CONTENIDO DEL INFORME TÉCNICO
CONSULTORES CALIFICADOS

1. Antecedentes de la Propuesta

Título: Apoyo experto para emergencia sanitaria por Influenza Aviar, en acciones de campo y de diagnóstico de laboratorio

Código: FIA-CO-V-2002-1-P-07

Entidad Responsable: Servicio Agrícola y Ganadero (SAG)

Coordinador: Rubén Moreira Zúñiga

Nombre y Especialidad del Consultor

Dra. Ilaria Capua

Viróloga experta del Laboratorio de Referencia de la Oficina Internacional de Epizootias (O.I.E.), para la Enfermedad de Newcastle e Influenza Aviar. Istituto Zooprofilattico Sperimentale delle Venezie, Laboratorio Virología.

Dr. Stefano Marangon

Epidemiólogo experto, Jefe de la Unidad Regional de Epidemiología. Istituto Zooprofilattico delle Venezie.

Dra. Janice Pedersen

Microbióloga del National Veterinary Service Laboratories (NVSL). Ames, Iowa, USA.

Lugar de Origen del Consultor (País, Región, Ciudad, Localidad)

Dra. Capua: Italia, Región Veneto, Ciudad de Legnaro.
Dr. Marangon: Italia, Región Veneto, Ciudad de Legnaro.
Dra. Pedersen: USA, Iowa, Ames

Lugar (es) donde se desarrolló la Consultoría (Región, Ciudad, Localidad)

Región Metropolitana: Santiago
VI Región: Rancagua
V Región: San Antonio
Región Metropolitana, Santiago, Pudahuel, Laboratorio de Lo Aguirre SAG.

Fecha de Ejecución

17 al 25 de julio de 2002 (Dra. Capua y Dr. Marangon)
06 al 11 de agosto de 2002 (Dra. Pedersen) (**)

(**) por razones de servicio de la Dra. Pedersen, hubo de retrasar su arribo al país, razón por lo cual, en vez de concluir el 9.08.02, esta concluyó el día 11 de agosto de 2002.

Proponentes: presentación de acuerdo al siguiente cuadro:

Nombre	Institución/Empresa	Cargo/Actividad	Tipo Productor (si corresponde)
Hernán Rojas Olavarría	SAG	Jefe Departamento Protección Pecuaria	
Rubén Moreira Zúñiga	SAG	Jefe Campaña Emergencia Influenza Aviar	
Patricia Avalos Moreno	SAG	Jefa Subdepartamento Laboratorio y Estación Cuarentenaria Pecuaria	
Christian Mathieu Benson	SAG	Virologo	

Problema a Resolver:

Auditar las actividades que desarrolla el Servicio Veterinario Oficial (SAG), en el marco de la emergencia sanitaria de Influenza Aviar, en sus aspectos de acciones de campo, estrategia de control y erradicación, apoyo en diagnóstico de laboratorio y técnicas implementadas, agilizando el diagnóstico de Influenza Aviar, al realizar la sub-tipificación en el país y no enviar muestras para estos fines a laboratorios de referencia, con el consecuente retraso en los resultados.

Objetivos de la Propuesta

Apoyar al SAG en la estrategia de abordaje de la emergencia sanitaria por Influenza Aviar, a partir de la experiencia de destacados expertos internacionales en el tema. Además de fortalecer la capacidad y diversidad diagnóstica de la enfermedad, montando las técnicas de Inhibición de la Hemoaglutinación (H5, H7 y H9) e Inhibición de la Neuroaminidasa (N2 y N3) para la subtipificación de virus y sueros para la Influenza Aviar

2. Antecedentes Generales:

La Dra. Capua tiene una destacada trayectoria en el diagnóstico de enfermedades virales en aves, siendo actualmente la Jefa del laboratorio de Referencia de la OIE para la Influenza Aviar y Enfermedad de Newcastle. Su trabajo ha destacado en las áreas de virología, diagnóstico y biotecnología, correspondiéndole participar activamente en los brotes de Influenza Aviar que afectaron a Italia.

El Dr. Marangon, es epidemiólogo y Jefe de la Unidad Regional de Epidemiología de la Región de Veneto (Italia), participando como responsable en las campañas de Influenza Aviar en Italia en 1997 y 1999-2000. Tiene una gran experiencia en control y erradicación de enfermedades.

La consultora, Dra. Pedersen es la profesional a cargo de la subtipificación de los sueros y virus Influenza Aviar en la sección de Virología Aviar de los laboratorios federales del USDA, localizados en Ames Iowa. La Dra Pedersen ha desarrollado su carrera en esta institución por varios años y ha estado trabajando en este campo en todos los focos de Influenza Aviar de su país incluyendo el foco actual en el estado de Virginia.

3. **Itinerario desarrollado por el Consultor**: presentación de acuerdo al siguiente cuadro:

Dra. Capua y Dr. Marangon				
Fecha	Ciudad y/o Localidad	Institución/ Empresa	Actividad Programada	Actividad Realizada
17-07-02	Santiago	SAG	Arribo al país de los consultores	realizado
	Santiago	SAG	Reunión de trabajo con epidemiología SAG	realizado
18-07-02	Santiago	SAG	Reunión de trabajo con epidemiología SAG	realizado
19-07-02	Santiago	SAG	Reunión de trabajo con epidemiología SAG	realizado
20-07-02	San Antonio	SAG	Visita a planteles de zona amagada por Influenza Aviar	realizado
	Malvilla – San Juan	Ariztía	Reunión de trabajo con staff técnico y directivo de la empresa en predios Miltil y Tremolén	realizado
	Pelancura	SAG	Visita a Centro de Operaciones de Zona de Emergencia Influenza Aviar	realizado
22-07-02	Rancagua	Agrosuper	Reunión de trabajo en oficina Central de empresa con staff técnico y directivo, además de visita a matadero de aves Lo Miranda.	realizado
	Santiago	SAG	Visita a laboratorio de diagnóstico de Influenza Aviar, Complejo Lo Aguirre, SAG.	realizado
	Santiago	SAG	Charla a profesionales y técnicos de Laboratorio Lo Aguirre sobre la experiencia de Italia de I.A. y comparación con la situación de Chile	realizado
23-07-02	El Paico	Ariztía	Visita a matadero de aves El Paico	realizado
	Santiago	Centrovet	Visita a laboratorio de diagnóstico privado	realizado
	Santiago	Amevea – Asohuevo	Charla sobre la experiencia italiana en I.A.	realizado
24-07-02	Santiago	SAG	Reunión de trabajo. Evaluación y Conclusiones preliminares	realizado
	Santiago	SAG	Reunión de trabajo con Comité Público-privado de Emergencia Avícola	realizado
25-07-02	Santiago	SAG	Regreso a Italia	realizado

Dra. Pedersen				
06-08-02 al 11-08-02	Santiago, Pudahuel	Laboratorio de Lo Aguirre	Montaje de IH e IN durante toda su estadía.	realizado

4. Resultados Obtenidos:

La evaluación de los consultores permitió objetivizar las debilidades o deficiencias que presentaba la estrategia de control. La sistematización de la información epidemiológica y los componentes de una campaña de esta naturaleza, inédita para esta especie en el país, implicaba el desarrollo de opciones de manejo de riesgo y gestión, del cual no se tenía experiencia previa, para la estructura veterinaria pública, así como para la actividad privada.

Por otra parte, la validación de los procedimientos y técnicas diagnósticas que se utilizaban en la campaña, así como la interpretación epidemiológica de los resultados diagnósticos, permitió establecer con mayor grado de certeza las distintas decisiones que se adoptaron.

Se montó la técnica de IH para la subtipificación ya sea de suero positivo a Influenza aviar tipo A por técnica serológica de Inmunodifusión o virus Influenza Tipo A. Esto fue posible para las siguientes hemoaglutininas: H5, H7 y H9. También se montó la técnica IN para subtipificar las neuroaminidasas N2 y N3 a partir también de virus o de suero positivo a Influenza Aviar tipo A. No era la finalidad de incorporar la subtipificación de todas las hemoaglutininas y neuroaminidasas existentes sino las más comunes e importantes de determinar. Se adjuntan fotografías.

5. Aplicabilidad:

La experiencia trasmitida de los consultores sobre el control de la enfermedad, sin duda es el gran valor de esta consultoría, que permitió visualizar los puntos críticos de la emergencia, comparados con los resultados obtenidos en Italia.

Si bien es cierto, tanto la conformación de la industria avícola como su integralidad, son distintas en su forma en la situación italiana, la que presenta un mayor número de planteles por unidad de superficie, lo que redunda en que la estrategia necesariamente debe ser distinta a la situación en Chile, permitió conocer el comportamiento de la enfermedad y su diseminación en la industria avícola.

Las pautas generales de control y monitoreo de la enfermedad, son universales en su esencia, por tanto debe ser aplicadas en el contexto nacional, de acuerdo a nuestra realidad.

Las herramientas que se utilizan en una estrategia de emergencia avícola, necesitan de un componente de diseño y apoyo financiero para la adopción de nuevas tecnologías diagnósticas, así como de aplicación de conceptos de manejo de la enfermedad, cuya experiencia a nivel nacional era muy limitada, al inicio del problema.

La tecnología de IH será aplicada en el país para agilizar el diagnóstico serológico y virológico de la Influenza Aviar en Chile ante una futura emergencia sanitaria o en la eventualidad de diagnósticos sospechosos.

6. Contactos Establecidos: presentación de acuerdo al siguiente cuadro:

Institución /Empresa	Persona de Contacto	Cargo/ Actividad	Fono/ Fax	Dirección	E-mail
AGRÍCOLA ARIZTIA	Marcelo Ariztía B.	Director	8323169	Los Carrera 444 Casilla 90, Melipilla	mariztiab@ariztia.cl
AMEVEA	José Miguel Correa	Presidente	2093473	Av. Italia 10 45, Providencia	jmcorrea@avicolaelmonte.cl
ASOHUEVO	Luis Andrade R.	Gerente	2330949	Santa Magdalena 75, Providencia	landrade@asohuevo.cl
AGROSUPER	Felipe Ortiz	Gerente	238448	Camino La Estrella 401, Sector Punta de Cortés, Rancagua	fortiz@agrosuper.cl

7. Detección de nuevas oportunidades y aspectos que quedan por abordar.

Se detectaron algunos aspectos que son necesarios abordar en el corto plazo, que dicen relación con capacitación de los cuadros técnicos en materias que tiendan a manejar con mayor eficiencia los eventuales escenarios de reemergencia de la enfermedad.

Se obtuvo de los consultores la posibilidad de visitar sus centros de trabajo y laboratorios, a objeto de conocer las metodologías de trabajo existentes en esos lugares, asi como la de capacitarse en áreas aún no cubiertas por nuestra experiencia en lucha sanitaria de enfermedades, adquiriéndose una nueva visión frente a este campo.

En el tema laboratorio se ofreció la posibilidad de poner a punto técnicas que están implementadas en nuestro laboratorio.

La experiencia en áreas de recopilación, procesamiento e interpretación de datos de vigilancia epidemiológica, es otro aspecto que puede ser abordado en conjunto con los centros colaboradores de referencia.

Por otra parte, esta la posibilidad concreta de utilizar esos centros que son de referencia mundial para las enfermedades de las aves de la lista A de la O.I.E, tales como la Influenza Aviar y la Enfermedad de Newcastle.

Es recomendable realizar una estadía de 1 o 2 semanas en los laboratorios del DVL, NVSL en Ames Iowa y en Italia, con el fin de aquilatar mayor experiencia y práctica en esta tecnología, ver como trabajan ellos con sus condiciones, ver las otras subtipificaciones que no se montarán en el país por razones de recursos principalmente

8. Resultados adicionales:

El apoyo de esos centros italianos de referencia mundial para el diagnóstico de enfermedades aviares. En general, siempre ha existido buena comunicación con la organización norteamericana del punto de vista diagnóstico viral en aves y en mamíferos, esta capacitación logra afianzar más aún lazos de comunicación e intercambio técnico entre las instituciones, quedando abierta la posibilidad de interactuar con los laboratorios de Italia.

9. Material Recopilado:

Tipo de Material	Nº Correlativo (si es necesario)	Caracterización (título)
Compact Disc		Avian Influenza, 1999-2000 Italian Epidemic
Manual		Manuale Operativo in caso di Influenza Aviaria, anno 1999.

10. Aspectos Administrativos

10.1. Organización antes de la llegada del consultor

a. Conformación del grupo proponente

____ muy dificultosa __X__ sin problemas ____ algunas dificultades

(Indicar los motivos en caso de dificultades)

b. Apoyo de la Entidad Responsable

__X__ bueno ____ regular ____ malo

La entidad responsable entregó todas las facilidades operativas y administrativas para el éxito de la consultoría.

c. Trámites de viaje del consultor (visa, pasajes, otros)

__X__ bueno ____ regular ____ malo

d. Recomendaciones (señalar aquellas recomendaciones que puedan aportar a mejorar los aspectos administrativos antes indicados)

10.2. Organización durante la consultoría (indicar con cruces)

Ítem	Bueno	Regular	Malo
Recepción del consultor en el país o región	X		
Transporte aeropuerto/hotel y viceversa	X		
Reserva en hoteles	X		
Cumplimiento del programa y horarios	X		
Atención en lugares visitados	X		
Intérpretes	X		

11. Evaluación del consultor:

En opinión del staff técnico del grupo proponente, la visita de los consultores ha sido de gran ayuda para el desarrollo de la campaña de emergencia, que ha permitido adoptar una estrategia acorde con nuestra realidad, y ha permitido manejar mas antecedentes para una mejor decisión frente a complejas situaciones en las cuales nos hemos visto enfrentados. La experiencia gravitante de la situación vivida en Italia frente al mismo tema, sin duda ha permitido compartir y comparar nuestras acciones, de las cuales vemos con gran satisfacción, que de acuerdo a lo manifestado por los consultores, la emergencia ha sido manejada adecuadamente.

La opinión del sector privado frente a la presencia en Chile de estos consultores ha sido óptima, ya que reconocen en ellos, su gran capacidad y dominio técnico de la enfermedad. Situación que es reconocida en diversos ambientes y sociedades científicas.

La Dra. Pedersen demostró gran capacidad profesional en el desarrollo de su consultoría, los objetivos fueron cumplidos a cabalidad. La experiencia demostrada nos hizo darnos cuenta que la trayectoria de la Dra. Pedersen en este diagnóstico es amplia.

12. Informe del Consultor:

Se adjuntan reportes de consultoría.

13. Conclusiones Finales

Para la campaña de emergencia de Influenza Aviar, es del todo satisfactoria la asistencia y el apoyo entregado por los consultores que nos visitaron. Las sugerencias y recomendaciones aportadas por los consultores, se enmarcan dentro de los parámetros internacionalmente reconocidos por la O.I.E.

La experiencia transmitida por los consultores y la proyección y adecuación a nuestra realidad avícola, sin duda marcan un hito en el abordaje exitoso de esta emergencia avícola, la cual ha sido un trabajo mancomunado entre el sector público y el privado.

El montaje de la técnica fue realizado en su totalidad por la consultora, Dra. Jan Pedersen, quién a través de su experiencia pudo demostrar su alto grado profesional y su exactitud y certeza en las técnicas implementadas. Cabe señalar que como bien se dijo antes, este fue sólo el montaje de la técnica, falta ahora adquirir mayor experiencia de los profesionales en Chile para poder así desarrollar esta tecnología con mayor dominio y seguridad, lo que podría lograrse teniendo en cuenta un futuro entrenamiento en los laboratorios donde trabaja la Dra. Pedersen por parte del personal del Laboratorio del SAG, asi como en los laboratorios de Italia.

Fecha: 09/09/02

Nombre y Firma coordinador de la ejecución: Rubén Moreira Zúñiga

AÑO 2002

ASISTENTES A ACTIVIDAD DE DIFUSIÓN DE LA CONSULTORÍA

FECHA:

Nombre	Actividad	Institución o Empresa	Teléfono	Firma
Experiencia italiana en los brotes de Influenza Aviar.	Charla	ASOCIACIÓN DE PRODUCTORES DE HUEVOS (ASOHUEVO)	2330949	
Experiencia italiana en los brotes de Influenza Aviar.	Charla	ASOCIACIÓN DE MEDICOS VETERINARIOS ESPECIALISTAS EN PATOLOGÍA AVIAR (AMEVEA)	2093473	
Experiencia italiana en los brotes de Influenza Aviar.	Charla	SERVICIO AGRÍCOLA Y GANADERO (SAG)	6886183	

ASOCIACION DE PRODUCTORES DE HUEVOS DE CHILE

Santiago, 27 de agosto de 2002

Señor
Hernán Rojas O.
Jefe Protección Pecuaria
Servicio Agrícola y Ganadero
Presente

De mi consideración:

En relación con la exposición técnica en torno al caso de Influenza Aviar (experiencia italiana), el día 23 de julio en el Salón Consejo de la Sociedad Nacional de Agricultura, en Santiago de Chile, los Empresarios Productores de Huevos y Asesores Técnicos, tuvieron la oportunidad de asistir en calidad de invitados especiales.

Al evento se registró la presencia de 22 productores y sus respectivos asesores, los que representaron el 72% de la producción de huevos en el país.

Sin otro particular de usted muy atentamente,

Luis G. Andrade R.
Gerente Asohuevo

Santa Magdalena #75 Oficina 407 - Providencia - Santiago - Fono/Fax: (56-2) 2330949 - 2333497
E-mail: asociacion@asohuevo.cl

Señores : Servicio Agrícola y Ganadero (SAG)

Atención Dr. Hernán Rojas , Director
Dr. Rubén Moreira , Jefe Campaña Influenza Aviar

Estimados Drs.

Por medio de la presente hacemos llegar a Uds. nuestros agradecimientos y felicitaciones por la reunión realizada el día 23 de Julio sobre "Influenza Aviar" con los Drs Stefano Marangon y la Dra. Ilaria Capúa en las dependencias de la Sociedad Nacional de Agricultura .

Creemos que la oportunidad , calidad de los exponentes y antecedentes entregados justifica plenamente el haber realizado esta reunión conjunta; la masiva asistencia de nuestros asociados (39 socios) así lo demuestra.

Reiteramos nuestro interés a participar activamente y en conjunto con las autoridades sanitarias del SAG de todas aquellas iniciativas y actividades que vayan en directo beneficio de nuestro quehacer profesional y de la avicultura chilena .

Se despiden de uds. atentamente

Oscar R Encina L (MV)
Secretario

José Miguel Correa M
Presidente

Agrupación de Médicos Veterinarios Especialistas en Patología Aviar
AMEVEA - CHILE
Av. Italia 1045 - Providencia
Santiago Chile

AS AGROSUPER®

CERTIFICADO

El suscrito **GERENTE DE PRODUCCION** certifica que con fecha 22 de julio del 2002 sostuvo reunión en Oficina Central de la Empresa ubicada en Rancagua, con el Dr. STEFANO MARANGON Médico Veterinario consultor en el Tema de Influenza Aviar.

A dicho evento asistió el staff técnico de la empresa y Médicos Veterinarios del Servicio Agrícola y Ganadero.

FELIPE ORTIZ
GERENTE DE PRODUCCION

Rancagua, 11 de septiembre de 2002

AGRICOLA SUPER LTDA.
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ARIZTIA

DESDE 1936

Melipilla Agosto, 23 de 2002.

Señor
Hernán Rojas O.
Director División de Protección Pecuaria
Servicio Agrícola y Ganadero
Fax: 6716184

Ref.. Visita de los Dres. Marangon y Capua.

Estimado Doctor Rojas:

De acuerdo a lo solicitado, le informamos que los Drs. Stefano Marangon e Ilaria Capua, junto a los Dres.Rubén Moreira y Jorge Fuller, del S.A.G. visitaron los planteles de Reproductoras de Pavos, Tremolén y el plantel de Reproductoras broiler Miltil, el día Sábado 22 de Julio de 2002.
En las reuniones se trataron los siguientes temas :

A) TREMOLEN

Staff Ariztía : Sres.: Marcelo Ariztía B. : Director.
José Ramón Villar : Gerente de Calidad
Juan Benolt : Jefe Granja
Sergio Espinoza : Médico Veterinario

1) Aparición de Influenza Aviar en Tremolén.
2) Hipótesis de aparición del brote.
3) Registro de fechas de reparto de alimento, ingreso de personal y choferes
4) Medidas de Bioseguridad y mapas de unidades epidemiológicas limpias y afectadas.
5) Implementación de medidas de Bioseguridad.
6) Se comenta que dentro de las medidas de bioseguridad está contemplado, que no se cruce el personal de aseo, mecánicos, eléctricos y camiones de alimento y gas.
7) Comentan que las distancias entre sectores , la distribución de éstos y las medidas de bioseguridad son adecuadas.

ARIZTIA

DESDE 1936

B) MILTIL.

Staff Ariztía : Sres. Marcelo Ariztía B. (Director)
José Ramón Villar (Gerente Calidad)
Rodrigo Pérez (Jefe granja).
Sergio Espinoza P.(Médico Veterinario)
Carlos Pèndola (Médico Veterinario)

1) El Médico Veterinario Dr.Sergio Espinoza explica los hechos ocurridos desde los primeros días de Mayo hasta la confirmación de la enfermedad.
2) Se describe la intoxicación por NH4 S04 más la cloración excesiva y el inicio de la mortalidad desde el 17 de Mayo en adelante.
3) Se desarrolla el tema, con mapas y se explica los sectores más afectados y el comportamiento de la crianza.
4) Se informa sobre los cruces de personal y de operación.
5) Los Drs. Marangon y Capua explican que el virus posiblemente estuvo circulando en la granja desde Abril y probablemente se pudo confundir con bronquitis ú otra patología inespecífica.
6) Se les señaló el plan de aseo, limpieza y desinfección de las 2 granjas (Miltil y Tremolén), los cuales consideraron adecuados.

Agradeciendo su atención, se despide atte.

Sr.Marcelo Ariztía Benoit
Director Empresas Ariztía

Dr.Sergio Espinoza Pimentel
Médico Veterinario Acreditado
Agrícola Ariztía Ltda.

Avian Influenza Diagnostic Training and Laboratory Review

At the Servicio Agricola Y Ganadero (SAG) Laboratory

August 6-11, 2002 by Janice C. Pedersen

Introduction

Highly pathogenic avian influenza, an economically devastating disease of poultry, was first detected in the San Antonio (V region) of Chile in May of 2002. For economic and export purposes Chile will need to continue surveillance programs and monitor for the presence of avian influenza virus (AIV) by testing for antibody to avian influenza (AI) as well as isolation of the virus. Any serum that is positive by the agar gel immunodiffusion (AGID) test for AIV antibodies needs to be tested by the hemagglutination-inhibition (HI) and neurmanidase inhibition (NI) tests for subtype determination. Historically, subtypes H5 and H7 AI viruses have been shown to have the potential to be highly pathogenic for poultry. Characterization of all hemagglutinating viruses by the HI test will enable SAG to determine if the viral isolate is of the H5 or H7 subtype. In order to control AI caused by H5 or H7 viruses, the SAG should have the capability to first identify hemagglutinating viruses and then to subtype AIV.

Subtyping or serotyping of AIV

Characterization of AIV surface antigens, the hemagglutinin (HA) and neuraminidase is accomplished by the hemagglutination-inhibition (HI) and neuraminidase-inhibition (NI) assays. The SAG laboratory will need the capability to

perform the HI and NI assays to identify serum positive for H5 and H7 AIV antibodies and to subtype AI viral isolates. Training was conducted at the SAG laboratory with Dr. Christian Mathieu in order to incorporate these assays and technical skills into SAG's diagnostic capabilities. Subtyping all 15 H subtypes and 9 N subtypes requires a large repository of diagnostic reagents. Full serotyping or subtyping capabilities would require the SAG laboratory to develop and maintain HI antigens and antisera for H1-H15 AIV and NI antigens and antisera for 9 different neuraminidases. SAG is not expected to acquire or produce reagents for all subtypes of AIV. Any isolate that cannot be identified with H5, H7, H9, N1 or N2 reagents needs to be transferred to a reference OIE laboratory for further characterization. Surveillance for AIV antibody and virus can be conducted with H5, H7 and H9 HI and N2 and N3 NI reagents.

Subtyping and Serotyping Recommendations

- Serum specimens to be tested by the HI test for detection of subtype specific antibody should be free of hemolysis. Serums that are heavily hemolized may be inferior in quality or unusable for the HI and NI tests. Any serum that is positive for AIV antibodies by the AGID test needs to be separated from the blood clot and stored at 4° C in an sterile test tube until it can be serotyped. All serum should be stored at 4° C after receipt until all test results are finalized. All AGID positive serum specimens should be separated from the clot as quickly as possible and collected in tubes or vials that have not been previously used for collection of avian serum.
- Serums that are weakly positive for AIV antibodies by the AGID test may not have sufficient antibody titer to be serotyped by the HI and NI test. Detection of antibody

and serotype identification should be viewed as a flock test. Not all serum from each flock needs to be tested; those with strong AGID reactions, in sufficient quantity and without hemolysis will make ideal specimens for serotype identification.

- Identification of AIV subtype for all viral isolates should be conducted in a Class II biological safety cabinet. It is imperative that the laboratory testing of all live viruses be conducted with adequate biocontainment to prevent laboratory contamination and cross-contamination. The allantoic-amniotic fluid (AAF) from all dead embryos should be harvested in a Class II biological safety cabinet that is designated for the manipulation and testing of inoculated embryos or hemagglutinating viruses. A separate Class II biological safety cabinet should be designated for the inoculation of field (unknown) specimens.
- Quantitation of the number of hemagglutinating units (HAU) is necessary for the identification of viral isolates by the HI test. It is strongly suggested that the HI test, used for the identification of hemagglutinating viruses, be conducted with 16 HA units/50 µl for PMV-1 and AIV. Currently, AAF harvested from inoculated embryos is tested by the HA test in a tissue culture plate (Petri dish) with a 1% suspension of chicken red blood cells. Equal volumes of AAF and 1% RBC are incubated with a circular rotation and observed for the presence of hemagglutination. The advantages of titrating the virus while conducting the HA test is: the number of HAU can be determined for the HI test and a cell control can be tested simultaneously to assure that the RBC suspension will form a button when not agglutinated.
- When conducting the HI test in a Petri dish with equal volumes of undiluted antiserum and non-quantitated hemagglutinating viruses, the possibility of a false

negative reaction exists. A false negative reaction would be detected when there are too many HAU's of a viral isolate for successful inhibition with a non-tittered antibody.

- Viral isolates (hemagglutinating) should be screened by the HI test for H5 and H7 AIV and PMV-1.
- Initial laboratory training for the HA, HI and NI tests were conducted with Dr. Mathieu. Time was a limiting factor for a thorough and complete training session. Further training is recommended. Additional knowledge and experience are recommended for trouble shooting skills and the identification of viral isolates. This training is available at the National Veterinary Services Laboratory in Ames, Iowa.
- Centrifugation of chicken erythrocytes for the HA and HI tests needs to be conducted in a calibrated centrifuge at a standardized RPM. Preparation or washing of the erythrocytes at a standardized RPM will pack the red blood cells to a standard density. Current procedures do not allow the cells to be packed to a standard density for the preparation of 0.5% suspension without a spectrophotometer.

Comments and Recommendation for the Improvement of Laboratory Biocontainment

Standard BL-3 laboratory facilities, as outlined by Biosafety in Microbiological and Biomedical Laboratories (U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention and the National Institutes of Health) are necessary for the isolation and identification of highly pathogenic avian influenza virus. The following

comments are made in relation to the layout, facilities and organization of the diagnostic laboratory.

Biocontainment Recommendations

- Showering facilities have been installed to upgrade the level of biocontainment for the diagnostic virology laboratory. This is a significant improvement in the level of biocontainment. These facilities are adequate. The amount of physical space for the showering and changing clothes has been challenged during the avian influenza outbreak due to the increase in laboratory personnel. Showering and the increased biocontainment procedures are a major change in standard operating procedure for all employees employed prior to the outbreak. It is imperative that the diagnostic laboratory maintain this increased level of biocontainment by requiring all employees, visitors and maintenance personnel to wear only laboratory clothing and undergarments while in the laboratory and for all clothing worn in the laboratory to be left on the laboratory (dirty) side of the shower for decontamination prior to leaving the laboratory. Contaminated laboratory clothing must be autoclaved before being removed from the laboratory for routine laundry services.
- All personal, laboratory-related or maintenance items entering the laboratory must be decontaminated by direct contact to formaldehyde gas for 6 hours before they are considered decontaminated. Any item that can not be properly fumigated should not leave the laboratory until decontaminated. Currently items are being passed through a small airlock which operates with a negative airflow system. These items need to be exposed to formaldehyde vapors for 6 hours before they

are considered decontaminated. Fumigation procedures recommended for a BL-3 facility can be provided by the National Veterinary Service Laboratories upon request.

- The number of Class II biological safety cabinets (BSC) is insufficient. Field specimens should be handled and inoculated in a dedicated Class II BSC that is not used for egg harvesting, testing unknown AAF by the HA test and identification of hemagglutinating viruses. At the current time there in an insufficient number of biosafety cabinets to allow for the complete separation of laboratory activities and testing. However, there is a physical separation (the cabinets are located in separate rooms) of the two BSC that are being used for the isolation of viral pathogens. One BSC should be dedicated to the handling of field specimens for inoculation and the other cabinet should be dedicated to the handling and testing of specimens that have been processed for virus isolation.
- All biological safety cabinets need to be adequately disinfected following each use. A laboratory standard operating procedure (SOP) should be drafted to cover the correct use and disinfection of biological safety cabinets. This SOP needs to outline laboratory procedures for the handling of infectious agents that will minimize the creation of aerosols. Disinfection of biological safety cabinets between different laboratory activities or testing needs to be emphasized.
- Due to the ease with which AIV can be cross-contaminated, only the embryos of one diagnostic specimen should be opened within the BSC at any one time. Embryos from which AAF has been harvested need to be deposited in a container that is not open to the air allowing aerosolization of the virus and leading to the

possibility of cross-contamination. All procedures used during the harvesting of AAF from inoculated embryos should be conducted by techniques that minimize aerosolization.

- The AAF from inoculated embryos should be harvested and stored in uniquely identified sterile test tubes and tested by the HA test after the AAF from all embryos is harvested. It is suggested that all harvested AAF should be tested for the presence of hemagglutinating virus by the HA test in a U-bottom microtiter plate. The plate should be sealed with an adhesive plate sealer after the addition of chicken erythrocyte suspension to minimize aerosolization and cross-contamination. Consumables (U bottom plates, plate sealers and filtered pipette tips) are necessary to reduce the possibility of cross-contamination. Pipettors and other related equipment should be dedicated for use with harvested AAF and viral isolates. Current pipettor inventory is insufficient to allow for the dedication of this equipment for virus isolation vs. serology. Filtered sterile pipette tips are necessary to prevent contamination of equipment and cross-contamination of laboratory specimens.

Conclusion

The virology unit of the SAG laboratory is commended for their efforts to improve the level of laboratory biocontainment and to implement the diagnostic tests necessary for identification of avian influenza virus and antibody. One of their assets is their goal for improved facilities and diagnostic capabilities. Results of all diagnostic tests (HA, HI,

NI, AGID, RT-PCR, chicken inoculation, blood agar) need to be viewed as a total picture before results are definitive. Pathogenicity testing need to be conducted on all newly isolated hemagglutinating viruses. Viral isolates identified as PMV-1 should be pathotyped by the intracerebral pathogenicity index (ICPI). All viral isolates identified as AIV should be pathotyped by the intravenous pathogenicity index (IVPI). This testing needs to be conducted in BL 3 facilities according to recommended OIE standards. All H5 or H7 AIV isolates should be sent to a reference OIE laboratory for sequencing and determination of the pathogenic potential.

Avian Influenza in Chile

Report of the mission carried out from 17 to 25 May 2002

by Dr. Ilaria Capua and Dr. Stefano Marangon

Introduction

Highly Pathogenic Avian Influenza (HPAI) has never occurred in South America, however, for export purposes, South American countries perform surveillance programs for avian influenza (AI) on birds reared for the external market. Chile has implemented a surveillance programme since year 2000, with 17196 samples tested in 594 flocks, in 2001 39001 samples were collected in 1209 flocks and in 2002 up to the end of April, 13050 samples were collected in 395 flocks, all with negative results. The first positive samples were those collected from a grand parent flock located in Rancagua (VI region) on 16.05.02.

Description of the outbreaks

To present date, only 2 outbreaks have been confirmed. The two farms, a broiler breeder (Miltil) and a turkey breeder (Tremolen) both belong to the company Ariztia and are located in proximity to each other (4km).

Index case (Miltil)

The premise houses 617 800 breeders, a hatchery and is located in proximity to a broiler operation containing 1 495 000 broilers (Malvilla location). The broiler farm is physically and functionally separated from the breeder operation. The water supply for the operation is obtained from surface water (natural pond). The water is chlorinated prior to the administration to the birds.

Prior to the emergence of avian influenza the Miltil premise was sampled as part of the surveillance programme for exports. Between January and May 2002, a total of 315 samples were collected and tested with negative results. The last sampling for this purpose was collected on the 10th of May.

A brief description of the events related to the AI outbreak follows.

During the end of April and the first week of May 2002, a clinical condition characterised by low mortality, slight drop in egg-production and salpingo-peritonitis appeared in selected sheds of the premise. On May 9th, 2002, birds belonging to sheds n° 35-37-47-52-54 were collected and submitted to a private laboratory (Centrovet) with the suspicion of infection with infectious bronchitis virus (IBV).

On the 23rd of May notification of a disease causing high mortality was forwarded by the Ariztia company, with the clinical suspicion of poisoning or intoxication. The following day an official inspection was carried out by Dr.G.R. Cancino Valenzuela, epidemiologist and by Dr.C.Jara Mayo, pathologist of SAG and the suspicion of HPAI was forwarded. Samples collected from 4/6 sheds resulted serologically positive to type A influenza by the AGID test. All swabs and organ samples collected resulted negative by virus isolation.

On the 25th of May 2002 serum samples were collected from additional 6 sheds with positive results. All sheds which were sampled with positive results were in lay. On the 26th of May 2002, an unidentified haemagglutinating agent obtained from the samples collected on May 9th (IBV suspicion) was sent to the official Lo Aguirre Laboratory. The isolate (A/ck/Chile/176822.02) was sent to NVSL, Ames Iowa and was characterised as an avian influenza virus of the H7N3 subtype of low pathogenicity (cleavage site sequence PEKPKTR*G, IVPI 0.0).

On the 29th of May additional 8 pullet breeder sheds were sampled, all with negative results.

Data on the sampling carried out on the remaining sheds containing live animals is not available.

Due to the high mortality and to the laboratory results, it was decided to depopulate the Miltil premise. During the depopulation of the premises (1-8 June), samples were collected from previously seronegative young stock for attempted virus isolation. Five samples resulted positive for the isolation of a haemagglutinating agent. The strains were sent to sent to NVSL, Ames Iowa and to VLA Weybridge and were characterised as avian influenza viruses of the H7N3 subtype of high pathogenicity. Two distinct strains were identified, which both exhibited an insertion of 30 nucleotides corresponding to 10 AA, which represents an unique event, never observed before. Two different cleavage site motifs were identified among the 5 strains, namely CSPLSRCRETR*G and CSPLSRCRKTR*G. Both genotypes exhibit IVPI values indicative of HPAI.

On the 20th of June, all the 116 000 hatching eggs still present in the hatchery were destroyed. All the chicks hatched up to that date were sent to broiler operations.

The data on the mortality rates observed in the farms was correlated to the egg-production curves and to serological and virological results for a significant number of sheds on the premise.

Comments

The analysis of the correlation between data on the mortality rates observed in the farms and the egg-production curves and with the serological results indicate that:

- An H7N3 low pathogenic avian influenza (LPAI) virus was introduced in the premise of Miltil presumably prior to the middle of April 2002, on the basis of the data available it is not possible to estimate the date of introduction.
- The LPAI virus mutated to the HPAI virus in one of the sheds of the Miltil premise, however it is not possible to establish in which shed.
- The correlation between the serological, clinical and epidemiological data indicates that the HPAI virus has probably circulated in a seropositive population for a while.
- The circulation of the HPAI virus has occurred primarily in the sheds containing adult birds, and only subsequently in farms containing young birds. This is probably due to the higher number of contacts at risk in the sheds containing the adult birds (egg collection).
- The results of the laboratory at times appear to be inconsistent with the clinical data observed in the sheds. This could be due to the absence of data on the subunit (pabellon /galpon) from which the samples were collected.

Secondary outbreak (Tremolen)

The secondary outbreak was identified on a turkey breeder farm located in Fundo Termolen. The premise holds 4 dark houses (DH) for young birds containing a total of 26000 birds, 4 breeding houses containing 24000 birds and a hatchery.

In general biosecurity measures were regularly applied in the premise. With regards to staff, all subunits had dedicated personnel that carried out rearing operations except for DH2 and 3 that shared the same staff.

On the 1st of June 2002, clinical signs indicative of HPAI were observed in DH3. Serum samples collected on the 3rd of June resulted AGID positive. Stamping out was completed on the 7th of June and the carcases were buried on the spot.

On the 11th of June clinical signs were observed in DH2 and the animals were depopulated from the 15th to the 19th of June. Carcases were disposed of as above. The barns were completely sealed off without disposal of the litter.

Two HPAI viruses identical to ones obtained in Miltil were isolated from DH3.

From the 3rd of June, organisational procedures were modified, with the enforcement of stringent biosecurity measures. To date, all the serological and virological tests enforced on a regular basis by SAG have resulted negative on all the remaining birds on the premise.

Comments

- The results of the laboratory at times appear to be inconsistent with the temporal development of the clinical data observed in the sheds.
- The biosecurity measures rapidly enforced following the HPAI outbreak in DH3 presumably were able to avoid further spread of infection. However, infected litter is still present in DH 2 and 3 and this must be appropriately disposed of after maturation.
- Monitoring of the live animals present on the farm must continue to ensure that there is no AI virus circulating any longer.

General comments on the two outbreaks

- Adequate control and eradication measures have been enforced on the outbreak sites to depopulate, clean and disinfect the affected premises and to avoid further spread of infection.
- Surveillance measures on poultry farms located in the restriction zones have been planned and enforced on a regular basis. All the seropositive farms were put under official surveillance and adequate prevention measures were implemented.
- There is a lack of epidemiological data on the two outbreaks. Since a complete epidemiological investigation was not carried out, and no tracing exercise has been performed in either outbreak, it was not possible to identify at risk farms and to precisely define the area at risk of infection.

Monitoring programme

A national monitoring programme has been carried out from the 1st of June 2002 in all poultry farms with the exception of ostrich farms located in the 11th region. Until 15th July 2002, 79834 serum samples were tested with the AGID assay for the detection of antibodies to AI. During the same period a number of 2265 registrations and reports were performed and issued by the laboratory. For the sake of comparison approximately 6500 registrations and reports were issued and performed by the laboratory during the whole of year 2000. The majority of the samples foreseen for the monitoring programme has been collected and processed during the month of June. All poultry farms on the Chilean territory have been checked al least once. Up to the 9th of July a total of 438 poultry farms were tested. Serological positivity was detected in 32 farms located only in the Vth, VIth and RM regions. Of these 15 belonged to the company "Agrosuper"; 10 belonged to the company Aritztia (including the index case of Miltil and the secondary outbreak of Tremolen); 4 belonged to Agricola Chorombo; 1 Pablo Massaud; 1 Agricola Lo Herrera and one Champion.

A total of 1042 positive samples (1.31%) were detected. Of these 984 /72186 (1.36%) were in chickens while 48/6516 (0.73%) in turkeys.

On each seropositive group, 28-60 samples were collected for serological investigations once a week for at least three weeks. In addition, cloacal swabs were collected for virological investigation from the seropositive flocks. To date seronegative farms have not been officially tested following the first round of the monitoring programme. Some farms have been serologically tested on a voluntary basis in the affected regions.

It should however be emphasized that in the majority of cases (and particularly in meat-type farms) the samples have been collected by veterinarians associated with the companies under the indirect supervision of SAG.

Agrosuper

A total of 28seropositive flocks (sectores) were detected in 15 farms.

Broiler farms

Serological positivity was sporadic in the order of 1-2 positive samples out of 30-80 sampled birds.

5 of the seropositive flocks were broilers and 2 of them have been subsequently tested at slaughter with no evidence of additional seroconversion.

Grand Parent farms

Sheds 1,4,6,8 exhibited at least one serological positivity, however 1 and 8 exhibited only 1 positive sample on one occasion. Shed 4 resulted positive at 5/6 subsequent samplings with up to 10/70 positive samples. The total number of samples found positive was 23/368. Shed 6 resulted positive in 6/6 occasions with a maximum of 11/56 samples, with a total of 50/324 positive samples.

56 sera were tested by the HI test at VLA Weybridge, and all resulted negative at antibodies to the H7 subtype of AI whereas 31/56 resulted positive (>= 1:8) for antibodies of the H5 subtype. Titers ranged from 0 to 1: 256. For the sake of completeness additional testing performed at NVSL Ames indicates that the seropositivity was to a virus of the H5N2 subtype.

A sample collected on the 5th of June resulted positive to the isolation of H7N3 HPAI, in absence of any clinical signs to date.

Broiler Breeder farms

AGID serological positivity was detected in 9 broiler breeder farms with the same type of serologic positivity as above. Among these 75 AGID positive samples collected in one breeder farm were sent to V.L.A. Weybridge for confirmation of positivity. These samples were all negative to H7 antibodies while 32 of these contained antibodies to the H5 antigen. For the sake of completeness additional testing performed at NVSL Ames indicates that the seropositivity was to a virus of the H5N2 subtype. Furthermore two AGID positive samples collected in 2 other breeder farms were sent to NVSL, Ames. These sera were negative to H7 antibodies and positive to H5N2 antibodies. However, the H7N3 HPAI virus has been isolated in 4 breeder flocks in absence of any clinical signs to date and in absence of serological positivity to H7.

Comments

The company Agrosuper has performed a wide serological survey in order to establish the presence of serologic positivity in their farms. A total of 617 serum samples collected from the 10 seropositive premises were sent to NVSL, Ames and VLA Weybridge. None were positive to H7 and 102 were positive (>= 1:8) to H5 antibodies. From the data available it appears that the positivity is caused by a virus of the H5N2 subtype. Since there is no evidence of any clinical signs or isolation of this virus it is reasonable to think that the seropositivity could be related to a contaminated vaccine.

This hypothesis appears to be supported by epidemiological data, since seropositivity has been related to the use of an identified batch (1242) of an inactivated avian inclusion body hepatitis vaccine produced in Mexico. In fact among the 23 seropositive breeder flocks: 14 had been vaccinated with the above mentioned batch of vaccine, four resulted HI negative for both H5 and H7 subtypes and 5 resulted negative at the retesting of the flock. The evolution of the serologic positivity was clearly related to the inoculation of the above mentioned batch of vaccine.

Since no H7 antibodies or clinical signs were detected, and the isolate obtained is indistinguishable from the isolates obtained from Miltil and Tremolen, the risk of contamination cannot be ruled out.

Ariztia

All the 8 farms which resulted positive by the surveillance were meat type farms. Since no data are currently available on the AI strains responsible for positivity, it is not possible to draw any conclusions on the secondary spread of the H7N3 virus. However from the data presented, seropositivity appears to be scattered and sporadic and no evidence of seroconversion has ever been detected.

It is strongly advisable that further investigations are carried out promptly aiming at the establishment of the nature of the serological positivity.

Pablo Massaud

This is a broiler breeder and broiler fattening company. Variable serological positivity was detected to the H5N2 virus only in breeder flocks. Since there is no evidence of any clinical signs or isolation of this virus it is reasonable to think that the seropositivity could be related to the use of a contaminated vaccine. This is supported by the fact that the same lot of presumably contaminated inclusion body hepatitis vaccine (batch 1242) has been used. Virological tests always resulted negative.

Other farms

No additional testing aiming at the establishment of the subtype causing seropositivity was performed on the other AGID positive farms, with the exception of 1 broiler farm (Chorombo , La Marquesa) for which the serological AGID positivity was not confirmed on 1 sample. From the data presented, also for these farms, seropositivity appears to be scattered and sporadic and no evidence of seroconversion has ever been detected.

Backyard flocks

Surveillance on backyard flocks has been performed by SAG taking into account some risk factors related to living and social habits of the staff working either at Miltil or at Tremolen premise.

No evidence of spread of infection has been detected to date. In the restriction zone a total of 4835 blood samples were collected and tested with negative results.

General comments

- All the seropositive farms were put under official surveillance and adequate prevention measures were adopted.
- The number and characteristics of the sampling procedures appear to be appropriate. However the serological monitoring should be repeated at least in the risk area and in the farms functionally connected with the outbreaks. Samples should be collected by official veterinarians.
- The serological techniques performed by the laboratory should be integrated with the HI test with the objective of establishing the virus subtype responsible for the positivity.
- The data obtained from the company Agrosuper indicates that this positivity is caused by a virus of the H5N2 subtype. The issue of a contaminated inclusion body hepatitis vaccine has been raised and must be clarified.
- The nature of the serological positivity in the broilers of the company Ariztia should be established, taking into account that chicks originating from the hatchery in Miltil have been distributed in broiler operations up to the 20th of June.
- There is no evidence of AI infection in backyard flocks in the area at risk or elsewhere.
- Epidemiological data on the monitoring programme are managed at local/regional and national levels. Some information available at the SAG central office appear to be inconsistent with the data supplied by the local/regional Authorities.

Laboratories

SAG Laboratory (Lo Aguirre)

An inspection was carried out in the laboratory at Lo Aguirre. The facility is appropriate in general, however with reference to the layout of the facility and to the general situation it must be emphasized that:

- Generally speaking the number of rooms, laminary flow cabinets and space in general are insufficient at times to handle the great number of samples submitted, thus increasing the risk of cross contamination,
- In particular there is not enough separation between the clean and dirty parts of the PCR area
- More safety cabinets should be available in order to avoid cross-contamination of samples
- Disposable glassware and test tubes should not be recycled
- All disposable glassware and other consumables and durables which are necessary to speed up and increase the reliability of the diagnostic result should be made available and prioritised

With reference to the methodology used for the diagnosis of AI:

- Standard procedures should be applied for the selection, preparation and processing of samples, with reference to virus isolation and identification techniques,
- The AGID test should be integrated with the HI test in order to assess the nature of the seropositivity
- The PCR test results should be considered as valid only after the test has been used for a reasonable amount of time and the risk of contamination has been ruled out

Centrovet

It was not possible to evaluate the productive activity of this lab since the vaccine production and diagnostic lab have been closed. The lab records were made available and it appears that:

- This laboratory has a poor and unreliable diagnostic virology facility, often there is no follow up of evidence that could be indicative of the isolation of a virus, and therefore the isolation of the influenza virus was totally casual
- From the observation of the lab records however it appears that between January and May 2002 at least 12 submissions of meat turkeys affected by a clinical condition compatible with LPAI were analysed. Although this clinical condition may also be caused by a series of other

pathogens, due to the unreliability of the diagnostic virology section, the presence of LPAI in meat turkeys prior to the first isolation (May 9th) cannot be ruled out.

Data management system

Although the peripheral crisis units are well organized and undertake a great number of activities in the field and liase with the laboratory and SAG central headquarters, the management of data should be upgraded. In fact the peripheral crisis units, the diagnostic laboratory and the SAG headquarters work on different information systems which are not sufficiently compatible, thus resulting in a lack of the necessary information to support decision makers at SAG.

Conclusions

In Chile there is no legal basis for the compensation of farmers for animals stamped out in case of an outbreak of LPAI or HPAI. This situation can jeopardise any effort put in place by the veterinary authorities to promptly detect and eradicate the disease. Due to the absence of a compensation policy farmers could try to hide the presence of the infection in order to avoid the huge economic losses determined by the application of stamping out measures on their premises. This may occur particularly with LPAI virus infections which can spread without causing overt clinical symptoms in the affected flocks.

Based on the results of the monitoring measures implemented in all poultry farms on the Chilean territory, **the spread of the AI virus appears to be limited**. In fact, only two outbreaks have been identified to date in Miltil and Tremolen premises. Nevertheless, **the epidemiological situation is not completely clear** due to:

- the majority of samples were not collected directly by an official veterinarian;
- it was not possible to identify, inspect and test adequately the farms at risk of infection, since an incomplete epidemiological investigation was performed on the two outbreaks;
- due to the enormous pressure on the laboratory and to the type of diagnostic techniques implemented, the results of the investigations can not be considered as completely reliable;

- not all the farms connected with the outbreaks were monitored and tested following the evaluation of an appropriate incubation period for avian influenza infections.

On the basis of this evaluation the presence of LPAI infected poultry flocks cannot be ruled out and therefore all efforts should be made to assess the epidemiological situation. In addition and for this reason, biosecurity measures should be improved among poultry flocks. Possibly, a minimum set of biosecurity measures should be laid down, and their application verified by official veterinarians visiting the flocks.

With reference to the issue of vaccination, the implementation of this method of control does not appear to be necessary at present on the basis of the data collected in the field. However, should the situation evolve in a different manner, where the spread of LPAI is not under control, a vaccination plan should be drawn and considered.

General recommendations

Evaluation of the field situation with AI

Monitoring program – As foreseen in the "Fichas Tecnicas Acciones de eradication de influenza aviar" issued by the SAG – Departamento de proteccion pecuaria in July this year, **a survey should be implemented in order to evaluate the presence of avian influenza in the intensively reared poultry in Chile**. Taking into account that all the outbreaks site were depopulated in June the above-mentioned survey should be carried out not prior to the beginning of August. Due to the established presence of antibodies to at least two subtypes of AI (H5 and H7), **the monitoring programme should include the HI test to identify the strain responsible for serological positivity**.

It is strongly advisable to carry out the monitoring activities under strict official control and to apply well defined sampling procedures as reported in the "recommendations for sampling procedures".

With regards to meat-type poultry farms, samples can also be collected at the abattoir.

Sampling procedures - Blood samples, organs (pooled trachea and lungs and pooled intestines) and swabs should be collected by an official veterinarian, appropriately identified, inserted in a sealed, identifiable sample transportation container (bag) and sent to the official laboratory with a service car in refrigerated conditions. Additional manpower should be made available and all precautions should be taken to avoid the swapping of samples.

Data management system – A national register of poultry farms should be constituted and regularly updated, possibly in cooperation with poultry producers. Each farm should be identified by a unique code, and the farm code should be utilized to register all the relevant information related to the monitoring activities at local, regional and national (laboratory and central epidemiology unit) levels. A specific software to store and manage laboratory data should be implemented and its integration with the system used for epidemiological analyses guaranteed.

Diagnostic laboratory

Serology - **The diagnostic laboratory should implement the HI test in order to establish the subtype of AI responsible for the positivity**. The HI can be performed on all sera or on sera that have resulted positive at the AGID test, and must be performed with the H5 and H7 antigens. Reference reagents should be used and a first batch of reagents has already been supplied to the laboratory by IZSVE. In addition, IZSVE is willing to perform testing on a reasonable number of samples, free of charge to support the diagnostic activity of the laboratory.

Virus isolation techniques must be performed in accordance to OIE or internationally recognized standard procedures, and advice should be sought only from experts that have had significant experience in the field of the diagnosis of avian influenza infections..

All efforts should be made to prioritise the upgradement of the laboratory.

Manpower must be made available for supporting the staff in administrative and technical procedures.

Emergency plan

The contingency programme recently issued by SAG must be effectively put in force. In particular, strict measures must be applied on seropositive/suspect farms, and clear rules for the controlled marketing of these flocks should be defined, taking into account the natural shedding curve of avian

influenza infections. Epidemiological investigations should be performed on all affected premises, using a standardised questionnaire.

Training

It would also seem advisable that staff from SAG directly involved in diagnosis and data management should visit some foreign, experienced institutions in order to upgrade and make more efficient the level of their performances.

Vaccination

Considering the present epidemiological situation and the actions already undertaken by SAG, **a vaccination programme should be drawn and planned for**. This vaccination programme should be discussed with the industry and with the trade partners and should take into account:

- Type of strategy and type of vaccine to be used
- Availability of vaccine and duration of programme
- Geographical area and type and number of farms and animals included in the vaccination campaign
- Minimum biosecurity measures to be applied on the production circuit
- Monitoring measures to be implemented on: vaccine distribution, vaccinated/unvaccinated flocks, vaccine efficacy and vaccination schedules
- Actions to be enforced in cases of breaks
- Restriction and sanitary measures to be enforced on the movement of live birds and vehicles inside and outside the vaccination area
- Restriction measures to be applied to the trade of fresh poultry meat and table eggs originating from vaccinated and unvaccinated premises located in the vaccination zone

Finally, the Istituto Zooprofilattico Sperimentale delle Venezie as OIE Reference Laboratory is willing to give any additional advice to SAG concerning aspects of the epidemiology, diagnosis and control of avian influenza which may arise in the future.

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