with global consumption projected to increase nearly 30 percent by 2030. Not only do small ruminants provide meat for domestic consumption and milk that reduces child malnutrition, but these animals also represent a valuable safety net for families in crisis. Sheep and goats can be sold for cash to purchase staple foods and other commodities when required.

Moreover, access to and control over small ruminants affords many rural women a crucial opportunity to develop small businesses through the sale of milk, meat and hides. These income sources enable women and marginalized groups to feed their families and improve their quality of life more effectively.

Fortunately, we've never been more prepared to meet the challenge of PPR. In 2011, FAO and OIE marked the end of rinderpest, a cattle disease similar to PPR. The two organizations led the international community in this first-ever global eradication of a livestock disease. FAO and OIE stand ready now to apply to PPR the lessons learned from the rinderpest success.

Thanks to rinderpest and decades of experience in animal disease prevention and control, FAO and OIE have the knowledge and tools necessary to beat PPR. We know PPR's epidemiology and how it spreads. We have access to the necessary tools, including vaccines and disease control strategies. What we lack is political will.

The successful implementation of the PPR control and eradication programme requires not only political will, but also enhanced partnerships with researchers, the private sector and farmers' organizations.

FAO and OIE are counting on the international community's full commitment to and economic ownership of this initiative. We will need to work together, across borders and with shared resources to eradicate this disease for ourselves and our children to come.

Like rinderpest before it, eradicating PPR fits squarely into FAO's Strategic Objectives for achieving a world without hunger by: i) establishing an enabling environment for poverty alleviation and food security; ii) providing producers with policy support services in overcoming barriers to sustainable animal production, economic growth and more efficient use of natural resources; iii) raising awareness and improving rural employment for reducing poverty; iv) meeting international standards in animal health and food safety, thus promoting trade in live animals and their products; and v) building the resilience of agricultural communities.

With adequate political, financial and in-kind support, PPR can be eradicated by 2030. Ending PPR will mean better food security, nutrition and livelihoods for vulnerable farming families the world over, and eradicating the disease will represent major progress on the post-2015 Sustainable Development Goals and the Zero Hunger Challenge.

Together, we can achieve this important feat. It is my sincere hope that we all call on our governments to support FAO, OIE and partners in the eradication of PPR.

For a world without hunger.

Helena Semedo

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A herder taking a herd of sheep to a cistern for a drink of water, West Bank

©FAO/Kai Wiedenhoefer

SURVEILLANCE

Overview of FAO's coordination of peste des petits ruminants control activities

Contributors: Felix Njeumi, Bouna Diop, Khadak Singh Bisht, Ibrahim Wora Salami, Mohammed Bengoumi, Nacif Rihani, Markos Tibbo and Abdul Baqi Mehraban[1]

In most parts of Africa, the Middle East and Asia, sheep and goats are an essential part of the economy of the rural poor, providing significant contributions to quality nutritious food via milk and meat. Furthermore, they provide additional income from skins and wool throughout the year. For farmers with crops, small ruminants (total global population of 2.1 billion animals) provide insurance against crop failure and drought. Several infectious diseases of high impact affect small ruminants in these regions and can seriously influence the livelihoods, nutrition quality and resilience of these communities. The occurrence of peste des petits ruminants (PPR) – with up to 90 percent mortality rates – can be most devastating. The global eradication of rinderpest in June 2011 – a disease of cattle similar to PPR – was only the second time in history that the world was able completely to free itself of an infectious disease, the first disease being smallpox.

The 37th FAO Conference held in June 2011 *"Encouraged FAO to take full advantage of the rinderpest eradication achievement and apply the lessons learned to prevent and control other diseases impacting food security, public health, the sustainability of agriculture systems and rural development."* Since then, FAO, the World Organisation for Animal Health (OIE), the International Atomic Energy Agency (IAEA) and other partners have been working closely to develop the Peste des Petits Ruminants (PPR) Global Control and Eradication Strategy.

Consultations through both regional workshops and e-consultations with a broad range of stakeholders have resulted in very positive feedback and support to this initiative. This paper reviews activities undertaken by FAO since the 37th Conference.

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Village small herders, the Niger

The position paper on *FAO's approach for supporting livelihoods and building resilience through the progressive control of PPR and other small ruminant diseases* was issued in 2012 (FAO, 2012). Based on this paper, countries and regions were assisted in formulating national PPR strategic control plans and regional PPR roadmaps. FAO also assisted countries in developing better understanding of their epidemiological and socio-economic situations, vaccine delivery systems and knowledge gaps relevant to PPR control. Several countries already had a national strategy for PPR control, but these strategies needed to be reviewed and linked to regional strategies and the global one.

GLOBAL ACTIVITIES

In October 2012 the Global Framework for the Progressive Control of Transboundary Animal Diseases (GF-TADs) Global Steering Committee requested that the activities of the GF-TADs Working Group be extended to PPR, with the aim of developing a PPR global control and eradication strategy (in four stages) and organizing an international conference to launch a PPR control and eradication programme. Since 2013, the working group has been meeting once a month. Several activities have been implemented jointly by FAO and OIE at the global and regional levels, and consistent with its mandate FAO has coordinated activities at the national level

An e-conference on the establishment of a PPR Global Research and Expertise Network (PPR-GREN) was organized by FAO and OIE and hosted by FAO from 3 February to 15 March 2014. The e-conference, with 302 participants, collected inputs from scientific and policy decision-makers and interested stakeholder communities as a prelude to the launch of a structured PPR-GREN. The concept of including other important diseases of small ruminants in an overall programme of disease control with PPR was largely supported as a more cost-effective approach

[1] Food and Agriculture Organization of the United Nations

to improving small ruminant health and associated livelihoods. The following matters stimulated considerable concern/debate: i) the efficacy of vaccines against different isolates of PPR virus; ii) the value of sero-monitoring; iii) vaccine quality control and potential advancements in thermostability; iv) the ability to differentiate serologically animals that have been vaccinated from those that have been infected and recovered (DIVA); v) the inadequate global supply of PPR vaccine; vi) the value of vaccination in outbreak conditions; vi) the number of vaccine doses per vial; and vii) the possible role that rinderpest and its control may have played in suppressing PPR virus activity, especially in Africa. Some of these concerns have already been addressed and others will be addressed in the near future.

In March to June 2014, FAO conducted a survey with PPR experts (including national disease control officials with responsibility for PPR control, and specialists in international institutions or regional disease control centres) to identify perceived risk factors for PPR transmission, spread and maintenance. The survey was conducted via an online form, supplemented by forms sent as e-mail attachments or paper copies to participants located in areas without reliable Internet access. In the first section of the questionnaire, participants were asked to list and rank the most important risk factors for the transmission, spread and maintenance of PPR. Responses from 210 participants (67 percent of those contacted) were categorized and frequencies of risk factors calculated. Activities associated with movement such as husbandry, trade, and grazing and watering practices were considered to be primary risk factors for both the transmission and spread of PPR. The responses noted that inadequate application of control measures such as vaccination and quarantine reduces the effectiveness of disease control programmes, allowing the disease to be maintained in the population. This study provided a unique perspective from experts who are currently working in the field of PPR and generated an important baseline of practical knowledge, which can be combined with technical evidence in the literature and lessons learned from rinderpest eradication.

The five major risk factors identified for each of the three aspects of the disease are listed in Table 1.

To obtain the support of FAO's governing body, a policy paper was prepared and, in October 2014, the FAO 24th Committee on Agriculture (COAG):

a) endorsed the joint establishment and implementation by FAO and the World Organisation for Animal Health (OIE) of the Global Peste des Petits Ruminants (PPR) Control and Eradication Programme in line with the proposed governance structure, including acting as FAO-OIE Secretariat in collaboration with other international and regional partners, such as African Union (AU), South Asian Association for Regional Cooperation (SAARC) and the Association of Southeast Asian Nations (ASEAN), among others;

b) recommended that FAO members support the implementation of the PPR programme and noted the emphasis on the need for a broad range of partnerships at national, regional and international level;

Table 1: Major risk factors

	Transmission	Spread	Maintenance
Risk factor	Movement	Movement	Control
	Husbandry	Trade	Husbandry
	Trade	Control	Trade
	Grazing and watering	Husbandry	Movement
	Contact	Grazing and watering	Susceptible animals

"This study provided a unique perspective from experts who are currently working in the field of PPR and generated an important baseline of practical knowledge"

c) looked forward to receive updates on programme implementation progress at future COAG sessions.

The COAG meeting was followed by an experts' workshop in October 2014 to revise the first draft of the PPR global strategy. From earlier consultations with a broad range of stakeholders through e-conferences and regional/national workshops the need for a dedicated vaccination campaign had been identified. Shortages in vaccine production, low compliance with quality assurances and the inability to deliver vaccines to meet needs in the field (thermostability, size of vials, etc.) represent risks to the planned campaigns and would constitute a major challenge to progress in the time-bound 2030 PPR-GCEP.

VACCINE

To address these challenges, 46 participants from 21 vaccine-producing laboratories attended an FAO-sponsored workshop in Kathmandu, Nepal from 1 to 3 December 2014. This PPR vaccines workshop was a timely opportunity for discussing and addressing challenges regarding the use, quality and quantity of PPR vaccines to ensure the programme's success. The workshop was supported by international technical and research institutions including FAO, OIE and IAEA and served in clarifying a number of issues regarding vaccine production, including vaccine quality, quantity and distribution and related future research.

First, participants recommended that to safeguard the quality of vaccines, manufacturers must comply with international OIE standards. They also suggested establishing a certification process for thermostable PPR vaccines and using the outcomes of "strengths, weaknesses, opportunities and threats" (SWOT) analysis to improve vaccine quality. The Pan African Veterinary Vaccine Centre (PANVAC) should increase its capacity to meet demands for international quality control of PPR vaccines by becoming the focal point for the submission and certification of batches of PPR vaccines. PPR-GCEP must also investigate the possibility of establishing another quality control laboratory through the OIE laboratory twinning programme.

Second, vaccine manufacturers will need to supply increased amounts of PPR vaccine in the near future. To predict the right quantities, countries must evaluate their PPR vaccine requirements for the next ten years in the context of the overall objective of eradicating the disease. Vaccine manufacturers need to prepare for increased production capacity and also consider the possibility of providing packaging with smaller numbers of doses.

A third concern was regarding the heat sensitivity of current vaccines and the need for their timely delivery. While manufacturers should continue producing the current vaccine, they should also consider adopting new formulations to provide thermostable products. All vaccines should be supplied with the appropriate diluent. Prior to the vaccination campaign, each country should establish a quality cold chain system and prepare standard operating procedures for the monitoring of vaccination and post-

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Goats grazing in a rice field, Viet Nam

vaccination procedures. The workshop participants also observed that more consistent and effective vaccine use can be fostered through closer engagement between vaccine producers and users.

Finally, to distinguish vaccinated from naturally infected animals, it is necessary to develop an effective and widely applicable differentiating infected from vaccinated animals (DIVA) vaccine with companion serological tests. Participants also recommended identifying other research areas, particularly for the final eradication phase.

Participants appreciated FAO's initiative of bringing together different vaccine producers and recommended that a similar workshop be organized annually to monitor implementation of the workshop recommendations. Countries participating in the workshop included Bangladesh, Botswana, Chad, China, Egypt, India, the Islamic Republic of Iran, Jordan, Kenya, Mali, Morocco, Nepal, the Niger, Pakistan, the Russian Federation, Senegal and the Sudan. Participating technical agencies included OIE, the Global Alliance for Livestock Veterinary Medicines (GALVMed), the Indian Veterinary Research Institute (IVRI) Izatnagar-India, IAEA, the International Livestock Research Institute (ILRI), Tufts University, the *Instituto de Biologia Experimental Tecnologica* (IBET), MERIAL, PANVAC and the Pirbright Institute.

REGIONAL/NATIONAL ACTIVITIES

In addition to global activities, regional and national activities have also been undertaken.

China and the Association of Southeast Asian Nations (ASEAN): The first incursion of PPR into Tibet Autonomous Region, China occurred in 2007, but information was lacking until mid-2013. Since then, in a series of consecutive outbreaks that continued into the second half of 2014, 22 of China's 31 provinces were reported infected. As a result, animals were slaughtered and vaccination (300 million doses) was conducted in 27 provinces. These control measures have significantly reduced the number of outbreaks and since May 2014, only eight sporadic outbreaks have been recorded. ASEAN countries are not infected.

> "It is necessary to develop an effective and widely applicable differentiating infected from vaccinated animals (DIVA) vaccine with companion serological tests"

SAARC: A regional roadmap, to be reviewed every two years, was jointly endorsed by FAO and the SAARC Secretariat in 2011. All SAARC countries with the exception of Sri Lanka have reported infection, although the disease has been reported only once and twice respectively in the Maldives and Bhutan. Each SAARC country has a national laboratory suitable for PPR diagnosis, and the leading regional diagnostic laboratory is located in Bangladesh. Surveillance and vaccination campaigns are ongoing in identified high-risk areas. Bangladesh, India, Nepal and Pakistan are producing PPR vaccines, although there is an urgent need to improve the quality and quantity of these vaccines to meet national and regional needs. During the regional roadmap review meeting in December 2013 several challenges were identified, including socio-economic impact assessment across the small ruminant value chain; development of a strategic plan and assured budget for its implementation; enhancement of technical expertise and skills; raising awareness among farmers; formulation and implementation of regulations regarding animal movement; availability and delivery of quality-assured vaccine; and harmonization of diagnostic assays/tests.

Central Asia and neighbouring countries: Currently only two countries in the Economic Cooperation Organization (Islamic Republic of Iran and Turkey) are producing vaccines, although vaccination has been used in several Central Asian countries. FAO is currently implementing PPR projects in Afghanistan and Pakistan (also SAARC members) with the following outputs: i) enhanced capacity for laboratory diagnosis and vaccine production; ii) improved disease surveillance; and iii) effective control through vaccination campaigns adapted to the production system. In Kyrgyzstan, a FAO Technical Cooperation Programme (TCP) project has been completed in which infected, at-risk and clean areas for PPR infection were identified. The example of Kyrgyzstan had shown how the stage 1 global strategy assessment can delineate the target vaccination areas for stage 2. If activities are well implemented in stage 2 (vaccination in infected areas), combined with surveillance in at-risk and clean areas, this can assist the country in moving directly to stage 4. In Turkey, PPR surveillance and vaccination using nationally produced vaccines are being implemented to prevent disease incursion into Europe.

Middle East: Only Egypt, Jordan and Saudi Arabia are vaccine producers. Surveillance and vaccination are ongoing in all countries with national and FAO support (Iraq, Jordan, Lebanon and the Syrian Arab Republic). A recent FAO and OIE workshop (under the GF-TADs umbrella) agreed on the need to formulate a regional roadmap.

East Africa: PPR surveillance is part of the general surveillance system in place at the country level and involves mainly public veterinary services with the participation of livestock owners and, to some degree, private animal service providers. Some countries (Ethiopia, Kenya, the Sudan, Uganda and the United Republic of Tanzania) have full capacities for PPR diagnosis, and PPR prevention and control are generally based

on vaccination, mainly focused around the outbreak (ring-vaccination). In response to introduction of PPR in Kenya and Uganda in 2006, FAO (TCP/RAF/3113(E)), the United Nations Development Programme (UNDP) and *Vétérinaires sans Frontières* (VSF)-Belgium supported the emergency control of the epidemic. Major activities implemented in 2008/2009 included surveillance, vaccination, capacity building and socio-economic assessment of the impact of the disease. In addition, the Government of Kenya mobilized national resources to respond to the disease.

Following a PPR risk assessment mission conducted in the United Republic of Tanzania in September 2010 by the FAO Crisis Management Centre – Animal Health (CMC-AH), an FAO TCP project (TCP/URT/3302(E)) was implemented from July 2011 to August 2013. The project implemented PPR vaccination in high-risk areas; pre- and post-vaccination monitoring; a socio-economic study; strengthening of national laboratories' capacity; and clinical and serological surveillance in non-vaccinated areas.

FAO continues to support the Somali Government in adopting a PPR control strategy. This encompasses establishing a cold chain in accessible locations, promoting engagement of relevant stakeholders, increasing awareness of the disease, developing incentives for livestock owners, and mass vaccination. Prior to this country-wide intervention, PPR outbreaks were frequent, with a sero-prevalence of 61 percent. The first vaccination round in 2012 covered about 19.7 million small ruminants out of an estimated population of 36 million. The second round of vaccination was carried out in 2013. Post-vaccination sero-surveillance (38 000 sera) carried out after the second round of vaccination revealed a 76 percent country-wide sero-prevalence, which is recognized as high enough to reduce significantly the circulation of the virus and to control the disease. As the programme achieved this early success in Somalia, absence of similar efforts in neighbouring countries threatens these gains. Current efforts aim to vaccinate at least 12 million animals every year, concentrating on young, unvaccinated herds and key border points with high movement. Consistency and collaboration in these efforts will ensure reduction and possible eradication of PPR in the Horn of Africa region.

In East Africa, small ruminants and small ruminant diseases often do not receive the attention that they deserve from decision-makers. Gaining support for PPR control requires demonstrating the tangible benefits delivered by small ruminants to rural livelihoods and along the value chain, and the socio-economic impact of the disease. Knowledge on the epidemiology of PPR, including its transmissibility (R_0) in different population settings and host species, needs to be improved. Varying farming systems, the high number of animals involved, inadequate cold chains, high turnover of small ruminants, and mild forms of PPR in endemic situations are other challenges to effective control.

Where PPR has established itself as an endemic disease or where epizootic PPR outbreaks are rare, farmers' awareness of the disease may fade over time. Where communication and public awareness programmes are neglected, poor acceptance of PPR vaccination campaigns by farmers will result. Most small farmers operate according to a low-input, low-output production system, which in any case leaves little margin for cost recovery in vaccination campaigns. In East Africa, PPR affects the livelihoods of sheep and goat farmers, many (if not most) of whom are women living under the poverty line (US$2.0 per day) and relying heavily on their stock for the provision of meat, milk, hides, wool, cash and other ecosystem services.

Status of the tools used in East Africa: Control strategies have been developed in some countries, requiring details of surveillance, vaccine coverage and strategies and other measures. However, because of low investments in PPR control by governments and development partners, the extent to which these strategies can be applied and their effectiveness are not clear. The current PPR vaccine based on the Nigeria 75/1 strain has been found to be safe and effective. However, improvements in vaccine thermostability would be advantageous. PPR vaccines used within the region are produced in Ethiopia, Kenya and the Sudan, but low production in the region means that additional vaccine is imported, mainly from the Middle East, particularly Jordan. To maximize farmers' interest, PPR vaccination programmes should be combined as much as possible with vaccinations against other national priority small ruminant diseases, such as contagious caprine pleuropneumonia (CCPP).

Central Africa: FAO has implemented PPR TCP projects in Cameroon, the Congo and the Democratic Republic of the Congo. As well as activities described below, laboratory equipment and suitable transportation for field activities were supplied. In Cameroon,

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Girls herding goats to the Juba River, Somalia

a national strategic plan was formulated, activities were conducted to improve vaccine production technology and capacity building on disease recognition and disease control was carried out.

Vaccination campaigns were undertaken in the three countries in targeted, at-risk areas. Nevertheless, post-vaccination monitoring demonstrated low coverage relative to national populations. Cross-border workshops were organized for surveillance coordination and knowledge sharing. FAO assistance was scaled up, with the International Fund for Agricultural Development (IFAD), the World Bank and other donors supporting the vaccination campaign and surveillance, specifically in the Democratic Republic of the Congo. Major problems encountered during project implementation included an inadequate vaccine cold chain and the existence of small-scale production systems with flock sizes of about ten head. This requires the use of small-size vaccine vials (of 50 instead of 100 doses), which increases vaccination costs.

Many problems were encountered in implementation: organizational problems; problems associated with constantly straying animals; and cold chain monitoring issues. Other constraints recognized were vaccination teams ignoring some localities because cold chain tools did not exist; a lack of appropriate resources to enable vaccinators to travel over the great distances involved; the rainy season, which was a great handicap for vaccinators because farmers were not available to assist; and poor roads.

Status of the tools used in Central Africa: The capability of national networks to carry out epidemiological surveillance of animal diseases has been strengthened; communication capacity has been improved; a diagnostic unit (*Laboratoire de Diagnostic Vétérinaire de Brazzaville*) has been established; sero-monitoring has been put in place; and zoo-sanitary measures, including the control of livestock movements, have been strengthened. To sustain the gains of the project, since 2011, the Congo has organized annual vaccination campaigns against PPR.

North Africa: A FAO regional TCP project aiming to assist Maghreb countries (Algeria, Libya, Mauritania, Morocco and Tunisia) in the prevention and control of PPR was implemented (2012–2013). The outputs included enhanced capacity for laboratory diagnosis and vaccine production; improved disease surveillance; better understanding of the socio-economic impact of PPR; and coordination/harmonization of PPR prevention and control in the Mediterranean Animal Health Network (*Reseau Mediterranéen de Santé Animale* – REMESA). Several training sessions (epidemiology, laboratory and socio-economics) were carried out. A regional PPR sero-survey was conducted and, although Morocco has been the only country in the region that has conducted vaccination campaigns, antibodies were not detected in unvaccinated animals in Morocco. In other countries (except Libya where field activities cannot be implemented) sero-prevalence varied from 37 to 62 percent. A socio-economic study in Mauritania and Tunisia has shown considerable impact of the disease. In December 2014, OIE conducted a workshop on the formulation of PPR control plans for all Maghreb countries.

West Africa: Currently, Mali, the Niger, Nigeria and Senegal are PPR vaccine producers. In several countries in the region, national governments, FAO and IAEA are supporting vaccination and surveillance activities that will soon be handed over to the Economic Community of West African States (ECOWAS).

> “The eradication of PPR is a public good and within the world’s reach, but it requires political will and financial support”

Southern African Development Community (SADC): Following an outbreak in a few countries, a regional PPR control strategy was developed. The main objectives were to: i) contain/control immediately the PPR virus circulating in Angola, the Democratic Republic of the Congo and the United Republic of Tanzania; ii) prevent the disease from spreading to Malawi, Mozambique and Zambia; and iii) propose a methodology for the long-term eradication of PPR from the SADC region (Angola, Botswana, the Democratic Republic of the Congo, Lesotho, Madagascar, Malawi, Mauritius, Mozambique, Namibia, Seychelles, South Africa, Swaziland, the United Republic of Tanzania, Zambia and Zimbabwe). Currently, only Botswana produces PPR vaccines. South Africa was declared PPR-free by OIE in May 2014. The current FAO regional TCP project aims to address the following outputs: enhanced capacity for laboratory diagnosis and vaccine production; improved disease surveillance; assessment of the socio-economic impact of PPR; and coordination/harmonization of PPR prevention and control in the region.

CONCLUSION

All these experiences have assisted in refining the global strategy. As with rinderpest, the eradication of PPR is a public good and within the world’s reach, but it requires the political will and financial support of countries, regional organizations and international resource partners; strategic partnerships with both the public and private sectors; and sustained commitment.

PPR-GCEP: The proposed PPR-GCEP will be guided by a PPR and Small Ruminant Health Advisory Committee (AC) assisted by the PPR-GCEP Secretariat to be hosted in FAO (Global Joint FAO/OIE Secretariat). The AC will provide strategic guidance and oversight on implementation of the programme. It will also play an important advocacy role with policy-makers, donors, national veterinary services and livestock owners. The main role of the Global Secretariat is to provide overall coordinated strategic directions and develop cost-effective control methodologies, tools, guidelines and training materials and networks to support implementation of the programme at the regional and country levels. Work at the regional level will be led by FAO decentralized offices in partnership with regional specialized or economic cooperation organizations – such as the AU-Interafrican Bureau for Animal Resources (IBAR), the Cooperation Council for the Arab States of the Gulf (GCC), SAARC and ASEAN – and non-governmental organizations where technical assistance and training to countries can be channelled.

PPR-GCEP and FAO Strategic Objectives: The programme is relevant to FAO’s five Strategic Objectives in: i) establishing an enabling environment for poverty alleviation and food security; ii) providing policy support services to producers to overcome barriers to sustainable animal production, economic growth and improved natural resource efficiencies; iii) providing outreach awareness/education and rural employment for reducing poverty; iv) meeting international standards in animal health and food safety, thus promoting trade in live animals and their commodities; and v) building the resilience of agricultural communities. [360]

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SURVEILLANCE

Seroprevalence of peste des petits ruminants virus antibodies in camels in Nigeria

Contributors: Timothy Y. Woma,[1,2] Melvyn Quan,[2] Dalan Bailey ,[3] Demo J.U. Kalla,[4] Hussaini G. Ularamu[1] and David Shamaki[1]

ABSTRACT

This study was undertaken to determine the seroprevalence of peste des petits ruminants (PPR) antibodies in camels in northern Nigeria to guide future disease control efforts. A total of 1 516 camel serum samples from four states (Borno, Kano, Katsina and Sokoto) were collected and seroprevalence was determined by PPR competitive ELISA. The individual state estimated prevalence rates of PPR in camels were 3.0 percent in Borno, 3.2 percent in Kano, 4.0 percent in Katsina and 3.3 percent in Sokoto. The overall prevalence for all samples tested was 3.4 percent (51/1 517). Among the variables examined, 3.8 percent of male camels and 2.4 percent of female camels were seropositive, along with 3.9 percent of younger camels and 3.4 percent of adults. The results indicated that PPR virus (PPRV) was circulating among camels in Nigeria. There is therefore need to collect tissue samples from camels with clinical signs of respiratory distress for molecular diagnosis and possible virus isolation from this species, in order to assess any further potential role in the maintenance of PPR in the host populations.

MATERIALS AND METHODS

Serum samples were collected from camels submitted for slaughter at abattoirs in Maiduguri (Borno State), Kano (Kano State), Katsina (Katsina State) and Sokoto (Sokoto State), Northern Nigeria between April 2010 and December 2012. These four states cover an area of 70 714.19 km^2. Estimates based on the National Agricultural Sample Survey (NASS 2011) by the National Bureau of Statistics and the Federal Ministry of Agriculture and Rural Development indicate that about 70 percent of the camel population in Nigeria is located in this study area. Daily maximum ambient temperatures vary from 28 °C to 39 °C throughout the year, the coldest month being January. The study subjects were of all ages and both sexes. A blood sample (approximately 10 ml) was collected from the jugular vein of each animal slaughtered into a vacutainer tube (Becton Dickson, United Kingdom of Great Britain and Northern Ireland). Each sample was labelled using codes describing the sex, age and body condition score of the camel. The serum was obtained after overnight storage at ambient temperature and centrifugation. The samples were transported to the laboratory on ice and stored at −20 °C until analysis.

A monoclonal antibody-based competitive enzyme-linked immunosorbent assay (c-ELISA), obtained from BDSL (United Kingdom of Great Britain and Northern Ireland) was used for detection of antibodies to PPR virus (Anderson, Mckay and Butcher, 1991). All serum samples were tested in duplicate and the optical density (OD) values and percentage inhibition (PI) were calculated as per the standard protocol of the manufacturer. Samples with PI greater than or equal to 50 percent were considered positive for PPR virus infection. Categorical data were analysed using the single factor analysis of variance (ANOVA) test of independence.

RESULTS

The overall seroprevalence of camels in the four states was 3.4 percent (51 of 1 517 samples). Of the 1 517 camels sampled, 984 (64.9 percent) were males and 532 (35.1 percent) were females. The highest seroprevalence rate of 4 percent was observed in Katsina State while the lowest rate of 3.0 percent was observed in Borno State. The proportion of camels in each category and the mean serum antibody prevalence profile of each variable investigated during the study are shown in Table1. Figure 1 shows the geographical area studied.

Table 1: Proportion of camels in each category and mean serum antibody prevalence profile of each variable investigated during a seroprevalence study for the presence of PPR antibodies in northern Nigeria (n = 1 517)

	No. of samples analysed	Percentage (%)	Prevalence (%)
States			
Borno	433	28.5	13 (3.0)
Kano	517	34.1	17 (3.2)
Katsina	296	19.5	12 (4.0)
Sokoto	271	17.9	9 (3.3)
Sex			
Males	984	64.9	38 (3.8)
Females	532	35.1	13 (2.4)
Body score			
Poor	42	2.7	2 (4.7)
Fair	846	55.8	26 (3.0)
Good	628	41.4	23 (3.6)
Age category			
Adult	1241	81.8	43 (3.4)
Young	275	18.1	8 (3.9)

[1] Viral Research Division, National Veterinary Research Institute, Vom, Nigeria

[2] Department of Veterinary Tropical Diseases, University of Pretoria, Onderstepoort, South Africa

[3] The Pirbright Institute, Woking, United Kingdom of Great Britain and Northern Ireland

[4] Animal Production Programme, Abubakar Tafawa Balewa University, Bauchi, Nigeria

Figure 1: Map of Nigeria showing the areas of sample collection in red

DISCUSSION AND CONCLUSION

The overall prevalence for all states studied was 3.4 percent, which is consistent with a seroprevalence rate of 4 percent (of 250 samples) in camels from Sokoto reported earlier (Daneji, Chafe and Tahir, 1997). An initial study in Nigeria estimated the PPR seroprevalence at 49.26 percent for sheep and 38.34 percent for goats (Shamaki, 2002). Abubakar *et al.* (2008) reported 38.5 percent seroprevalence of morbillivirus antibodies in camels in Maiduguri, Borno State using the complement fixation test (CFT) while Ambali *et al.* (1995) using the same CFT and agar gel immunodiffusion (AGID) techniques reported 11 percent seroprevalence of rinderpest antibodies in imported camels in the same study area. The differences in seroprevalence compared with this study might reflect variation in infection rates and in the sensitivity and specificity of the tests used. There was no significant difference (F test) in the seroprevalence rates for the different variables examined.

The PPR antibodies observed in the present study could only have come from a natural infection of the camels, as there is no documented evidence that camels are being vaccinated against PPRV in and around Nigeria. The study area was along the northern border of the country, which is considered an important route for legal and illegal animal transportation among Nigeria, Chad, Cameroon, the Niger and North African countries. The nature of the camel husbandry system, which allows camels to co-mingle with other ruminants at grazing and drinking points and in live animal markets, means that the camel population can serve as a source of PPRV infection to ruminants and vice versa. It is therefore important that camels be included among the group of animals to be monitored for PPR virus presence and to define their role in the epidemiology of the disease in Nigeria and elsewhere. Further work in Nigeria will concentrate on tissue sample collection from camels with respiratory distress for further research and continued surveillance.

ACKNOWLEDGEMENTS

This work was supported by a grant from the International Atomic Energy Agency (IAEA) CRP No: 14618, and the Agricultural Research Council of Nigeria through the Competitive Agricultural Research Grant Scheme (RFA 2 No. 48). The authors sincerely appreciate the support of all staff of the Morbilliviruses Research Laboratory, National Veterinary Research Institute (NVRI), Vom, and Mr Elisha Tiyagnet for technical assistance. 360

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Camels at the entrance to Sokoto Municipal Abattoir, Nigeria, July 2010

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SURVEILLANCE

Peste des petits ruminants in Turkey: findings between 2010 and 2013

Contributors: Seval Bilge Dagalp,[1] Ahmet Sait,[2] Ali Reza Faraji,[3] H. Mahur Altay[1] and Aykut Ozkul[1]

INTRODUCTION

The first declaration of peste des petits ruminants (PPR) in Turkey was made in 1996 based on pathological findings in goat cases (Alcigir, Vural and Toplu, 1996). The first genetic characterization of the local virus was reported using a local isolate recovered from an outbreak in Sakarya in 2000 (Ozkul *et al*., 2002). Between July 2002 and September 2003, clinical cases of PPR infection were diagnosed in seven villages located near the city of Bursa, which is in the Marmara region of western Turkey (Yesilbag *et al*., 2005). In the following years, the disease showed a tendency to spill over into the most southern parts (e.g. Mugla and Aydin Provinces) of the Aegean region (Toplu, 2004). In 2007 a PPRV outbreak was reported in Kirikkale Province, Central Anatolia (Kul *et al*., 2007) and before its final dissemination the infection was reported in sheep flocks in the central and eastern Black Sea region in 2008 and 2009 (Figure 1).

There are 27 million sheep and 5 million goats in Turkey and each year large numbers of neonates and young animals die from PPR. Veterinarians in the field are familiarized with the disease and can recognize it easily. Ear tag identification in small ruminants was not used in Turkey until the recent EU-supported PPR project (with €44.6 million over three years) that commenced in 2011. According to the new protocol started under this project, individual identification of small ruminants using ear tagging is being performed in parallel to routine countrywide PPR vaccination. This article describes temporal changes in the status of PPRV in Turkey after 2012.

DISEASE OCCURRENCE

PPR has been a continuous threat to small ruminant health countrywide for the last five years. Numbers of laboratory-confirmed and government-declared cases of PPR were recorded as 63 in 2010, 218 in 2011 and 59 in 2012 (Table 1). In 2010 and 2012, the disease was reported in 27 of Turkey's 81 provinces (33.3 percent). The number of provinces, however, in which outbreaks were officially reported reached 50 (61.7 percent) in 2011. Most outbreaks occurred between the months of May and September, coinciding in part with the hot, dry season.

While initially the disease was more common in eastern Turkey (before the period shown in Figure 1), it quickly became endemic countrywide owing to a number of factors. The most important factor helping such rapid dissemination was uncontrolled (or illegal) animal movements within the country, particularly just before Eid-al-adha celebrations. Illegal movements of livestock among endemic countries have been established as an important factor for continuing PPR outbreaks in most countries, leading to the continuous cycling of the virus throughout the region. As a result, official regulations and

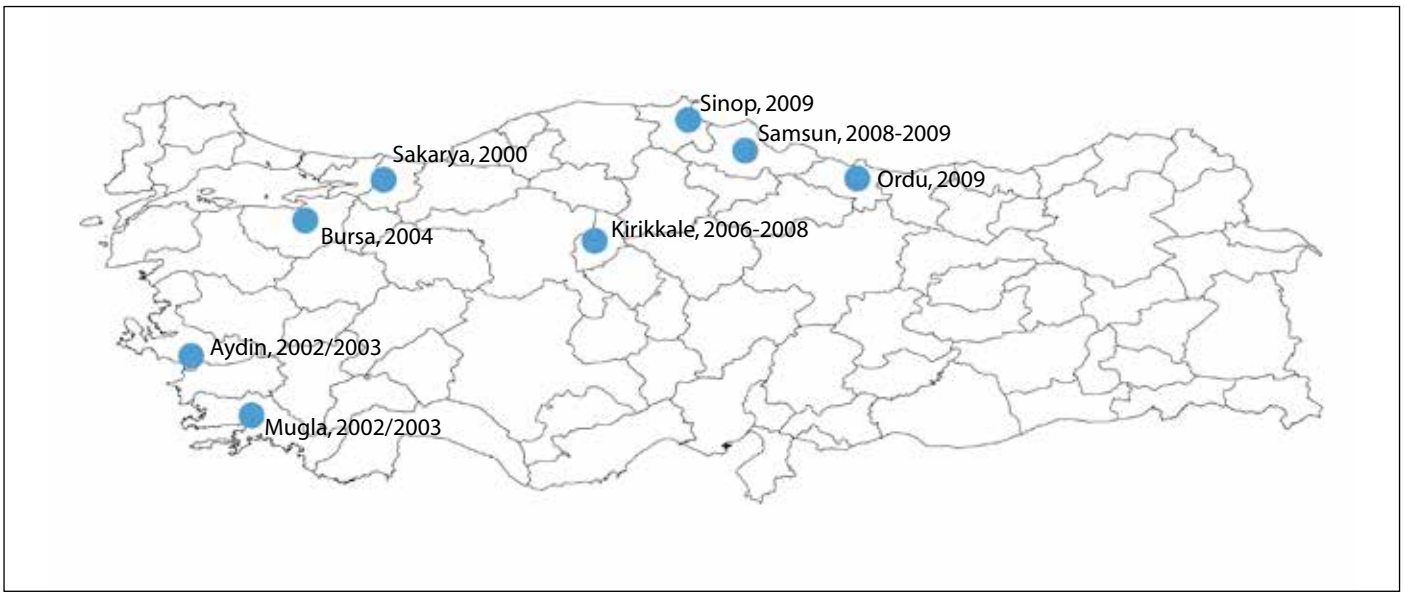

Figure 1: Milestone occurrences of PPR infection in western Turkey

[1] Ankara University, Faculty of Veterinary Medicine, Virology Department, Ankara, Turkey
[2] Pendik Veterinary Control Institute, Laboratory for Viral Diagnosis, Istanbul, Turkey
[3] Sekans Animal Health Laboratory, Ankara, Turkey

Table 1: Monthly numbers of infected provinces and PPR outbreaks detected in Turkey, 2010–2012

Month	2010		2011		2012	
	Province	Occurrence	Province	Occurrence	Province	Occurrence
J	6	9	4	4	7	12
F	3	6	6	8	4	5
M	8	9	5	10	9	11
A	1	1	12	15	2	3
M	2	2	14	18	3	4
J	6	9	17	26	1	1
J	7	7	23	50	7	7
A	8	11	22	50	2	2
S	6	6	11	17	1	1
O	2	2	9	9	1	1
N	0	0	6	6	5	9
D	1	1	4	5	3	3
Total		63		218		59

Table 2: Comparison of the occurrences of protective post-vaccination neutralizing antibody titres in sheep and goats with three PPR vaccines produced in Turkey

	Group 1			Group 2			Group 3		
	Day 0 (%)	Day 18 (%)	8th month (%)	Day 0 (%)	Day 18 (%)	8th month (%)	Day 0 (%)	Day 18 (%)	8th month (%)
Goats	0/10 (-)	7/10 (70.0)	9/10 (90)	0/20 (-)	20/20 (100)	20/20 (100)	1/20 (5)	20/20 (100)	20/20 (100)
Sheep	2/50 (4.0)	7/50 (14)	6/28 (21.4)	1/50 (2)	2/50 (4)	4/22 (18.2)	2/50 (4)	19/50 (38)	10/23 (43.5)

penalties regarding animal movements were developed to reduce international and national dissemination of livestock infectious diseases in Turkey. This will assist in reducing the spread of new variants of the virus not only among countries but also within the country. Highway checkpoints were established where veterinary and trade officials collaborate on routine document controls (on animals' origins and vaccination status) during animal transport within the country. In addition to these control measures, massive administration of PPR vaccine in endemic areas of Turkey has been used in an attempt to reduce the incidence of the disease.

> "The efficacy of three PPR vaccines was tested - The results showed that protective titres were less common in sheep than goats"

VACCINATION

Approximately 30 million doses of Nigeria 75/1 strain PPR vaccine prepared in Vero cells were produced and consigned to the field. These vaccines were produced by three laboratories: the Central Veterinary Institute, Ankara, and two private vaccine companies located in Adiyaman and Sanli Urfa. Vaccination campaigns are conducted in two rounds each year: the first in the period between March and May, and the second in the period between September and November. In a field study conducted in 2012 (Turan *et al*., 2012), the efficacy of three PPR vaccines was tested in a group of animals that were from routinely vaccinated flocks. The results showed that protective titres were less common in sheep than goats (Table 2).

PHYLOGENETIC ANALYSIS OF THE LOCAL VIRUS

The neighbour-joining analysis of some of the viruses detected from a variety of locations over consecutive years (2010–2012) is presented in Figure 2. These viruses all belonged to lineage 4, the same as the virus characterized in 2000. Other viruses detected and/or isolated in 24 provinces across the country during outbreaks in 2012 and 2013 were also found to be members of lineage 4 (not shown).

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Data presented here were derived from the Ph.D. theses of Ahmet Sait DVM, Alireza Faraji DVM and Mahur Altay DVM, and from records of departmental diagnostic services of Ankara University. 360

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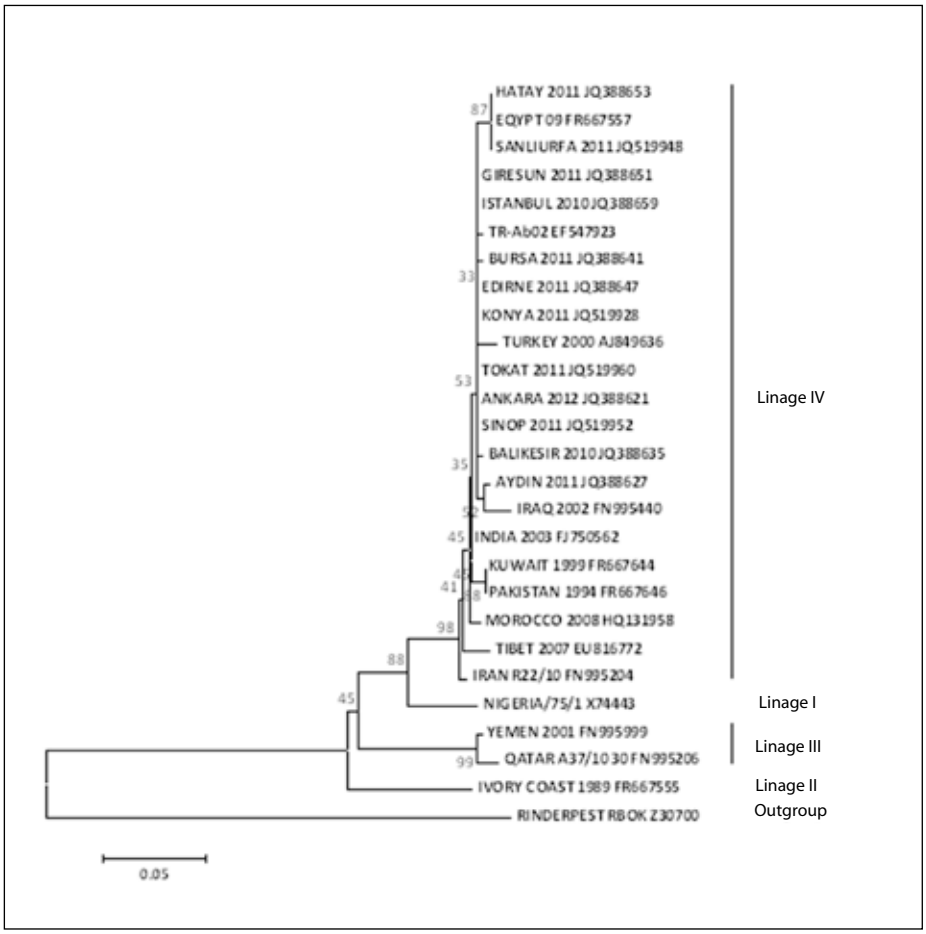

Figure 2: Partial F-gene sequence-based phylogenetic analysis (neighbour-joining) of PPRV strains detected between 2010 and 2012 in Turkey

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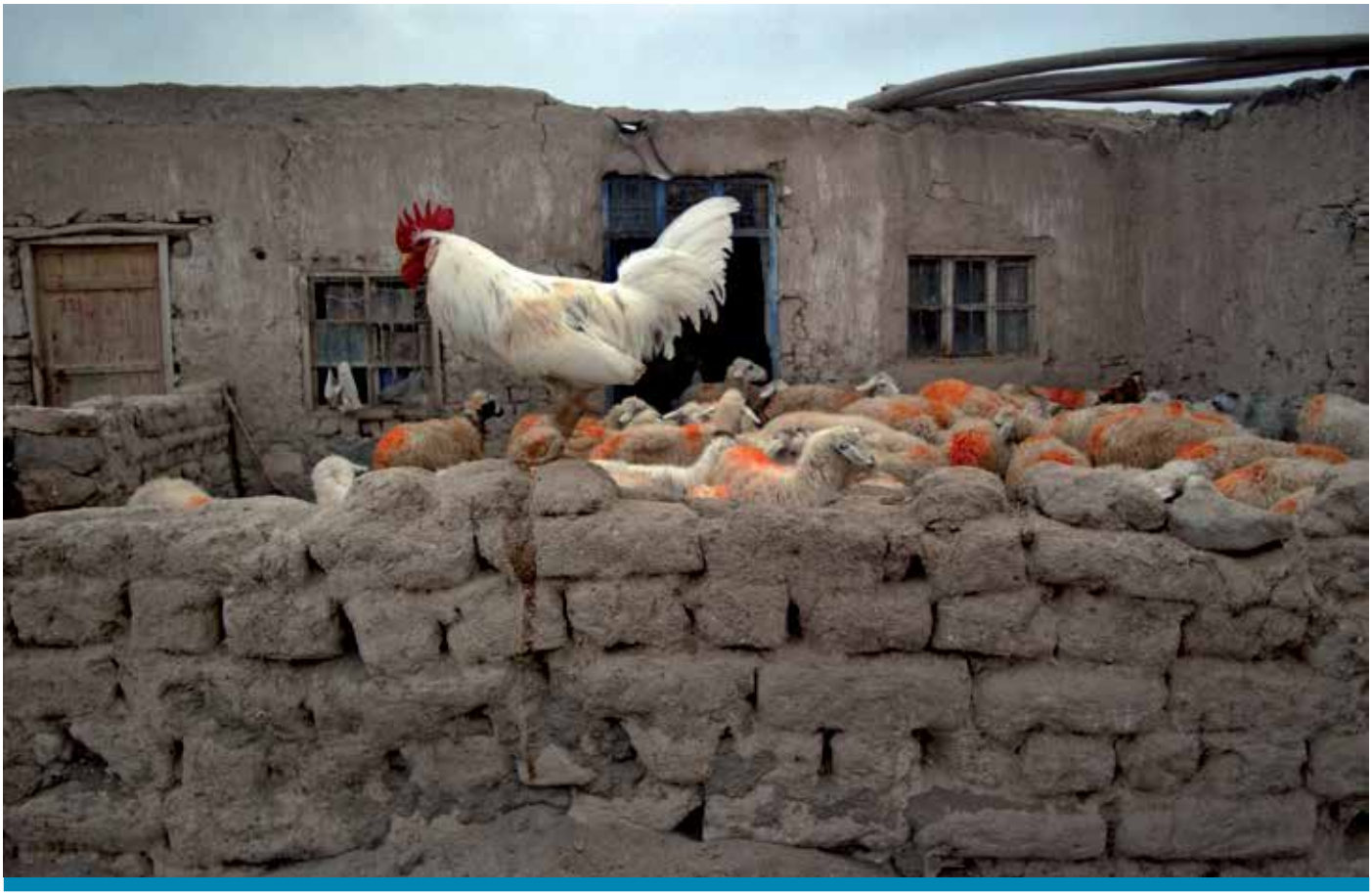

A barnyard holding sheep, Turkey


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ANALYSIS

Molecular detection and genotyping characterization of PPRV in various areas of Pakistan, 2007 to 2012

Contributors: Waqas Ashraf,[1] Qaiser Mahmood Khan,[1] Ameena Mobeen,[1] Hira Kamal,[1] Muhammad Moazam Jalees,[2] Muhammad Farooq,[1] Adama Diallo[3] and Hermann Unger[3]

First confirmed in Pakistan by reverse transcription polymerase chain reaction (RT-PCR) in 1994, peste des petits ruminants (PPR) is now considered endemic and is a major constraint to efficient production of sheep and goats. Effective control of PPR in Pakistan would require robust and efficient diagnosis and continuous surveillance of the disease. This report outlines a study of the molecular epidemiology of PPR in Punjab Province of Pakistan during 2007 to 2012. Clinical samples (swabs, blood and tissues) were collected from suspected PPR outbreaks occurring during this period in Sheikhupura, Sargodha, Faisalabad, Sahiwal, Mianwali, Multan, Dera Ghazi Kahn, Chakwal and Arifwala districts of Punjab Province and analysed using conventional RT-PCR. RT-PCR-positive samples were processed for isolation of peste des petits ruminants virus (PPRV) using the CHS-20 cell line with the ovine signalling lymphocyte activation molecule (SLAM) receptor expressed on the cell surface. Subsequently, PPRV isolates were genetically characterized based on highly conserved sequences of the fusion (F) gene, and more variable sequences of the nucleoprotein (N) gene. Real-time RT-PCR was established using the N gene (using fluorescent probes with IAEA collaboration). An inexpensive, one-step, single-tube, reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay was optimized for the rapid detection of PPRV based on M and N genes from both culture supernatants and clinical samples (this work was done as part of the CRP-IAEA project contract no. 14567).

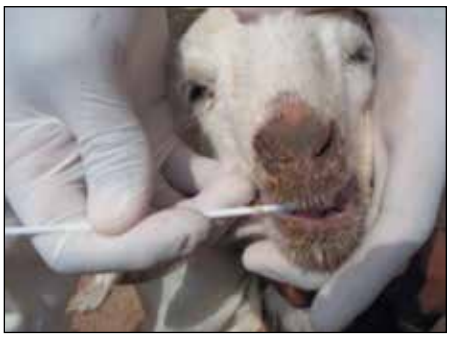
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Figure 1: Collection of dead epithelium by oral swab for PPRV detection


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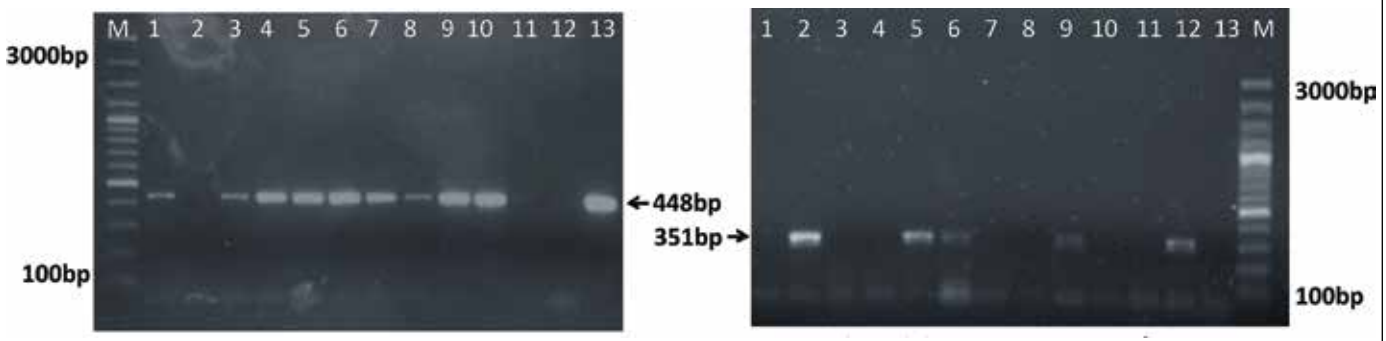
Figure 2: Collection of nasal swab from goat for the detection of PPRV

PCR-BASED DETECTION OF PPRV

Clinical samples including nasal/oral swabs, blood and tissues were collected from suspected PPR outbreaks in Punjab Province of Pakistan (Figures 1, 2 and 8). The samples were processed for RNA isolation and RT-PCR for PPR virus detection (Figure 3). One region of nucleoprotein gene using NP3/NP4 primers (351bp) and the other of fusion protein gene using F1b/F2d primers (448bp) were amplified for genetic characterization of these viruses.



RT-PCR-based detection using F1b/F2d primers

RT-PCR-based detection using NP3/NP4 primers

Figure 3: Reverse transcription PCR

[1] Molecular Diagnostic Group (MDG), Soil and Environmental Biotechnology Division (SEBD), National Institute of Biotechnology & Genetic Engineering (NIBGE), Faisalabad, Pakistan
[2] Institute of Microbiology, University of Agriculture, Faisalabad
[3] Animal Production and Health Section (APHS), Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria

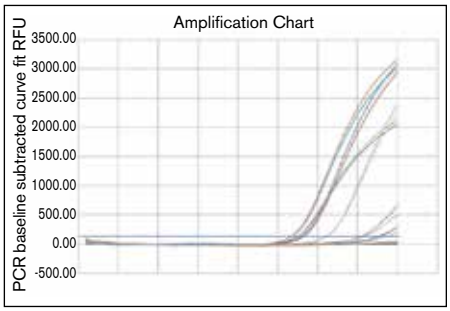


Figure 4: A real-time RT-PCR-targeting N gene of the PPR virus
Note: Amplification signals rising sufficiently above the threshold indicate that samples are positive for PPRV.

Real-time RT-PCR was optimized using TaqMan primers/probes targeting the N gene of PPRV (Adombi *et al.,* 2011) (Figure 4). The test was highly sensitive (100–105 percent PCR efficiency with detection limit up to 20 copies of viral cDNA), specific and reproducible.

RT-LAMP was established for on-site detection of PPRV in the clinical samples and from extracted RNA (CRP-IAEA contract no. 14567) (Figures 5, and 6).

VIRUS ISOLATION

RT-PCR-positive samples were processed for isolation of PPRV using the CH-20 cell line expressing ovine SLAM receptor on the cell surface (Adombi *et al.,* 2011) (provided by the Animal Production and Health Section [APHS] of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture). The CHS-20 cell line was found to be very efficient for virus isolation and the virus cytopathic effect (CPE) was observed sometimes within two days after inoculation of clinical samples, but most often within a week. Virus growth was generally fully established by the third passage in these cells.

PHYLOGENETIC ANALYSIS

Relevant N gene sequences of PPRV were retrieved from GenBank for the phylogenetic analysis of viruses detected in districts of Punjab Province (Figure 7). It showed that lineage IV of PPRV is prevalent in these districts. Within lineage IV, the isolates divided into two major groups: A (further divided into A-I, A-II and A-III); and B. Group A has homology of 99.2 percent with Dubai_2009 PPRV (Kinne *et al.,* 2010) (A-I) and 98 percent to 99.2 percent with Tajikistan/2004 (Kwiatek *et al.*, 2007) (A-III), and group B has homology of 97.2 percent with Shiraz101_2011 PPRV from the Islamic Republic of Iran.

CONCLUSION

This study was the first report from Pakistan on highly efficient isolation of PPRV using CHS-20 cells and detection by RT-LAMP

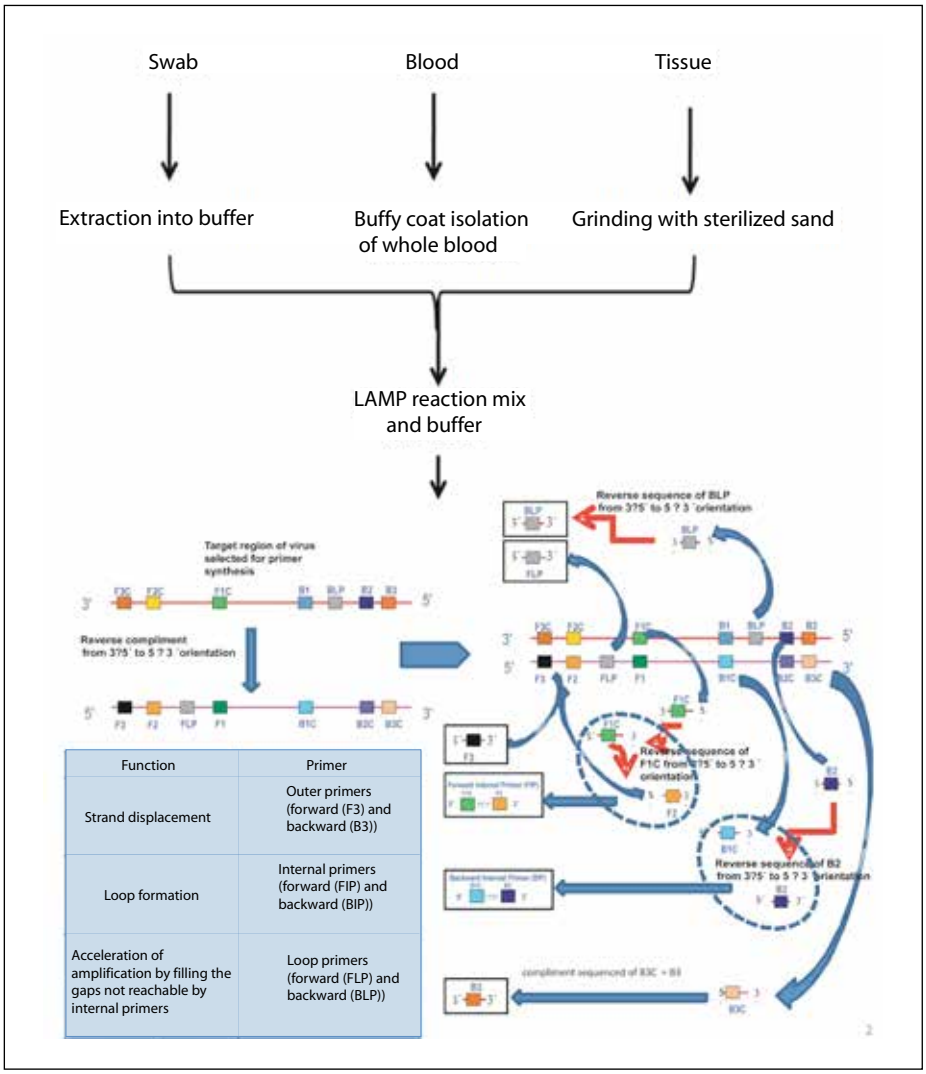


Figure 5: Outline of PPRV detection in clinical samples by RT-LAMP assay
Note: Processed swabs, blood or tissue samples were added to the sample buffer and then mixed with ready-to-use reaction mixture to carry out rapid highly specific loop-mediated isothermal amplification assay. For high specificity, a set of six primers was designed to amplify the target regions of N (CRP-IAEA contract no. 14567) and M genes (Bao *et al.* 2010) of PPRV.

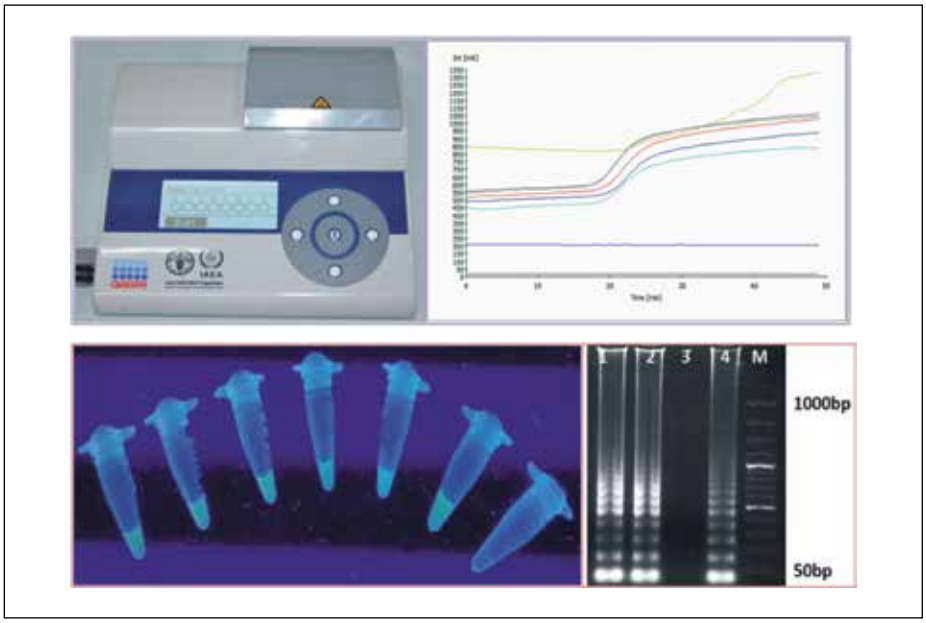


Figure 6: ESE Quant tube scanner (Qiagen) designed to carry out LAMP assay
Note: The software plots the amplification signal (mVolt) against time interval for 60 minutes. A positive test can be detected by slope validation (> 30 mvolts), and confirmed later if necessary by examination for colour change under UV and gel electrophoresis.

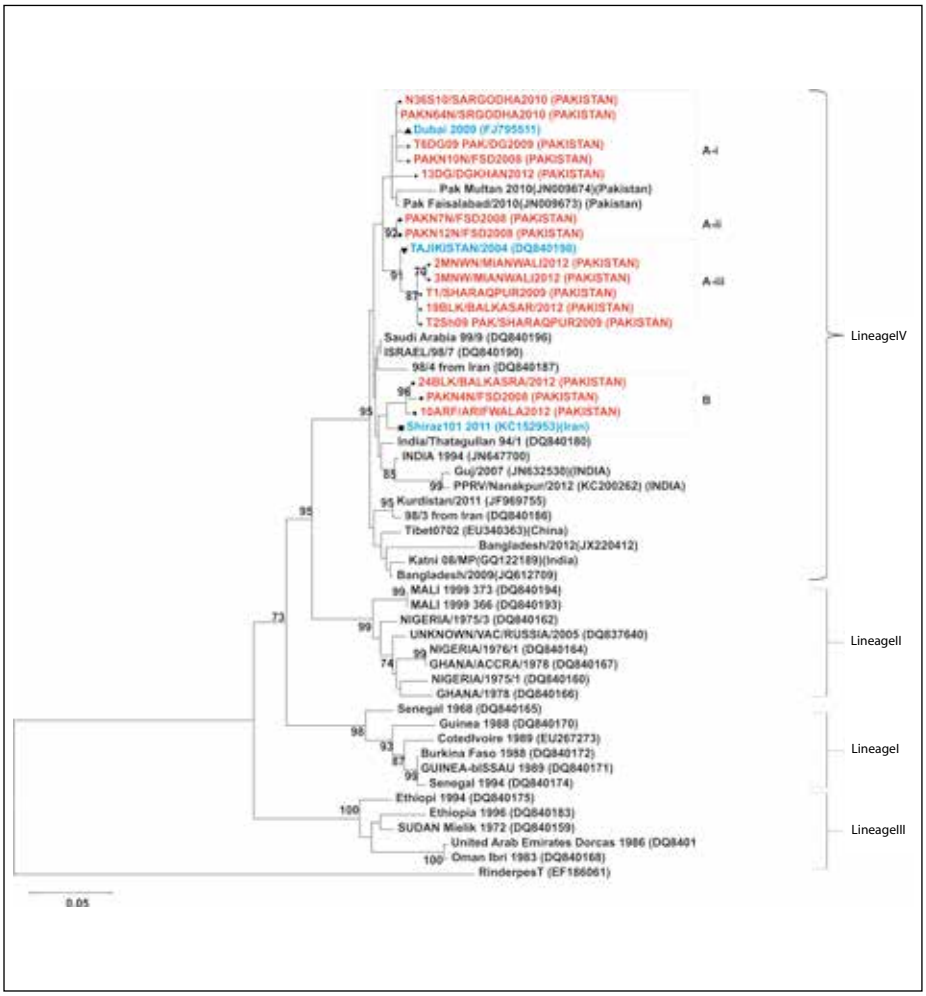


Figure 7: Neighbour-joining tree (2 000 iterations) of PPRV isolates detected in Pakistan
Note: Only > 70 percent bootstrap values are shown in the tree. All four lineages grouped separately from each other (70–100 percent bootstrap values). PPRV isolates detected during the study are in red, while those from neighbouring countries are in blue. These isolates divided into two major groups: A and B within lineage-IV (this grouping pertains to PPRV identified in districts of Punjab, Pakistan during the study). Group A is divided into A-I (closely related to Dubai_2009 PPRV), A-II (central position relative to A-I and A-III) and A-III (closely related to Tajikistan_2004 PPRV). Group B isolates are closely related to isolates from the Islamic Republic of Iran (Shiraz101_2011).

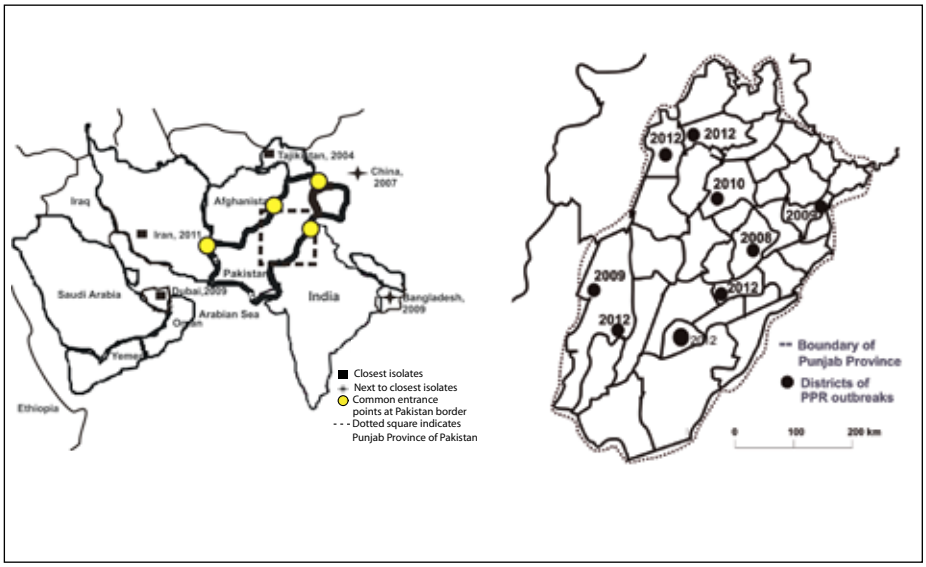


Figure 8: 8: PPR outbreaks in Pakistan and neighbouring countries with phylogenetic relationship
Note: The most closely related isolates from Tajikistan, Dubai and the Islamic Republic of Iran are named, with the year of the outbreak, in a solid box. Yellow circles indicate entrance points from neighbouring countries into Pakistan. The dotted square indicates Punjab Province, which is detailed in the right half mentioning the year of each PPR outbreak, indicating districts of outbreak with solid circles.

assay. It suggests the transboundary spread of PPRV through nomadic animal movement or trade in the region (Figure 8). Therefore, coordinated efforts are required for effective control of the disease. The applicability of RT-LAMP assay for rapid, cost-effective on-site diagnosis of PPRV antigen offers a very useful molecular diagnostic tool for PPR surveillance under field conditions. The PPRV strains isolated using CHS-20 cells are a permanent stock available for future studies especially on host-virus interaction.

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[2] Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan

[3] Animal Production and Health Section (APHS), Joint Food and Agriculture Organization of the United Nations (FAO)/International Atomic Energy Agency (IAEA) Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria

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ANALYSIS

Molecular analysis of peste des petits ruminants viruses from current outbreaks in Nigeria

Contributors: Timothy Y. Woma,[1,2] Melvyn Quan,[2] Dalan Bailey,[3] Pam D. Luka,[1] Hussaini G. Ularamu,[1] Dauda G. Bwala,[1] Olaiya D. Olalekan,[1] Samuel E. Mantip,[1] Benjamin B. Dogonyaro,[1] Solomon C. Chollom,[1] Gideon Bature,[1] Nenfa D. Tom,[1] Dyek Y. Dyek,[1] Haruna M Kazeem,[4] Adama Diallo[5] and David Shamaki[1]

Peste des petits ruminants (PPR) is of great importance in Nigeria, where small ruminants form an integral component of agricultural food production. PPR has remained endemic in Nigeria despite the use of a Vero cell-adapted live attenuated vaccine in small ruminants. This study was conducted to characterize PPR virus (PPRV) from an emerging wave of outbreaks in different parts of Nigeria. Most outbreaks occur between February and May (the dry harmattan season) or between July and September, in southeastern and southwestern parts of the country. All the PPRV identified in Nigeria from the 1970s until 2000 belonged to lineage II (Shamaki, 2002; Kwiatek *et al*., 2007; Banyard *et al*., 2010). However, following reports of the circulation of lineage IV in Cameroon and the Central African Republic, it has become necessary to reassess the epidemiological situation of PPRV in Nigeria, especially in states on the borders with neighbouring countries.

MATERIALS AND METHODS

Post-mortem samples consisting of spleen, trachea, lung, liver and lymph nodes were collected between April 2010 and October 2012 from 140 sheep and goats with symptoms suggestive of PPRV infection

A: Matted eyelids

B: Diarrhoea (sheep)

C: Diarrhoea (goat)

D: Mucopurulent oculo-nasal discharge

E: Raised hair, depression, increased rectal temperature

F: Frothy salivation, death

©Timothy Yusufu Woma, all photos

Figure 1: Clinical signs observed in Nigerian PPR outbreaks

[1] Viral Research Division, National Veterinary Research Institute, Vom, Nigeria
[2] Department of Veterinary Tropical Diseases, University of Pretoria, Onderstepoort, South Africa
[3] The Pirbright Institute, Woking, United Kingdom of Great Britain and Northern Ireland
[4] Department of Veterinary Microbiology, Ahmadu Bello University, Zaria, Nigeria
[5] International Atomic Energy Agency (IAEA)/Food and Agriculture Organization of the United Nations (FAO) Agriculture and Biotechnology Laboratory, Vienna, Austria

(Figure 1). The samples originated from multiple outbreaks at different locations in Nigeria (Figure 2). Adamawa State samples were from Gulak and Njobli; Taraba State samples were from Jalingo, Wukari, Kassa, Maihula and Garbabi; Plateau State samples were from Angwa Kurma in Jos; Yobe State samples were from Yusufari; Kano State samples were from Dogongora and Kano Municipal; Ondo State samples were from Akure and Idanre; Kwara State samples were from Baruten; Akwa Ibom State samples were from Uyo; Imo State samples were from Eziama-Obaire and Iho; Anambra State samples were from Adazi-Ani, Eziora, Umuchi and Amada; and Oyo State samples were from Iregba, Bodija and Ijebu-ode. Samples were transported on ice to the laboratory, where 20-percent homogenates of each tissue were prepared using phosphate-buffered saline solution. These homogenates were centrifuged and ribonucleic acid (RNA) was extracted from the supernatants using the QIAamp Viral RNA kit (spin protocol) from Qiagen (Limburg, the Netherlands) according to the manufacturer's instructions. Five microlitres (µl) of the extracted RNA was reverse-transcribed to obtain complementary deoxyribonucleic acid (cDNA) using Omniscript™ Reverse Transcriptase Kit (Qiagen) according to the manufacturer's instructions. The diagnostic nucleoprotein (NP) 3 and NP4 primer pair targeting the nucleoprotein gene (Couacy-Hymann *et al.*, 2002) was used to perform polymerase chain reaction (PCR) using 5 µl of the cDNA preparation, 35 µl of water, 5 µl of 10x PCR buffer, 0.5 µl of tag DNA polymerase and 1.5 µl each of deoxyribonucleotide triphosphate (dNTP) and the reverse and forward primers. The cycling conditions consisted of 95 °C for 5 minutes, 35 cycles of 95 °C for 30 seconds, 55 °C for 30 seconds, 72 °C for 1 minute and a final extension of 72 °C for 25 minutes. The product mixture was immediately incubated on ice and 10 µl of the PCR products was resolved on a 2-percent agarose gel stained with ethidium bromide. The gel photo revealed the expected band size of 350 bp. Representative amplified products from each outbreak were directly sequenced by a commercial company (Inqaba Biotech, South Africa). The sequences obtained were edited using the Molecular Evolutionary Genetics Analysis (MEGA) version 5 software and aligned with other selected sequences from different countries available in GenBank. A neighbour-joining phylogenetic tree was constructed using MEGA (Tamura *et al.*, 2007).

> "Some of the Nigerian strains from the current outbreaks grouped with other isolates in lineage IV, while some grouped with previously characterized Nigerian isolates in lineage II"

RESULTS

In this study, PPRV was detected in 81 (58 percent) of the 140 samples tested, confirming PPRV in all the locations. Most of these outbreaks occurred between December and early April (dry season). Phylogenetic analysis of the 350 bp fragments of the N gene sequences in this study with others from GenBank are shown in Figure 3. Some of the Nigerian strains from the current outbreaks grouped with other isolates in lineage IV, while some grouped with previously characterized Nigerian isolates in lineage II.

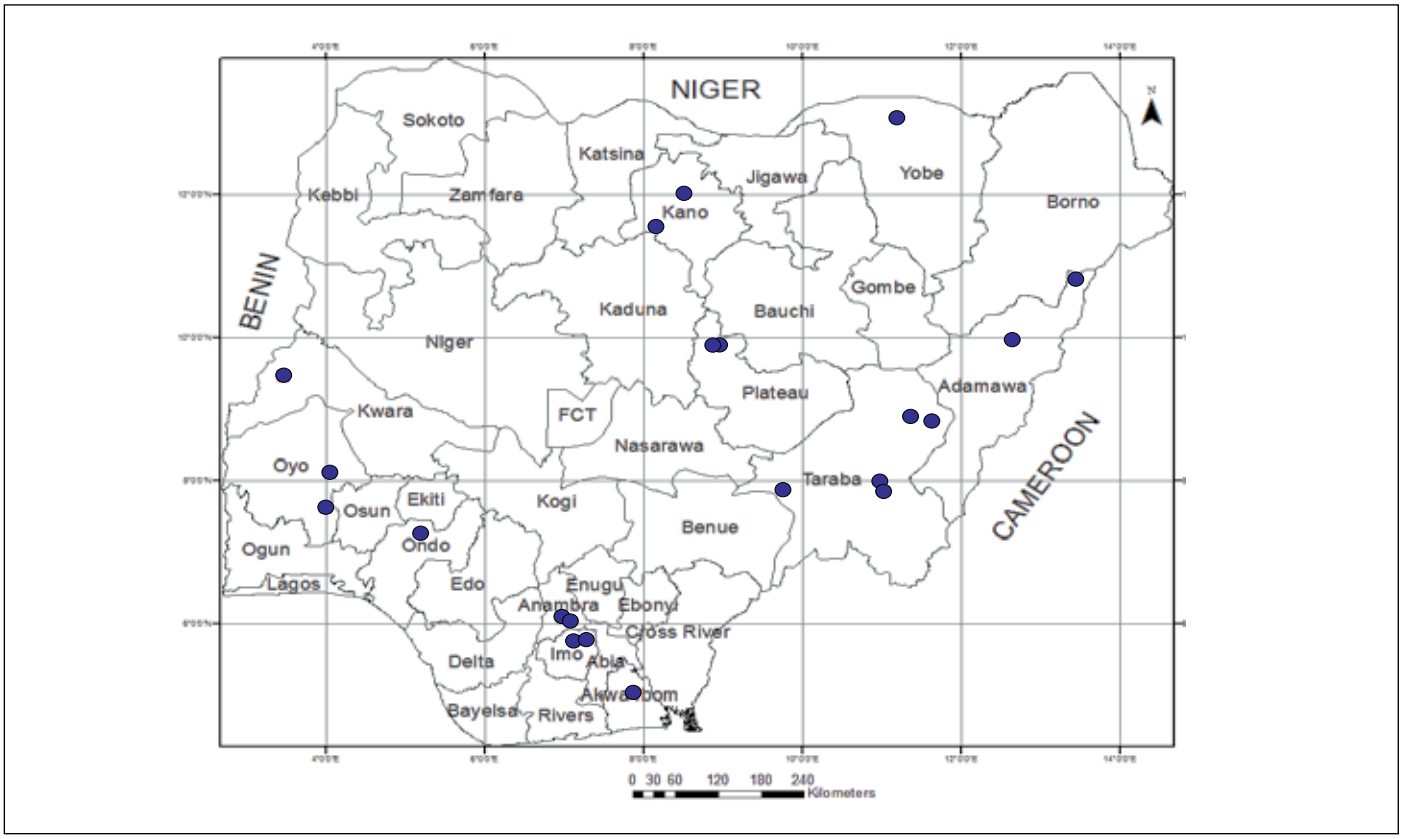

Figure 2: Map of Nigeria showing the states and villages where positive PPR samples were collected
Adamawa State samples were from Gulak and Njobli; Taraba State samples were from Jalingo, Wukari, Kassa, Maihula and Garbabi; Plateau State samples were from Angwa Kurma in Jos; Yobe State samples were from Yusufari; Kano State samples were from Dogongora and Kano Municipal; Ondo State samples were from Akure and Idanre; Kwara State samples were from Baruten; Akwa Ibom State samples were from Uyo; Imo State samples were from Eziama-Obaire and Iho; Anambra State samples were from Adazi-Ani, Eziora, Umuchi and Amada; and Oyo State samples were from Iregba, Bodija and Ijebu-ode.

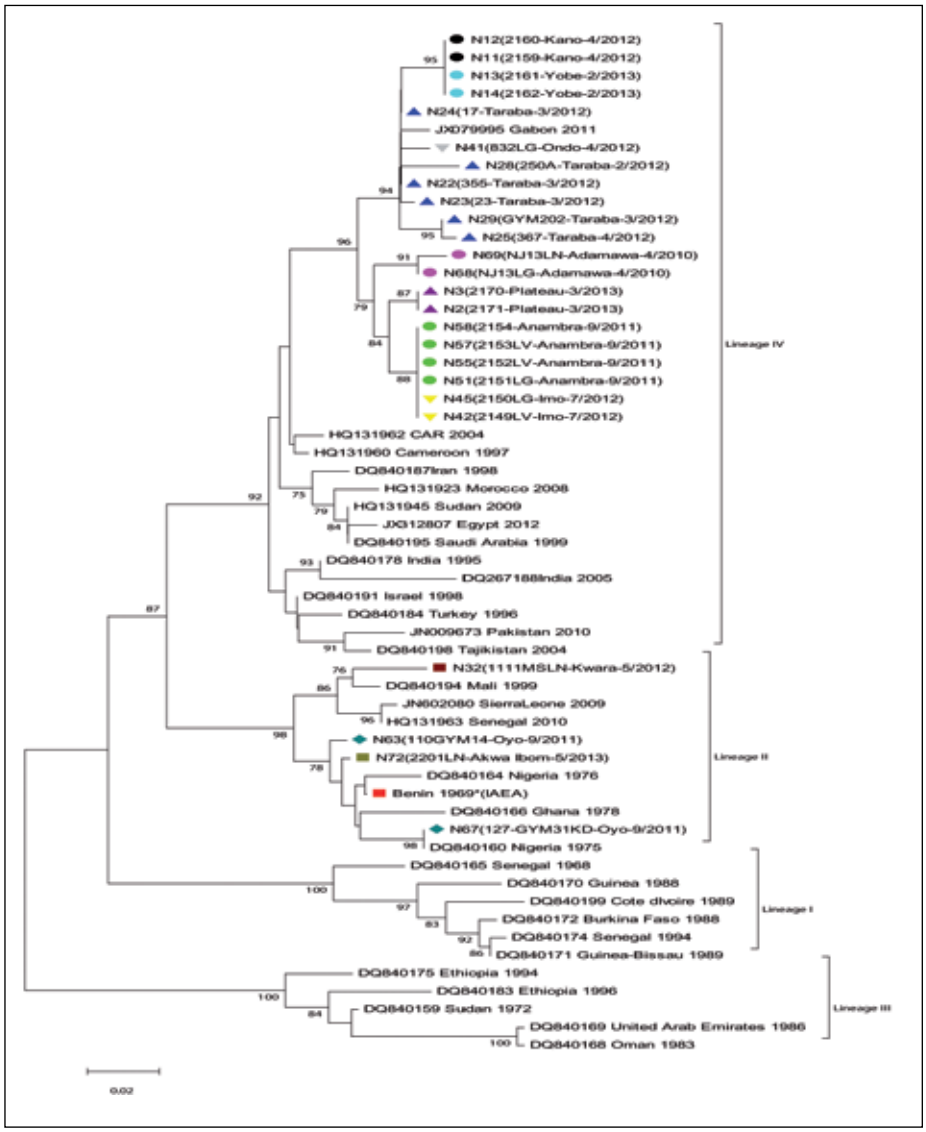

Figure 3: PPRV N gene phylogenetic analysis
An unrooted neighbour-joining phylogenetic tree showing relationships between sequences from this study (indicated by coloured dots) and those from GenBank (indicated by ascension numbers, countries of origin and years of isolation). The tree is based on the variable N gene region (255 bp) constructed in MEGA version 5 at bootstrap value of 1 000 replicates; only values of more than 70 percent are shown.

DISCUSSION AND CONCLUSION

The result of this study confirmed PPR endemicity in all the agro-ecological zones of Nigeria and showed that two lineages (II and IV) of PPRV are currently circulating in the country. PPRV of lineage IV is also circulating in neighbouring Cameroon (Banyard *et al*., 2010), which shares a long, porous border with Nigeria. Sheep and goats play an integral role in sustainable agriculture and employment in Nigeria, thus the control and eradication of PPR is a priority in easing poverty and improving the health and husbandry of animals kept by resource-poor people in this developing country.

The future plan is to isolate PPR viruses from these tissues using the recently developed monkey CV1 cell line expressing the sheep-goat signalling lymphocyte activation molecule (SLAM), and to sequence greater portions of the viral genomes to elucidate the genomic relationships of currently circulating viruses in Nigeria.

> “The control and eradication of PPR is a priority in easing poverty and improving the health and husbandry of animals kept by resource-poor people in this developing country”

ACKNOWLEDGEMENTS

This work was supported by a grant (RFA 2 No.48) from the Agricultural Research Council of Nigeria through the Competitive Agricultural Research Grant Scheme (CARGS) and the Animal Production and Health Section (APHS), Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Coordinated Research Programme (CRP) contract no. 14618/R3 (to D. Shamaki). The authors sincerely thank staff of the Morbillivirus Laboratory of the National Veterinary Research Institute, Vom, Nigeria and all the field staff who helped with sample collection and other logistics. 360

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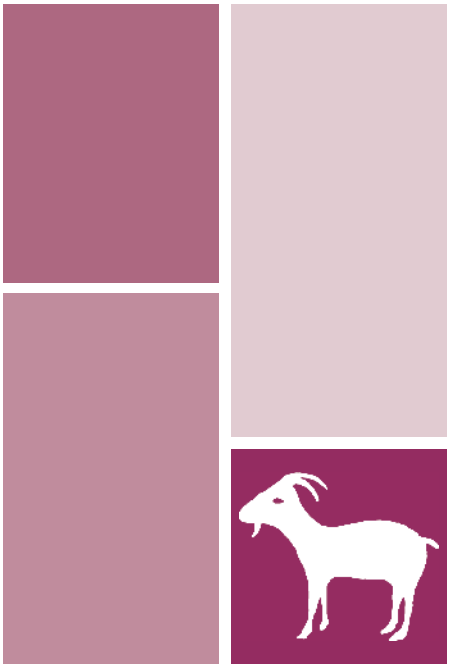

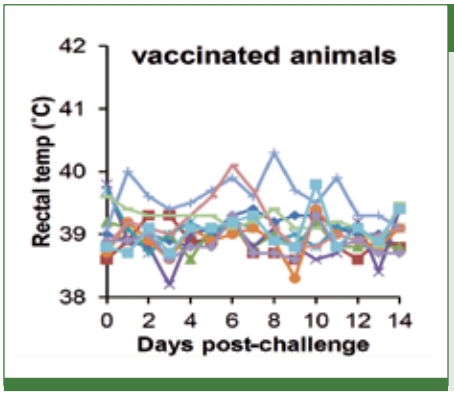

TOOLS

Improved diagnostics and vaccines for control of PPR

Contributors: Rebecca Herbert,[1] Jana Baron,[1] Carrie Batten,[1] Geraldine Taylor[1] and Michael D. Baron[1]

Peste des petits ruminants virus (PPRV) is a paramyxovirus that belongs to the genus *Morbillivirus*. It is widely distributed in Africa, the Near East and Asia, and has a major economic impact on livestock keepers in developing countries. Control of PPR is currently through vaccination with live attenuated strains of PPRV. It is therefore not possible to distinguish vaccinated animals from those that have recovered from natural infection. A differentiation of infected from vaccinated animals (DIVA) vaccine/test would improve epidemiological data by allowing tracking of infection in areas where there has been partial vaccination. Another problem for control programmes is that the clinical signs of PPR are so similar to those of other diseases of sheep/goats that conclusive diagnosis requires laboratory tests, primarily to identify the viral proteins using enzyme-linked immunosorbent assay (ELISA) or the viral genome using gel-based or real-time polymerase chain reaction (PCR). A simple, reliable and rapid field test could considerably speed up the implementation of disease control measures.

BASIS OF THE DIVA VACCINE/ TEST

The main protective antigens of morbilliviruses are the surface glycoproteins F and H, while the most immunogenic antigens are the H and N proteins. Expressing either of the H or F proteins in a heterologous virus-vectored vaccine has been shown to be protective for several morbilliviruses, including PPRV. The authors constructed a version of replication-defective human adenovirus type 5 that expresses PPRV H (Adeno-PPRV-H) and tested its immunogenicity and ability to protect goats from virulent PPRV.

Figure 1 depicts the position on the PPRV genome of the genes encoding the different viral proteins, the predominant antibodies elicited by PPRV infection or live PPRV vaccine (left-hand side) and the DIVA vaccine (right-hand side). Animals that have been infected are detected by the presence of antibodies to the N protein, while vaccination coverage can be assessed by the presence of antibodies to the H protein in the

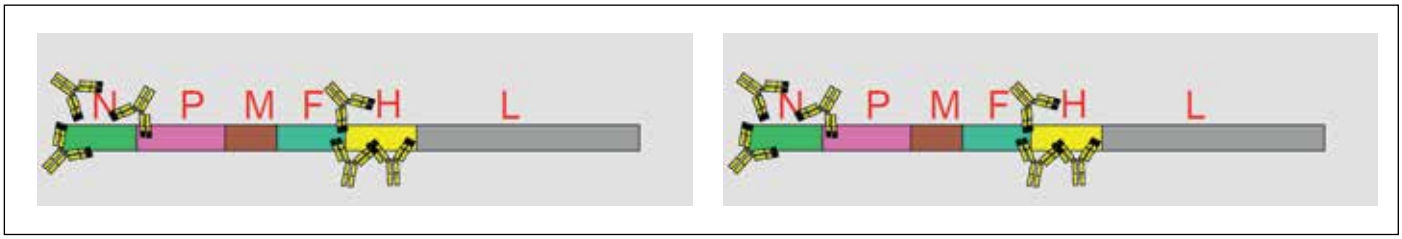

Figure 1: The position on the PPRV genome of the genes encoding the different viral proteins

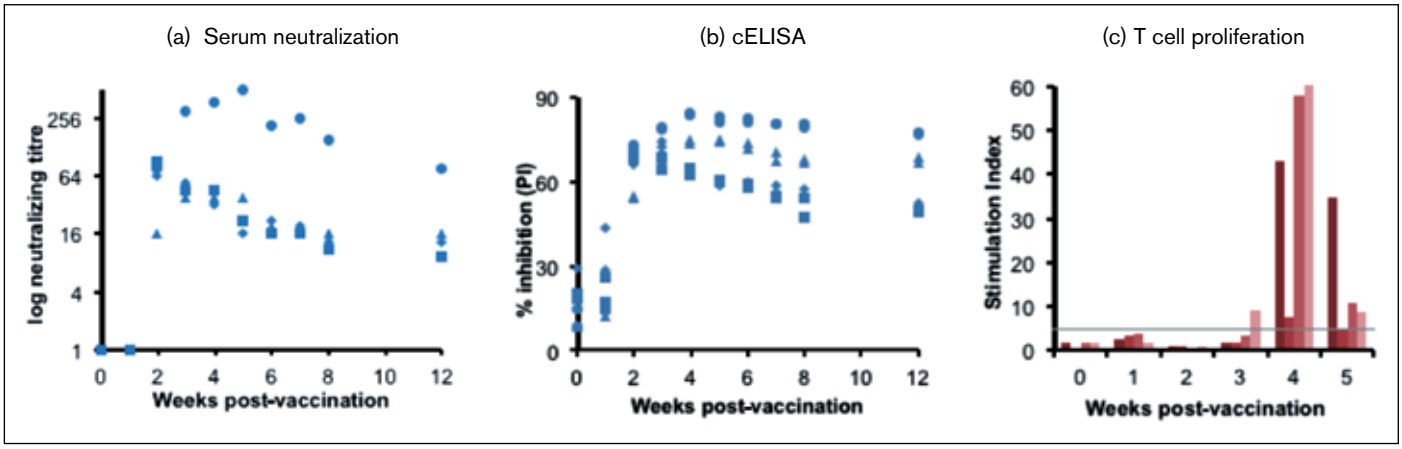

Figure 2: Immune responses in four goats vaccinated with the Adeno-PPRV-H vaccine

[1] The Pirbright Institute, Woking, United Kingdom of Great Britain and Northern Ireland

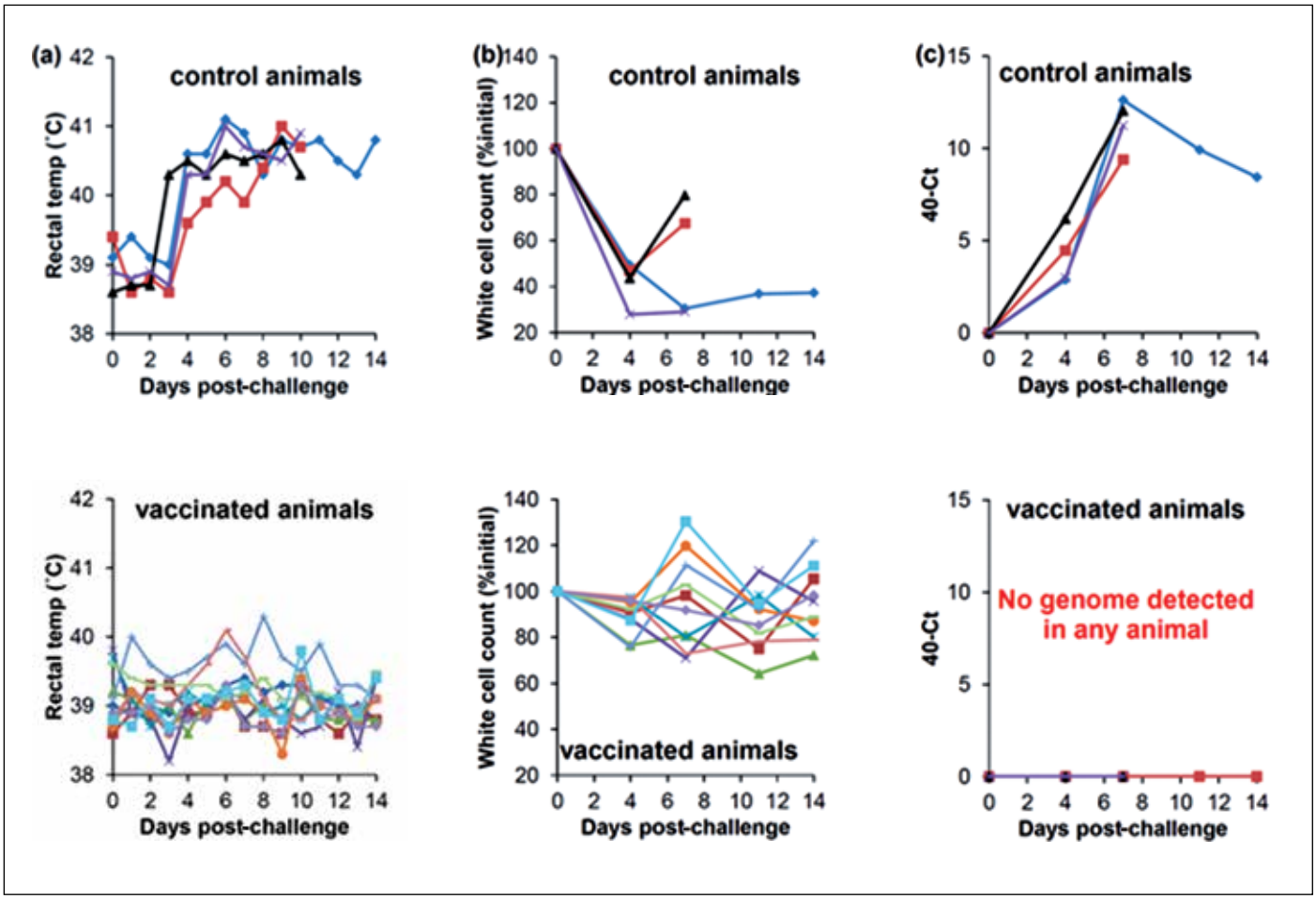

Figure 3: Results of challenging vaccinated goats with pathogenic PPRV

absence of antibodies to the N protein. Well-characterized tests for both types of antibody are already available

IMMUNOGENICITY OF THE VACCINE

In a preliminary experiment, Adeno-PPRV-H was used to vaccinate four goats and the immune response was monitored for 12 weeks. Strong antibody responses were detected using either a serum neutralization test (Figure 2a) or the commercial competition ELISA (cELISA) that detects antibodies to the PPRV H protein (Figure 2b). Cell-mediated immunity was demonstrated by a PPRV H-specific T cell proliferation assay (Figure 2c).

The immune responses in Figure 2 were examined by: (a) PPRV neutralization titre; (b) percentage inhibition of binding of monoclonal anti-PPRV-H antibody in cELISA; and (c) stimulation index for peripheral blood mononuclear cells stimulated with H-protein-specific peptides. The grey line in (c) indicates stimulation index = 5, below which results are considered negative.

PROTECTION AGAINST PPRV CHALLENGE

Groups of goats were mock vaccinated (four animals) or vaccinated (ten animals) with Adeno-PPRV-H. The animals were challenged with 10^5 $TCID_{50}$ (tissue culture infective dose) of a pathogenic PPRV strain at 16 weeks post-vaccination. All the vaccinated animals were fully protected (three of the four control animals were euthanized at seven days post-challenge because of the severity of their clinical signs).

> “Vaccination coverage can be assessed by the presence of antibodies to the H protein in the absence of antibodies to the N protein”

The results of challenging vaccinated goats with pathogenic PPRV shown in Figure 3 were monitored by: (a) rectal temperatures; (b) white cell count; and (c) real-time PCR of virus genome in whole blood after challenge of control (top row) and vaccinated (bottom row) animals. Real-time PCR results as expressed as 40-Ct (threshold cycle).

BASIS OF THE LATERAL FLOW DEVICE (LFD) PEN-SIDE TEST

The pen-side test is based on a simple immune-chromatographic system in which any virus particles in the sample are bound to antibody-coated coloured beads (Figure 4b); when the sample is carried along the test strip by the flow of buffer, the bead-antigen complex is trapped by the antibody in the test line (Figure 4c), which binds to the same virus particles.

ASSESSING THE PEN-SIDE TEST IN THE LABORATORY

A number of prototype test strips were prepared by a commercial specialist in the technology (details can be obtained from the authors) using different combinations of chromatographic filter material, buffer and antibody-coated beads. Prototype strips were tested to find a configuration that detected as little as 10^3 $TCID_{50}$ of cell culture-grown virus, which is equivalent to the amount of virus detected by real-time PCR in swabs from infected animals. Figure 5 illustrates three prototypes from the final batch.

The successful prototype was also shown to detect 10^3 $TCID_{50}$ of different strains of virus (Figure 6).

TESTING DURING EXPERIMENTAL CHALLENGE

Nasal swabs were taken from animals undergoing experimental challenge with different strains of PPRV (Baron *et al*., 2014b). Swabs were eluted in buffer and tested on LFDs. Viral genome was measured in each eluate by real-time PCR to estimate the amount of virus in the sample. The test was able to detect excreted PPRV even before clinical signs were visible. Although the LFD result and the PCR test on the same swab agreed in general, they did not correlate perfectly in quantitative terms (e.g. compare samples 1 and 6 in Figure 7b), a point that is more fully discussed in Baron *et al*., 2014a.

In each of the cases shown in Figure 7, the result (Ct value) of real-time PCR for the PPRV genome is given for the same samples; samples that did not reach the threshold by cycle 40 (no Ct) would normally be considered negative by this test

CONCLUSIONS

The recombinant Ad5 vaccine is effective and would act as a DIVA vaccine. The full validation study has been published by Herbert *et al*. (2014). Further studies are ongoing to determine minimum effective dose and whether addition of an Adeno-PPRV-F recombinant improves the protective response. Eventually, long-term studies will be required to determine the duration of protection, and therefore the vaccination intervals under field conditions.

The lateral flow device pen-side test for PPRV has undergone larger-scale trials to validate its utility in the field and is sensitive and specific (Baron *et al*., 2014a).

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Jenner Institute, Oxford, United Kingdom, which made the recombinant adenoviruses, and the assistance of the animal units at the Pirbright Institute. This work was supported by the United Kingdom Department for International Development and the Biotechnology and Biological Sciences Research Council. 360

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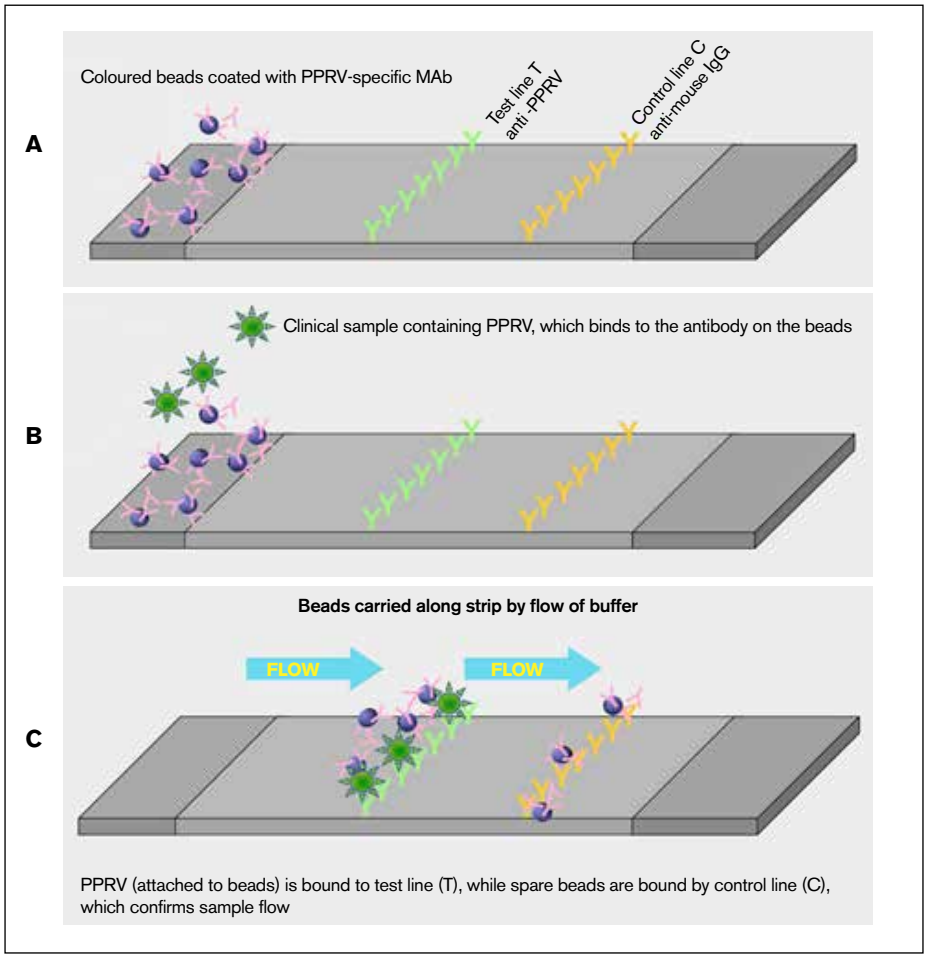

Figure 4: The pen-side test for PPRV
IgG = immunoglobulin G. MAb = monoclonal antibody.

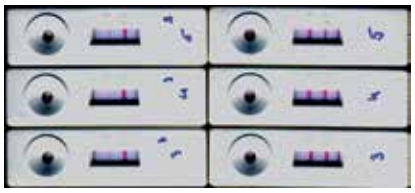

Figure 5: Detection of laboratory-grown PPRV by prototypes 3, 4 and 5

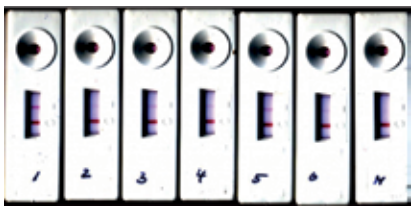

Figure 6: Detection of 10^3 $TCID_{50}$ of various strains of PPRV (representing all four lineages) by the pen-side test: 1 to 6 show different isolates of PPRV; M shows medium only

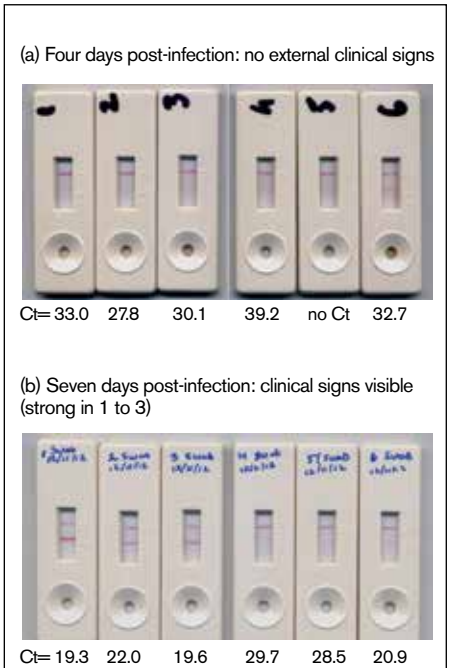

Figure 7: Testing of the pen-side test with infected animals using samples taken at: (a) four and (b) seven days post-infection

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TOOLS

Short communication: Peste des petits ruminants vaccine (Nigerian strain 75/1) confers protection for at least three years in sheep and goats

Contributors: Aamer Bin Zahur,[1] Hamid Irshad,[1] Aman Ullah,[1] Muhammad Afzal,[2] Asma Latif,[1] RiasatWasee Ullah,[1] Umer Farooq,[1] Muhammad Humayoon Samo,[2] Muhammad Jahangir,[1] Giancarlo Ferrari,[3] Manzoor Hussain[2] and M. Munir Ahmad[4]

SUMMARY

Peste des petits ruminants (PPR) is endemic in Pakistan and vaccine (PPRV strain 75/1 Nigeria) is used in sheep and goats to control the disease. The duration of immunity and protective efficacy of PPR vaccine were evaluated by experimentally vaccinating sheep and goats stationed at Barani Livestock Production Research Institute (BLPRI), Kherimurat, Pakistan. Post-vaccination antibody titres persisted beyond three years in vaccinated animals, which also withstood challenge with virulent PPRV and did not show any clinical signs or shed virus.

OBJECTIVE

The objective was to study the duration of immunity and protective efficacy of PPR vaccine in sheep and goats.

EXPERIMENTAL DESIGN

The studies was conducted on a total of 70 vaccinated animals, 5 and 6 animals were challenged at 24 and 36 months post-vaccination respectively using 10^3 50 percent tissue culture infected dose ($TCID_{50}$) per animal of a virulent field isolate. A total of 35 animals served as the control group. Serum samples were collected from both groups at monthly intervals for the first year, at two-monthly intervals in the second year and at three-monthly intervals in the third year. Levels of antibody to PPRV were determined using competitive enzyme-linked immunosorbent assay (c-ELISA). PPR virus shedding from challenged animals (control and vaccinated) was studied by examination of oral, ocular and nasal swabs and faecal material using RT-PCR for ten days post-challenge.

Table 1: Experimental groups for PPR vaccine study

Group	Sheep	Goats	Total	Dose
Vaccinated full dose	15	20	35	1.0 ml S/C
Vaccinated half dose	15	20	35	0.5 ml S/C
Unvaccinated control	15	20	35	Nil

Vaccine contained 102.5TCID50/ml of PPRV strain 75/1 Nigeria.

RESULTS

No statistical difference was observed using multiple regression analysis in the c-ELISA mean percent inhibition (PI) values between the animals that received a half dose or a full dose of PPR vaccine. No clinical evidence of PPR was seen when animals were challenged at 24 or 36 months post-vaccination in the vaccinated group. No virus shedding was detected in vaccinated animals following experimental challenge. However, virus shedding was observed in the control group until the end of the observation period (day nine post-challenge). All unvaccinated control animals developed clinical signs resembling PPR, and three out of four died, showing typical PPR lesions (erosions in the lower gums, pneumonic lungs, haemorrhages in the large intestine). PPRV was recovered from the spleen of one of the three dead animals in the unvaccinated control group.

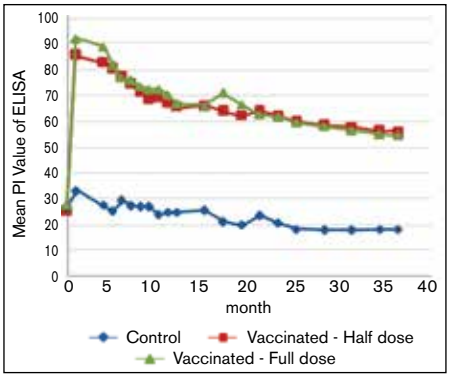

Figure 1: Mean percent inhibition (PI) values of PPR c-ELISA in sheep and goats following PPR vaccination experimental study

Table 2: Results from experimental study after challenging with PPR virus at 24 months post-vaccination

Group	ELISA title at the time of challenge (PI value)	Animals showing virus shedding	Animals with clinical signs	Mortality
Vaccinated	57, 60, 62	0/3	0/3	0/3
Unvaccinated	21, 18	2/2	2/2	2/2

Table 2: Results from experimental study after challenging with PPR virus at 36 months post-vaccination

Vaccinated	42, 49, 53, 57	0/4	0/4	0/4
Unvaccinated	16, 27	2/2	2.2	1/2

CONCLUSIONS

These preliminary results indicate that under experimental condition the vaccine used was potent and protected for at least three years. Larger trials are required to test vaccine efficacy under field conditions, but the current study is a good indication for the efficacy duration of the Nigerian strain 75/1 PPR vaccine against a recent Pakistan field isolate.

ACKNOWLEDGEMENTS

The authors acknowledge the assistance of FAO projects GCP/PAK/127/USA "Progressive Control of Peste des Petits Ruminants in Pakistan" and GTFS/INT/907/ITA "Controlling Transboundary Animal Diseases in Central Asian Countries" in undertaking this study. 360

[1] Animal Health Research Laboratories, Animal Sciences Institute, National Agricultural Research Centre, Islamabad, Pakistan
[2] Food and Agriculture Organization of the United Nations (FAO) project GCP/PAK/127/USA, Islamabad, Pakistan
[3] Food and Agriculture Organization of the United Nations (FAO), Rome, Italy
[4] Divisional Diagnostic Laboratory, Livestock and Dairy Development Department, Multan, Pakistan

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NEWS

Interview: "If you control a disease of small ruminants it is also a part of the fight against poverty"

Contributors: Adama Diallo[1]

These days a lot of effort is going into the monitoring and control of a number of viral diseases that threaten the lives of millions of livestock worldwide. As the demand for food production increases in order to satisfy the world's growing population, the need to protect livestock health becomes more and more vital. Peste des petits ruminants – PPR for short – is a disease of concern. It is often referred to as "goat plague". It is a highly contagious disease affecting small ruminants (sheep and goats). The disease has severe implications on the livelihoods of farmers and overall global food security. Dr Adama Diallo, the former Head of the Animal Production and Health Laboratory (APHL), Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, provides insights into PPR control and eradication issues. Dr Diallo, who has been working on PPR since 1984, developed attenuated PPR vaccines and several PPR diagnostic tests.

Interviewer: There are many transboundary and emerging animal diseases in different parts of the world, why is PPR the priority?

Diallo: PPR has huge social implications. Most of the world's poor people live in sub-Saharan Africa and Asia (Figure 1). If you look at the map of small ruminant density distribution, in Africa and Asia, it's similar to that of poverty (Figure 2). So control of any diseases related to small ruminants is also part of the fight against poverty. It is very important. PPR is the most important infectious disease of sheep and goats. The second reason is the expanding trend of PPR distributions. Every year, we have an increase in the number of countries infected by PPR. So PPR is becoming more and more important.

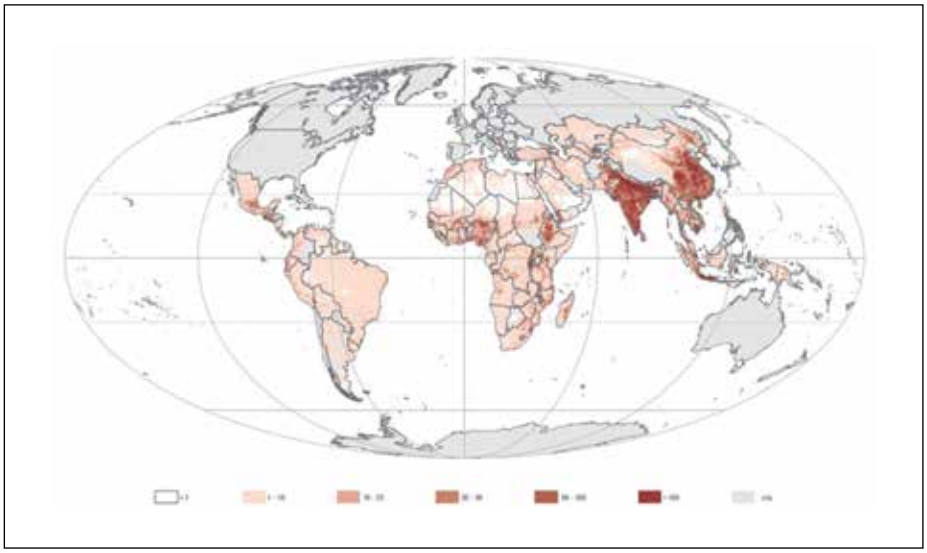

Figure 1: Distribution of poor population in developing countries, based on stunting among children
Source: FAO, 2010.

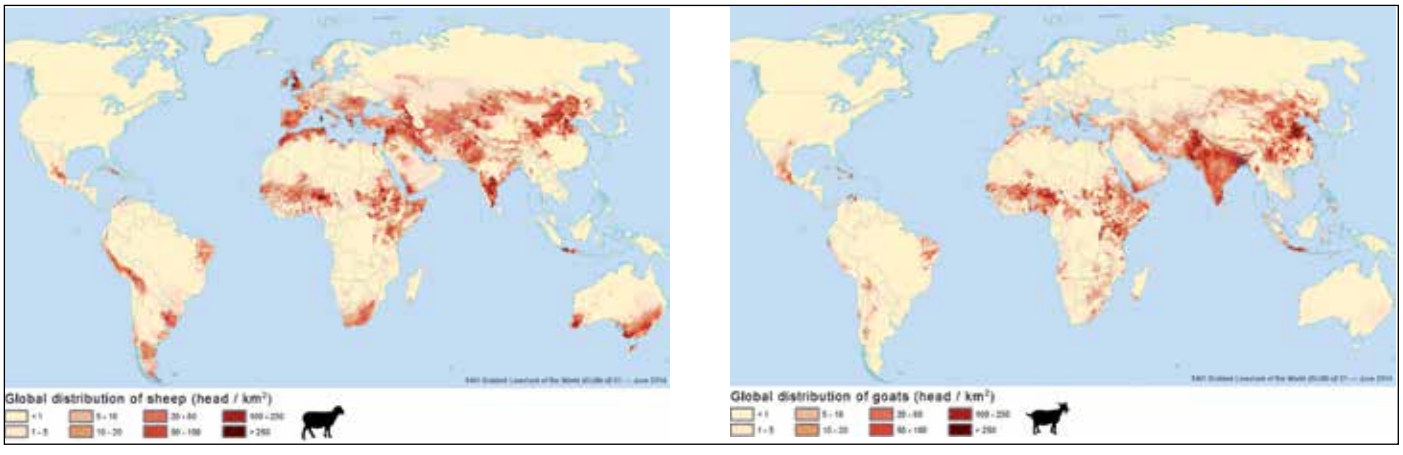

Figure 2: Distribution of sheep (left) and goats (right) from - Gridded Livestock of the World v 2.01
Source: FAO, 2014.

[1] Former Head, Animal Production and Health Laboratory (APHL), Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture

Children guarding a herd of sheep awaiting vaccination from a visiting veterinarian, Burkina Faso

Interviewer: How widespread is the disease?
Diallo: At the moment, more than 70 countries have declared PPR to the World Organisation for Animal Health (OIE). If you take this number, and the 80 or 90 countries at risk, more than 1 billion sheep and goats are at risk of PPR. It is a huge number.

Interviewer: FAO and OIE have joined forces once before to eradicate rinderpest also called cattle plague. Are there any lessons from the thirty year-long rinderpest campaign that will help PPR eradication?
Diallo: Yes, in fact it was more than thirty years, but the thirty years you are referring to is the last campaign against rinderpest. We considered the lessons from this campaign in launching the PPR eradication campaign. What are the lessons? First, let me mention the vaccine. The rinderpest vaccine has been successful (it provided life-long immunity); we have a similar vaccine against PPR. The technique for diagnosing rinderpest used in the rinderpest eradication campaign was very efficient, and we have a similar technique for PPR diagnosis. The most important lesson learned is the need for political commitment; and the second is the coordination of activities. The rinderpest campaign succeeded because we had very strong political commitment and we had also good coordination of activities led by FAO, with funds for the programme.

> “the demand for food production increases in order to satisfy the world’s growing population, the need to protect livestock health becomes more and more vital”

Interviewer: At this conference, FAO and OIE will launch the global initiative to eradicate PPR by 2030. What are the next steps for the conference participants?
Diallo: The next step is to convince all stakeholders that eradication is possible, to have political commitment. Some countries have started to control PPR. FAO and OIE will coordinate all these individual activities with partners. With support from donors, FAO and OIE will commence coordinating implementation of the strategy.

Interviewer: Dr Diallo, thank you very much for your time.
Audio available at: http://www.fao.org/news/audio-video/detail-audio/en/?uid=11056

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NEWS

PPR Roadmap Meeting for Eastern Africa

Contributor: Bouna Diop[1]

The PPR Roadmap Meeting for Eastern Africa was held in October 2014 in Kigali, Rwanda, within the framework of the FAO and OIE Global Framework for Transboundary Animal Diseases (GF-TADs) and in collaboration with the Government of Rwanda. Representatives from Burundi, the Democratic Republic of the Congo, Djibouti, Eritrea, Kenya, Rwanda, Somalia, South Sudan, the Sudan, Uganda and the United Republic of Tanzania, and participants representing the Intergovernmental Authority on Development (IGAD), the African Union Interafrican Bureau for Animal Resources (AU-IBAR), the African Union Panafrican Veterinary Vaccine Centre (AU-PANVAC), the Botswana Vaccine Institute (BVI), Merial and FAO attended the meeting. As the PPR eradication campaign is gaining a momentum that needs to be sustained, the meeting recommended that each country organize a national workshop with key stakeholders to ensure alignment of its national PPR plan with the regional and global strategy; organize national PPR consultations alongside implementation of its PPR control programme; and participate in an annual regional PPR consultation meeting to discuss progress made and identify constraints and challenges to be addressed. Countries where no PPR has been reported for more than three years should formulate a contingency plan and start preparing a dossier for recognition as PPR-free. The meeting recommended that FAO/AU-IBAR assist all Regional Economic Communities (RECs) on the harmonization of their PPR strategies, taking into consideration the PPR Global Strategy. The importance of socio-economic studies providing evidence of disease impact on vulnerable rural livelihoods was recognized. Studies can provide support and justification for investments in PPR control. Considering the importance of vaccine in PPR control, current capacity for PPR vaccine production of good quality in Africa was discussed. Countries should evaluate their needs for PPR vaccines over the next three years; at the same time, AU-PANVAC needs to sustain current efforts to ensure that good-quality PPR vaccines are produced and used. The meeting recommended necessary actions to be taken at the country level to ensure the participation of key national stakeholders in the PPR international conference in March 2015 in Côte d'Ivoire. Although the PPR progressive control phases outlined in the draft PPR global strategy need to be elaborated further, to develop the first PPR Roadmap for Eastern Africa, participants assigned themselves as follows: phase 2 (control and eradication) – the Democratic Republic of the Congo, Eritrea, Kenya, Somalia, South Sudan, the Sudan, Uganda and the United Republic of Tanzania; and phase 3 (verification) – Burundi, Djibouti and Rwanda. The meeting also discussed other small ruminant diseases to be considered with PPR control programmes in the region. The meeting recommended that in Eastern Africa, sheep and goat pox, CCPP and RVF be incorporated according to countries' needs. 360

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A pastoral farmer herding sheep, Kenya

[1] Food and Agriculture Organization of the United Nations, Nairobi, Kenya

CONTACTS @ HQ

FAO-EMPRES, Rome, Italy
fax: (+39) 06 57053057
e-mail: empres-animal-health @fao.org

Guillaume Belot
Veterinary Epidemiologist
tel: (+39) 06 57054878
e-mail: Guillaume.Belot@fao.org

Daniel Beltrán-Alcrudo
Animal Health Officer (Disease Ecology)
tel: (+39) 06 57053823
e-mail: daniel.beltranalcrudo@fao.org

Gwenaëlle Dauphin
Lead, EMPRES Laboratory Unit
OFFLU focal point
tel: (+39) 06 57056027
e-mail: gwenaëlle.dauphin@fao.org

Ian Douglas
Senior Officer
Crisis Management Centre - Animal Health (CMC-AH) manager
tel: (+39) 06 57055184
e-mail: ian.douglas@fao.org

Katinka de Balogh
Senior Officer (Veterinary Public Health)
tel: (+39) 06 57056110
e-mail: katinka.debalogh@fao.org

Ahmed El Idrissi
Senior Officer (Bacterial and Zoonotic diseases)
Lead, Programming Unit
tel: (+39) 06 57053650
e-mail: ahmed.elldrissi@fao.org

Giancarlo Ferrari
Animal Health Technical Expert
tel: (+39) 06 57056691
Giancarlo.Ferrari@fao.org

Akiko Kamata
Animal Health Officer
tel: (+39) 06 57054552
e-mail: akiko.kamata@fao.org

Sergei Khomenko
Disease Ecology / Wildlife
tel: (+39) 06 57056493
e-mail: sergei.khomenko@fao.org

Fairouz Larfaoui
Disease Intelligence / GLEWS/FAO
tel: (+39) 06 57053331
e-mail: fairouz.larfaoui@fao.org

Caryl Lockhart
Veterinary Epidemiologist / GLEWS/FAO
tel: (+39) 06 57054946
e-mail: caryl.lockard@fao.org

Raffaele Mattioli
Senior Officer (Non-Infectious Diseases)
tel: (+39) 06 57056078
e-mail:raffaele.mattioli@fao.org

Samia Metwally
Animal Health Officer (Virology)
tel: (+39) 06 57055838
e-mail: samia.metwally@fao.org

Béatrice Mouillé
EMPRES Laboratory Unit, Assistant Coordinator
tel: (+39) 06 57054456
e-mail: beatrice.mouille@fao.org

Cecilia Murguia
Information Management
tel.: (+39) 06 57056520
e-mail: cecilia.murguia@fao.org

Felix Njeumi
Animal Health Officer (Disease Management)
tel: (+39) 06 57053941
e-mail: felix.njeumi@fao.org

Elisa Palamara
Data management
tel: (+39) 06 57055124
Elisa.Palamara@fao.org

Julio Pinto
Animal Health Officer (Epidemiology)
Global Early Warning System (GLEWS/FAO)
tel: (+39) 06 57053451
e-mail: julio.pinto@fao.org

Claudia Pittiglio
Disease Ecology (Spatial Modelling)
tel: (+39) 06 57056371
e-mail: Claudia.Pittiglio@fao.org

Ludovic Plee
Veterinary Epidemiologist / CMC-AH
tel: (+39) 06 57055206
e-mail: ludovic.plee@fao.org

Eran Raizman
Senior Officer
Head, EMPRES
tel: (+39) 06 57052360
e-mail: Eran.Raizman@fao.org

Keith Sumption
Secretary
European Commission for the Control of Foot-and-Mouth Disease (EUFMD)
tel: (+39) 06 57055528
e-mail: keith.sumption@fao.org

Sophie von Dobschuetz
Veterinary Epidemiologist
tel: (+39) 06 57053717
e-mail: sophie.vondobschuetz@fao.org

James Zingeser
Veterinary Epidemiologist (zoonoses)
tel: (+39) 06 57055918
e-mail: james.zingeser@fao.org

CONTACTS @ AFRICA

Charles Bebay
Regional Manager a.i
Emergency Centre for Transboundary Animal Disease Operations (ECTAD)
Western and Central Africa
Regional Animal Health Centre for West and Central Africa
Bamako, Mali
tel: (+ 223) 20 24 92 93
e-mail: Charles.Bebay@fao.org

Berhanu Bedane
Animal Production and Health Officer
FAO Regional Office for Africa - West Africa Team
Accra, Ghana
tel: (+233) 0302675000/ 0307010930 ext. 3144
e-mail: behanu.bedane@fao.org

Mohammed Bengoumi
Animal Production and Health Officer
FAO Sub-Regional Office for North Africa
Tunis, Tunisia
tel: (+216) 71903236 ext. 236
mohammed.bengoumi@ fao.org

Oumar Diall
Animal Production and Health Officer
FAO Sub-Regional Office for Eastern Africa
Addis Ababa, Ethiopia
tel: (+251) 11 5517230/33
e-mail: oumar.diall@fao.org

Bouna Diop
Regional Manager
Emergency Centre for Transboundary Animal Disease Operations (ECTAD)
Eastern Africa
Regional Animal Health Centre for East Africa
Nairobi, Kenya
tel: (+254) 20 7625926
e-mail: bouna.diop@fao.org

Cheikh Ly
Animal Production and Health Officer
FAO Regional Office for Africa
Accra, Ghana
tel: (+233) 0302 675000 ext. 2502
e-mail: cheikh.ly@fao.org

Patrick Otto
Animal Production and Health Officer
FAO Sub-Regional Office for Southern Africa
Harare, Zimbabwe
tel: (+263) 4 253 655 – 8 ext.257
e-mail: patrick.otto@fao.org

CONTACTS @ Latin America and the Caribbean

Deyanira Barrero León
Regional Coordinator for Foot-and-Mouth Disease
Santiago, Chile
tel: (+56) 2 9232206
e-mail: deyanira.barrero@fao.org

Tito E. Díaz Muñoz
Senior Animal Production and Health Officer
FAO Regional Office for Latin America and the Caribbean. Santiago, Chile
tel: (+56) 2 3372250
e-mail: tito.diaz@fao.org

Cedric Lazarus
Livestock Development Officer
Sub-Regional Office for the Caribbean
Bridgetown, Barbados
tel: (+246) 4267110 ext. 245
e-mail: cedric.lazarus@fao.org

CONTACTS @ Asia

Carolyn Benigno
Animal Health Officer
FAO Regional Office for Asia and the Pacific,
Bangkok, Thailand
tel: (+66) (0)2 6974330
e-mail: carolyn.benigno@fao.org

Wantanee Kalpravidh
ECTAD Regional Manager
Emergency Centre for Transboundary Animal Disease Operations (ECTAD) Asia and the Pacific
Bangkok, Thailand
tel: (+66) (0)2 (697) 4231
e-mail: wantanee.kalpravidh@fao.org

Joachim Otte
Senior Animal Production and Health Officer
Secretary of the Animal Production and Health Commission Asia and the Pacific (APHCA)
FAO Regional Office for Asia and the Pacific,
Bangkok, Thailand
tel: (+66) (0)2 6974326
e-mail: joachim.otte@fao.org

CONTACTS @ Joint FAO/IAEA Division

Gerrit Viljoen
Head, Animal Production and Health Section
Vienna, Austria
tel: (+43) 1 260026053
e-mail: g.j.viljoen@iaea.org

CONTACTS @ Europe and Central Asia

Abdulbaqi Mehraban
Animal Production and Health Officer
FAO Subregional Office for Central Asia
Ankara, Turkey
tel: (+90) 312 3079500
e-mail: abdulbaqi.mehraban@fao.org

Andriy Rozstalnyy
Animal Production and Health Officer
FAO Regional Office for Europe and Central Asia. Budapest, Hungary
tel: (+ 36) 1 4612025
e-mail: Andriy.Rozstalnyy@fao.org

CONTACTS @ Near East

Nacif Rihani
Livestock Development Officer
Sub-Regional Office for the Gulf Cooperation Council States and Yemen
Abu Dhabi, United Arab Emirates
tel: (+971) 2 6586774
e-mail: Nacif.Rihani@fao.org

Markos Tibbo
Livestock Officer
Animal Production and Health Officer, OiC.
FAO Regional Office for the Near East.
Cairo, Egypt
tel: (+202) 3331 6143/6000 ext. 2803
e-mail: markos.tibbo@fao.org

Yilma Jobre Makonnen
Team Leader
ECTAD Egypt
Cairo, Egypt
tel: (+20) 23 3316000 (Ext. 2511)
e-mail: yilma.makonnen@fao.org

empres360 animal health CONTACT

The Emergency Prevention System (EMPRES) is an FAO programme, founded in 1994, with the goal of enhancing world food security, fighting transboundary animal and plant pests and diseases and reducing the adverse impact of food safety threats. EMPRES-Animal Health is the component dealing with the prevention and control of transboundary animal diseases (TADs).

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