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MOLECULAR EPIDEMIOLOGICAL STUDY OF AVIAN INFLUENZA AND NEWCASTLE DISEASE VIRUSES IN ETHIOPIA (GRIPAVI Project Proposal, France)

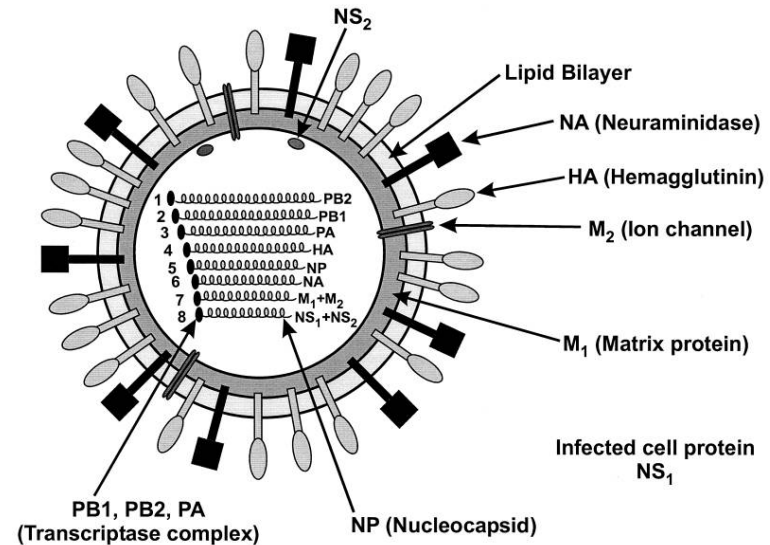
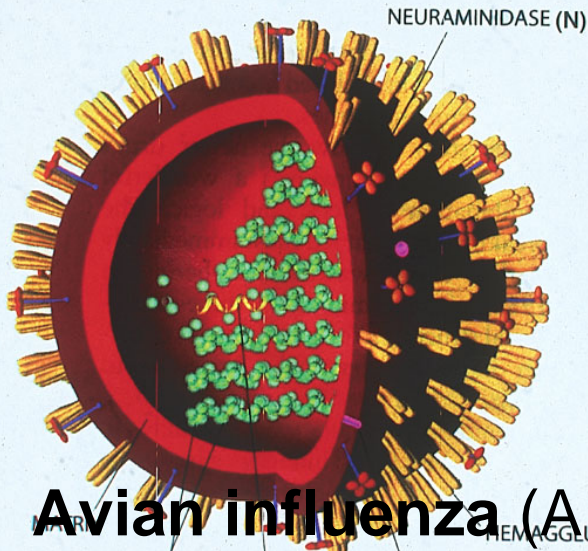
By

**Melesse Balcha, Mesfin Sahle, Abraham Gopillo and Fikadu Shiferaw
(NATIONAL ANIMAL HEALTH DIAGNOSTIC AND INVESTIGATION CENTER (NAHDIC))**

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Introduction

Influenza Virus

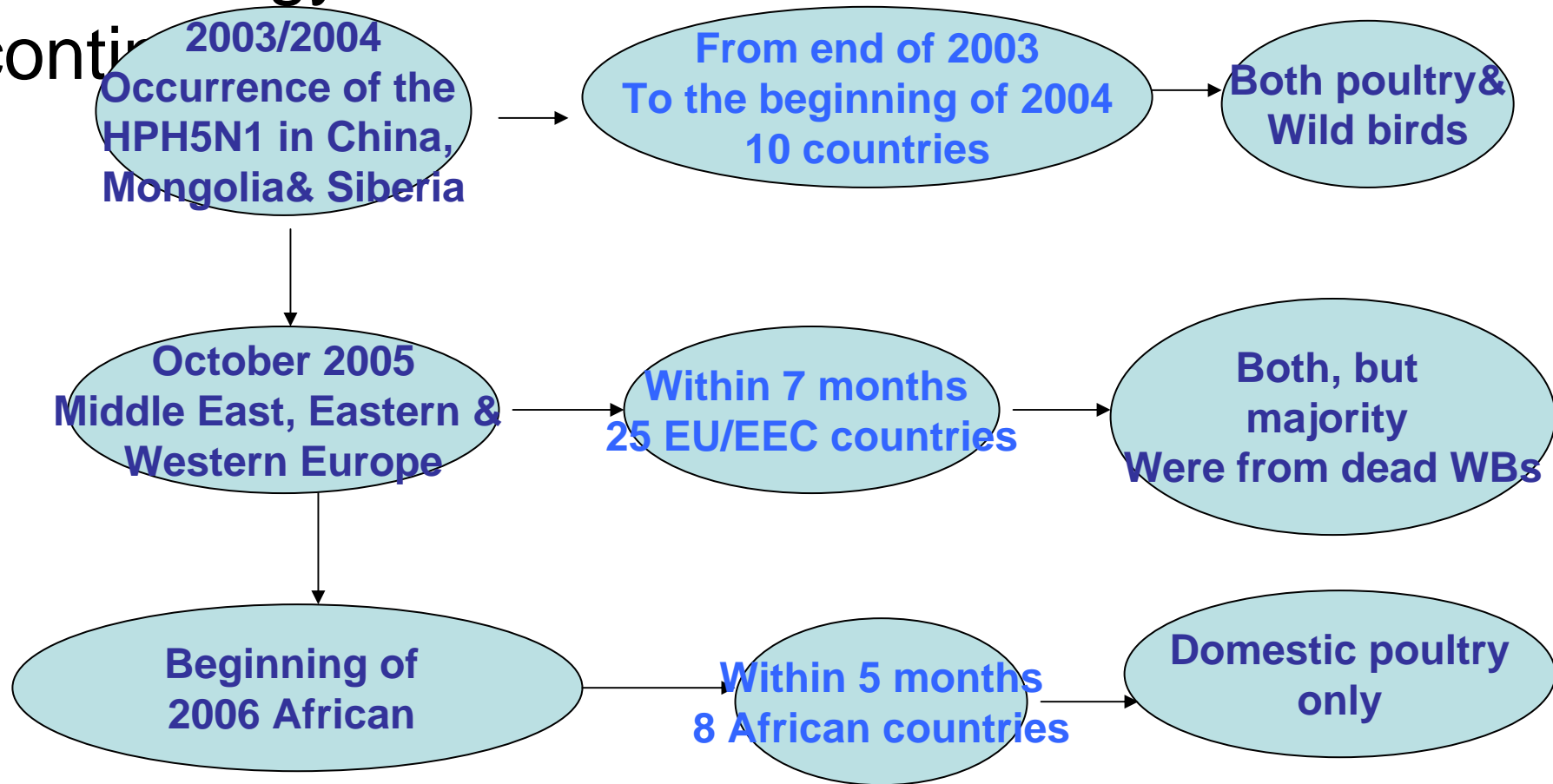


- **Avian influenza (AI)** is a disease of viral etiology ranging mild or even asymptomatic infection to an acute, fatal disease of chickens, turkeys, guinea fowls, and other avian species, especially migratory waterfowl. The virus particle has an envelope with glycoprotein projections with HA and NA activity.
- These two Ags (HA) and (NA): basis of the serologic identity of the AIV designated as H and N with the appropriate numbers in the virus designation.
- 16 HA and 9 NA antigens of Avian Influenza viruses.

Epidemiology

Distribution and occurrence

- Chronology of occurrence of H5N1 in 3 continents





Two major routes of dissemination of the virus is through

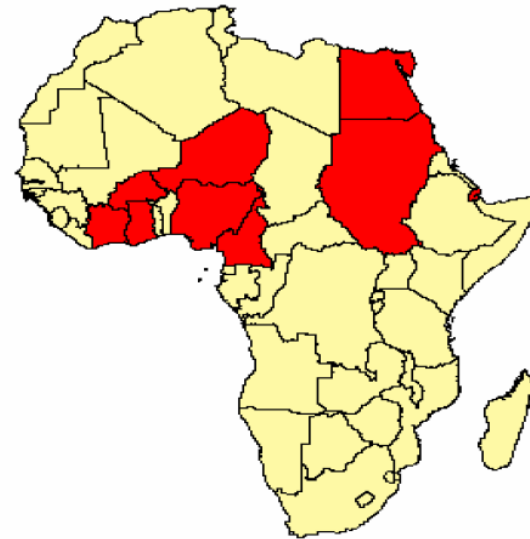
- international trade of wild birds, poultry, poultry products, etc and
- wild bird migration

Ethiopia with regard to HPAI is a non-infected but a country with high risk.

WHY? because of:

- Its **proximity to already infected** countries (look fig 2 above)
- Its **risky trade** relationships with high risk countries
- The **huge population and various species of migratory birds hosted** every year
- Limited capacity and infrastructure in the country to identify the disease early for prompt action.

Confirmed HPAI outbreaks in Africa
Since January 2006



- Burkina Faso
- Cameroon
- Cote d'Ivoire
- Djibouti
- Egypt
- Ghana
- Niger
- Nigeria
- Sudan



Poultry Production and Poultry trade in Ethiopia

- Poultry production systems

a. Village or backyard production:

98 -99% local breed.

Birds are owned by individual households and are maintained under a **scavenging system**,

The main purpose of production being:

eggs for hatching (51.8%),
sale (22.6%) and
home consumption (20.2%), and
sacrifice (healing ceremonies) (25%),

The level of biosecurity is low or nonexistent.

b. Small scale production system:

500 to 10000 birds indoor and outdoor with a low biosecurity level

c. Commercial poultry production system:

an average of 10000 birds kept in door with a medium to high biosecurity level. These are mostly privet.

There are **several poultry multiplication and rearing** centers from the government based in rural area. These are part of a genetic improvement program and they distribute to farmers pullets and day old chickens of improved breeds

Regional Poultry production and multiplication centers

Name	Region	Annual production / distribution			Feed in ton
		Fertile egg	Day old chicks	Pullets and cokes	
Mekele	Tigray	900,000	180,000	100,000	8000
Kombolcha	Amhara	3,500,000	360,000	140,000	15,000
Andassa	Amhara	1,080,000	180,000	46,800	8,000
Bedele	Oromia	627,544	108,838	27,000	8,000
Nathreth	Oromia	388,331	113,373	54,000	8,000
Adele	Oromia	397,485	113,939	18,000	
Awassa	SNNP	1,000,000	180,000	100,000	8,000
Sum		7 893 360	1 236 150	485 800	55 000

Poultry (day old chicks) rearing centers

Name	Region	Number / year
Fitcha	Oromia	7200
Ambo	Oromia	9000
Nekemt	Oromia	9000
Assela	Oromia	
Diredawa	Diredawa	3,000
Sodo	SNNP	9,000
Gubrie	SNNP	
Sum		37 200

TRADE POULTRY, Ethiopia

- Before outbreaks of H5N1 in Africa Ethiopia has been importing DOC from Netherlands, Kingdom of Saudi Arabia, Egypt, Kenya and Germany
- **Local trade** free movement & trade of chickens in the country.
- A chicken can have the chance to move for more than 250-500km in the country. But the chickens from multiplication centers are disseminated throughout the country.

Importation 2005-2006

Year	Month	Exporting country	Quantity	Year	Month	Exporting country	Quantity
2005	Jan	E	21 000	2005	August	KSA	16 640
	Feb	E	130 000			N	14 400
		N	15 500		September	K	90 000
		UK	60 000			E	5 000
		KSA	7 000			KSA	39 5000
	March	KSA	7 000		October	N	30 000
	April	N	15 440			KSA	39 500
		KSA	30 000			E	5 000
	May	E	12 000	2006	December	K	7 500
		N	25 008		Jan	K	7 500
2006	June	N	15 500			UK	11 000
		E	68 800		Feb	N	61 680
	July	E	68 800			E	8 400
		KSA	81 200			N	53 900

E = Egypt PB = Netherlands UK = Royaume Unis AS = Kingdom of Arabia Saoudia K = Kenya

Wet lands and Migratory birds in Ethiopia

- **Wetland areas of Ethiopia**
- Topographical variability and several wetlands; “Wetland” may mean fresh or saline lakes, rivers, springs, ponds, temporary or permanent swamps, mangrove forests and coral reefs.
- The Great Rift Valley passes through Ethiopia, creating a chain of lakes and ponds that serve as feeding and maintenance stations for its flying visitors “migratory birds”.
- **There are 69 identified major important wetland bird areas in the country. Out of which 45 are selected as focus area for HPAI disease prevention.**

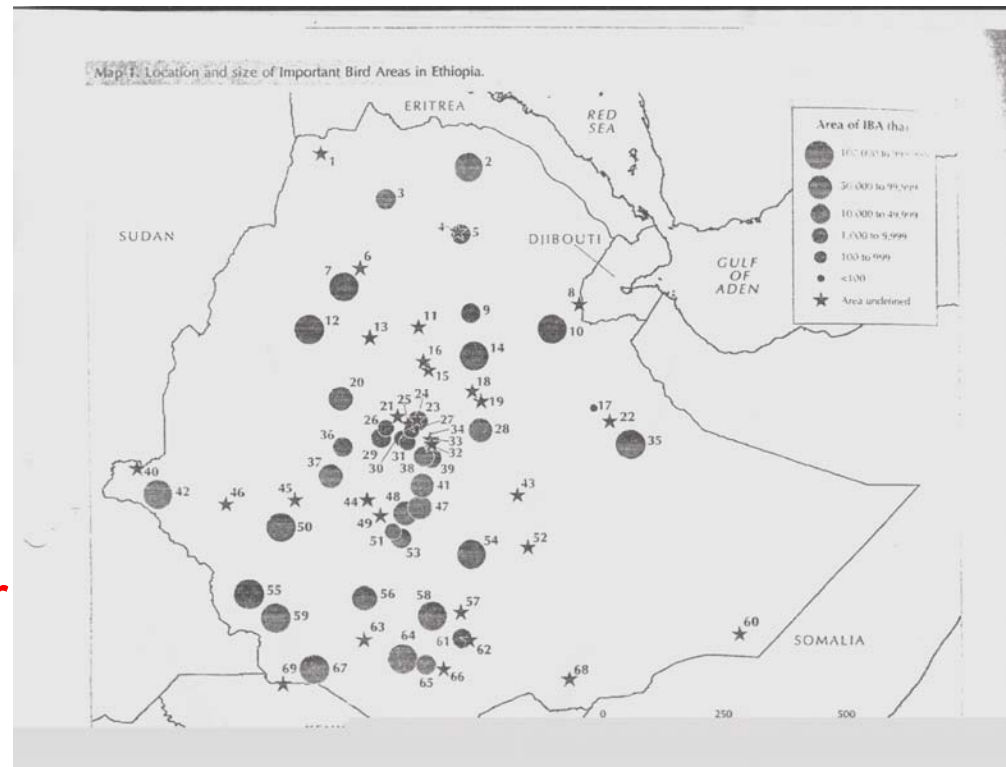


Fig 3: Wetland Areas of Ethiopia hosting the migratory birds

Migratory Routes and Birds

- **Wetland Birds of Ethiopia**
- Ethiopia has 862 identified bird species. Of these 177 are obligate WLS in one form or another.
- The wetland birds of Ethiopia fall into 11 orders, 31 Families, and 88 Genera.
- 90 wetland bird species are resident.
- 68 are Palaearctic migrants 8 of which have resident races.
- 4 of the total list are intra-African migrants.
- Lakes Abjata and Shalla possess about 50% of wetland birds of the country (MoARD, Wild Life Unit).



Activities related to HPAI surveillance and diagnosis in Ethiopia



- Since October 2005 two phases of surveillance conducted.
- phase I October 2005 to June 2006 about 1000 samples have been collected both from poultry and birds and all tested.
- Phase II 8156 samples were collected and 2600 of them were tested.
- No detection of isolate HPAI but only H8N4 from white faced whistling duck around the southern Ethiopia.

Available data on LPAI and Situation of NDV

- Only H8N4 is known to exist in the country
- 2005-mid2006 there was high mortality in pigeons of the country the cause of these was found to be Pigeon Paramyxovirus Type 1 (PPMV1) of pathogenic strain
- From other outbreaks in poultry west Ethiopia, Assossa two strains of NDV had been isolated > 97% identical to isolates in Saudi Arabia, Kenya and Sudan.
- There are only 2 other isolates of NDV known in the country, 1 is vaccinal and imported



Challenges

- At beginning there was lack of knowledge & skill to manage the dx but this has been gradually solved.
- Some problems still exist including:
- the incompleteness of a laboratory with required BSL to handle viruses,
- Incompleteness of SPF unit,
- knowledge and skill to handle, identify species and sample wild birds, etc.,
- Previous surveillances were financed by FAO/ USAID, but now in August the FAO project will terminate after
- This time there will not be any other dependable source of fund to undergo surveillance



- We appreciate the initiation from French GFA and CIRAD to assist our countries to combat HPAI
- This will be a blessed decision of this country to focus on Africa



Therefore

Molecular Epidemiology of AI & NDV was proposed

- **Objectives** will be:
- Early detection of any HPAI in the country for prompt action to be put in place
- Isolation and molecular characterization of AIV&NDV circulating in the country,
- Assessment of risk factors contributing for introduction, dissemination and maintenance of HPAI in Ethiopia
- Building Capacity of NAHDIC in diagnosis surveillance and communication of Scientific results/findings
- Experimental study of virulence of PPMV1 to poultry to determine if they can be the cause of outbreaks in poultry

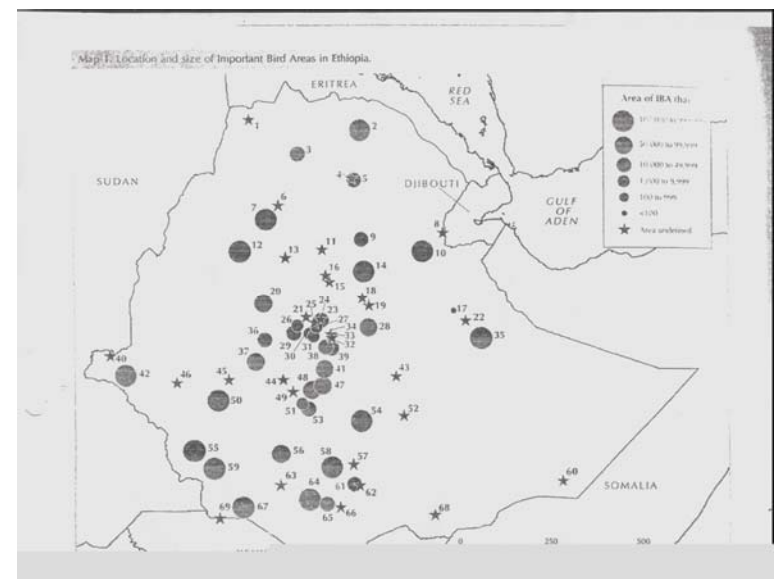
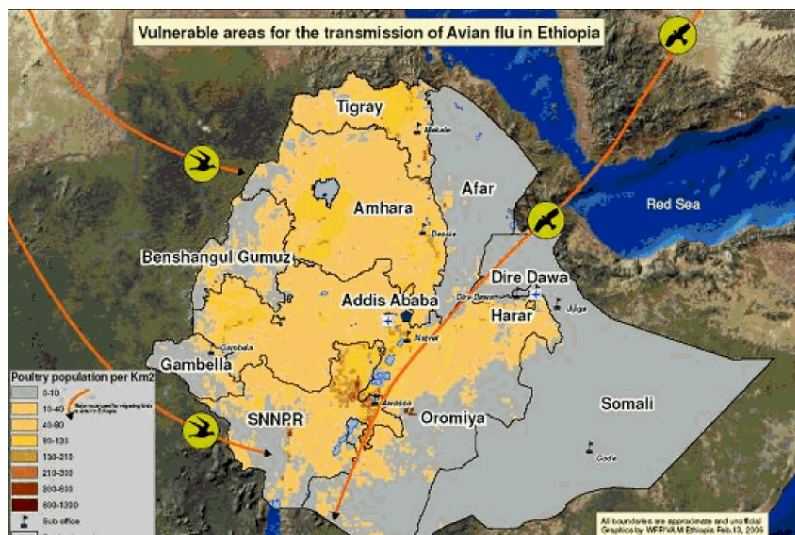
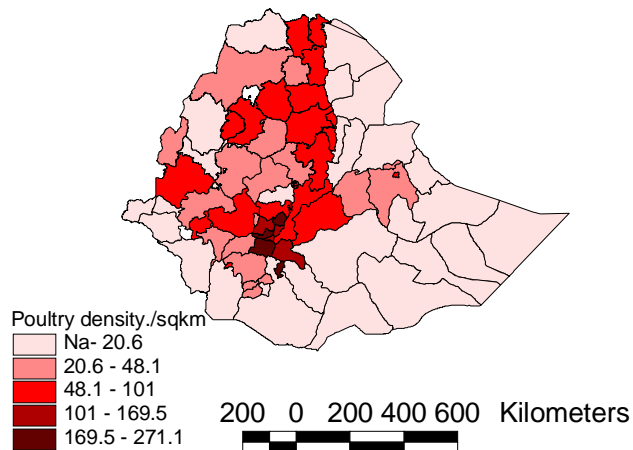


Forwarded Activities

the ff activities will be carried out:

- active & passive surveillance in domestic & non-domestic birds
 - Isolation of viruses of both Newcastle & AI (LPAI& HPAI)
 - Molecular Characterization of both NDV and AI viruses
 - Conducting experimental virulence study on chickens by challenging with PPMV1 isolates
 - Evaluating the protective capacity of the vaccines used in the country against ND viruses circulating in the country
 - Preparation of antigen and anti-sera from isolates and evaluation for using as diagnostic reagents
 - Risk Assessment on factors of introduction, establishment and dissemination of HPAI. Identification of wild birds gathering places, sites of high interaction with domestic chickens and multiple spp. Modeling with CIIRAD

Poultry density per square km area





Methodologies cont...

- **Study Animals**
- **Poultry** backyard, commercial farms and government poultry multiplication centers with focus in live bird market, poultry kept near wetland sites and poultry in selected border areas will be targeted.
- **Non-domestic birds** water fowls, non domestic resident and wild birds. For the random surveillance migratory water fowls will be targeted while for outbreak report based surveillance any of the non domestic birds will be targeted.
- **Study type**
 - I. Surveillance** (both active & passive)
 - Structured Random surveillance (expected hot spot areas)
 - Outbreak Report based surveillance
 - II. Risk Assessment:** both qualitative and quantitative risk with special emphasis on the assessment and quantification of epidemiological and managerial factors that contribute for the introduction, maintenance and spread of the disease in the country. Modelling will be done in collaboration.

METHODOLOGIES con...



III. Experimental study on virulence of the PPMV1 on poultry:

- For this SPF or antibody free chickens will be used, grouped into treatment and control groups. They will be challenged with the already existing and other isolates of PPMV 1 and followed and evaluated. This will be to determine if the pigeon NDV can cause outbreaks of NDV in poultry.

IV. Evaluation of the protective capacity of the NDV vaccines produced in the country against different isolates from the country. will be carried out in collaboration with the NVI experts.

V. Antigen and antisera preparation

This will be done in collaboration with CIRAD and NVI.

Sample Collection: will be following the recommendations of EU Directive 2005/94/EC using antibiotic medium /brain-heart-infusion medium. Both cloacal and tracheal samples in duplicate and a pool of 4-5. Fresh fecal droppings. Tissue samples will be preferable in case of mortality.



METHODOLOGIES con...

- **Data collection**

Clinical, epidemiological and Georeferenced data will be collected from every sampling area

Data Analysis

- **LABORATORY DIAGNOSTIC METHODS**

laboratory routines will be:

- Virus isolation followed by HA and HI tests and parallel to this RT PCR or rRT PCR.
- Those samples expected to be positive will be sent to CIRAD and Weybridge, UK, for confirmation and sequencing.

Budget request and schedule



THANK YOU