

SPECIFIC INFORMATION REQUESTED BY U.S.A. IN THE PROCESS OF RECOGNIZING CHILE AS A COUNTRY FREE OF CLASSIC PORCINE PEST (PPC).

FACTOR No. 1: Authority, organization and infrastructure of the Veterinary Services in the country.

- Which are the international regulations presently in force regarding waste food used for feeding swine?

Swine fed with waste food is prohibited in Chile.

Porcine products confiscated at international customs post barriers are thence forwarded for incineration once denaturing has been effected.

Waste food of animal products proceeding from aircraft kitchenettes are treated with heat and eliminated through the sewage.

Legal guidelines, related with waste food and swine being fed with waste food, were translated and forwarded to Dr. Goodman by email on September 24, 2002.

- Article No. 13 of the RRA 16, in English, was also included in the same document.
- Which is the budget the Department has for surveillance and control of diseases?

The 2002 surveillance livestock project has considered the following normal financial resources (in Chilean pesos): *(See Spanish version for data)*

Objectives (*)	Working performance				Total (1)	Total (2)
	Professionals	Technicians	Administrative personnel	Assisting personnel		
(1) Per diem and overtime						
(2) Operational expenditures						

(*) Objectives

- 1: Epidemiological Surveillance Plan for exotic and endemic diseases.
- 2: Immediate and prompt response in case of any detection of exotic disease.
- 3: Training of personnel to execute the emergency plan in the event of a sanitary problem.
- 4: Prepare Contingency Plans for each one of the diseases of interest to us: FA, PPC and ENC.

FACTOR No. 2: Disease Status

- Suspicions on Red Diseases received in Chile in 2002.

Laboratory results received for PPC and Erysipelas have shown negative results. Food poisoning and deficiencies in vitamins and minerals have been established as other definite diagnoses.

Table No. 1 No. Red Disease Suspicion. Chile 2002

Regions	No. of Suspicions
I	3
II	None
III	None
IV	None
V	None
VI	None
VII	None
VIII	2
IX	None
X	2
XI	None
Metropolitan Region	4
TOTAL	11

- When and in how many cases female carriers were associated to the transmission of the disease during the period 1996-1998?

Only in one focus of Classic Porcine Pest occurred in July 1996 in the area of Quillagua, Province of Tocopilla, Region II. This area had another focus in 1995.

The origin of this focus could have been associated to a female carrier that may have remained infested after the focus in 1995.

- Animals existing at the focus in 1995 were vaccinated?

YES.

FACTOR No. 3: Sanitary condition of neighbor countries.

- Based on the information provided by the OIE (HandiSTATUS II) situation of the PPC in Chile bordering countries is as follows:

Argentina: Without any occurrence of disease as from May 1999.

Peru: Disease is present and localized in some areas with two focuses of the disease in 2002, situated in regions of the Amazon and Puno.

Bolivia: Disease is present in this country. During the three first months of 2002 no occurrence has been reported.

Note: FAO web site can also be visited where reference is made to the continental Plan for the Eradication of PPC in America.

- Would there be a potential action of transporting infested wild swine from neighbor countries?

The above is improbable to occur in case of Peru and Bolivia, considering the fact that the echo-system existing in those countries only allows existence and development of South-American camelidae.

Some wild boars have been transported from Argentina to Chile, mainly in the area South-Austral of the country and according to the serologic samples performed this year in some wild boar operations in Chile results to ELISA PPC have been negative.

FACTOR No.4: Information details on the active program of disease control.

- Upon a suspicion on PPC, which are quarantine procedures and the time the operations remain under this status?

Upon a suspicion of PPC, the first action taken at the porcine industry under suspicion is an epidemiological investigation in order to become aware of the disease features presented and also the entry and exit of animals occurred during the past months.

At this point, we believe that the “clinical situation of PPC should be compatible with the response of a swine population highly sensitive to the disease and therefore, with proper morbidity, mortality and lethal conditions.”

Necropsies are performed and blood and organ samples taken to be forwarded to the Central Laboratory and the operation is submitted to the “pre-diagnosis quarantine” prohibiting the transfer of animals to other farms, fairs, or slaughterhouses, except those with a high bio-security level.

The farms where swine are originated and destined are investigated both at the location of the suspicious operation location or other SAG points in the country.

Quarantine is official once a Veterinarian from SAG, along with other specialists in pathological and epidemiological anatomy, detect clinical signals, or pathological findings compatible with PPC and with positive laboratory results to direct immune-fluorescence or viral isolation in PK 15.

Chilean policy regarding diseases indicated under List A is a sanitary sacrifice of the affected animal population, suspension of certification in exports, communication to the OIE and the veterinary services of those countries with which commercial trade is maintained.

** What is the reason for the differences in quarantine periods applied during focus in 1995 and 1996?

In PPC focus during 1996 the sanitary sacrifice of the affected animal population was applied resulting in a shorter quarantine period than the one applied in 1995.

** Which are the signs that allow ending of the quarantine period?

1. Absence of clinical and anatomy-pathological signs compatible with PPC.
2. Negative Laboratory results.
3. Favorable evolution of the mortality and productivity parameters in the operation.

FACTOR 5: Vaccination status in the region

** Which is the level of compliance with prohibition of anti-PPC vaccination?

Total

** What measures have been implemented in the country in order to impede vaccination?

The SAG suspended the laboratory control of the PPC vaccine and the production of vaccine in the country.

The Association of swine producers in Chile (ASPROCER) established an insurance system destined to pay an indemnity to its members in case of losses on account of PPC.

Vaccination was prohibited in Chile by means of Resolution No. 2928 dated October 6, 1997.

FACTOR 7: Details related with control of animals and by-products importation from regions of a higher risk level and bio-security level applied.

** Importation of animals and animal semen.

Table No. 2: Swine imports, Chile period 1998 - 2002 (to November 2002).
(See Spanish version for data)

Date - CDA No. (*) - Consignee - No. of swine - Country of Origin
CDA: Customs Certificate of Destination

Table No. 3: Imports of Porcine Semen, Chile 1998 – 2000 (to November 2002).
(See Spanish version for data)

Date - CDA No. (*) - Consignee - Dose No. - Country of Origin
CDA: Customs Certificate of Destination

FACTOR 9: Features and details on disease surveillance in the country

1. PPC surveillance

PPC epidemiological surveillance plan in Chile during 2002 pursued the following objectives:

1. Maintain an active surveillance of risky herds located between the First and XII regions in the country.
2. Maintain an active surveillance in farms that have shown positive serological result in industrial locations during previous monitoring actions.

Monitoring in risky herds

Each region involved in this monitoring must select the risky herds and perform a follow up on those during the course of the year, with visits every three months plus samples being taken. In general terms, the risky herds are those located near the borders, close to ports, airports and border crossing points, close to

garbage dumps and those fed with waste food. The number of samples taken in each visit must be calculated according to the table of critical in-farm 20% prevalence, i.e. 13 samples per herd as a maximum.

(See Spanish version for data)

Region																		Total
Farms																		
Samples																		

Maintain an active surveillance within industrial operations.

100% of the industrial farms with background of positive PPC serology, with a critical in-farm prevalence of 1% and with a maximum of 28 samples per location, will be sampled.

(See Spanish version for data)

Region																		Total
Farms																		
Samples																		

1.1 No. of Suspicions – Chile 2002.

Laboratory results from tests performed for PPC and Erysipelas have been negative, food poisoning and deficiencies in vitamins and minerals have also been established as other definite diagnoses. (See Factor 2)

1.2 Epidemiological surveillance on PPC – Chile 2002.

Surveillance in monitoring risky herds and industry facilities with positive serologic background on PPC have, for the first case, established a statistical sampling plan and a prevalence within farm limits of a 20% with a maximum of 13 samples per herd and 1% for the second case within industry facilities, with a maximum of 28 samples per industry facilities.

It is expected to continue with the progress in the serologic sampling plan associated to the epidemiological surveillance on PPC in Chile during the months of November and December.

1.3 Epidemiological surveillance on PPC in wild boars – Chile 2002. See Annex 4

107 blood samples for ELISA PPC have been taken from 8 wild boar industrial locations.

Results have been negative. These results have been complemented with clinical exams showing that these animals are found to be in excellent health conditions.

An interesting remark would be the fact that samples have been taken from animals at a location from which the others have originated

Another interesting note refers to the organizations, which have basically originated from the capture of wild animals and/or the purchase of them at established breeding places or zoos. This fact would indicate the absence of PPC in those animal groups, thus eliminating their potential effect as a factor of PPC introduction and dissemination in the country.

Furthermore, and complementing the above concept, bio-security measures applied at industrial herds prevent any contact between wild animals and domestic animals, making this contact practically impossible.

New samplings under process will support this favorable epidemiological situation for swine and wild boar population in our country. In addition, a complete census of organizations holding wild pigs that are under surveillance activities will be performed in areas where hunting is practiced.

Table No. 4 RESULTS OF SURVEILLANCE ACTIVITIES IN CLASSIC PORCINE PEST – CHILE 2002

Period: January to October
Source: Laboratories Department
(See Spanish version for data)

REGIONS	No. of Owners/ No. of IFD Samples Negative results	No. of Owners/No. of ELISA Samples Negative results	Total Laboratory Samples
	Domestic – Industrial	Hazardous- Industrial Herds	IFD – ELISA

Total owners -No. of samples

Table No. 5: No. of breeding places and wild boars being sampled as per regions in Chile, 2002.

Region	No. Breeding Locations	No. of Blood Samples	PPC- ELISA Results
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*One of these breeding places has been dedicated to the sale of reproductive material, therefore a number of other breeding locations have originated therefrom.

FACTOR 10: Laboratory diagnosis capability

- Diagnosis tests for PPC existing solely at the Central SAG laboratory are:
- Direct Immune Fluorescence (1979)*
- Viral Isolation in PK 15 (1983)
- ELISA (1995).

* Tests were established as from this year.

Tests for diagnosis mentioned above are developed following norm from OIE.

- Procedures in tests for diagnosis
- ELISA CLASSIC PORCINE PEST (IDEXX)
 1. Add 50ul of diluting material of the Sample to all pipettes to be used in the Test and to Control pipettes.
 2. Add 50ul of Positive and Negative Control to duplicate appropriate pipettes. Do not use the same test-tube points in other samples. Mix contents of the micro-pipettes carefully revolving the plate or by means of an agitator of plate of micro-titling. Incubate the sealed plates with a retracting plastic sheet or inside a humid chamber, at room temperature during two hours.
 3. Eliminate contents of pipettes and wash three times with Washing Solution (1/10).
 4. Add 100 ul of anti CSFV:HRPO conjunction to each pipette. Incubate plates sealed with plastic material or in humid chamber, during 30 minutes at room temperature.
 5. Wash pipettes repeating point 3. Add 100 ul of Substrate Solution to each pipette and incubate 10 minutes at room temperature in the darkness.
 6. Stop reaction after 10 minutes, adding 100 ul of a stopping solution to each pipette.
 7. Measure absorbing capacity of samples of controls at 450 nm.
 8. Calculate the average of absorbing values for each unknown sample submitted to the test and for the controls.

Calculations:

$$\text{Blocking \%} = \frac{\text{OD neg} - \text{OD test}}{\text{O D neg}} \times 100$$

Validation of Test:

The average O D value of the negative control should have an optic density over 0.50. Positive Control should present a blocking percentage over 50%.

Interpretation of Results:

The problem sample is Positive (containing antibodies) if it shows a blocking percentage over or equal to 40%.

Problem sample is Negative (non-containing antibodies) if it shows a blocking percentage below or equal to 20%. If the blocking percentage of the sample is found between 20 and 30% the animal will have to be submitted to the test some weeks later.

• **PPC DIRECT IMMUNE FLUORESCENCE**

1. A piece of tonsil, spleen or ganglion of approximately 1 cm. is cut.
2. This piece is placed in the cryostat at 20°C.
3. Once the piece of organ is frozen, cuts of 4u should be made.
4. Cuttings of 4u are placed on slides where they must be fixed in acetone during 15 minutes at 4°C.
5. Slides are let dry at room temperature.
6. A drop of conjugation is placed on each sample and spread over the tissue.
7. It is placed in a humid chamber and taken to a heater at 37°C for 30 minutes.
8. The slide is placed in a bath carbonate/bicarbonate tampon 0,5 M ph 9,0 during 5 minutes along with mild agitating action.
9. Same procedure is repeated in a bath similar to point 8.
10. It is then taken to a distilled water bath softly submerging all slides 20 times.
11. The slide is taken out and carefully dried to avoid damage of the tissue.
12. A glycerin drop is placed and covered with a slide cover.
13. The tissue is observed at the ultra-violet light microscope.
14. The positive samples to this technique are confirmed with virus-culture in PK 15 cells.

• **PPC VIRUS CULTURE IN PK 15 CELLS**

Viral culture is performed in Leighton tubes. These bring a lamina with one lay of PK 15 cells.

1. Maceration of the organs under study is prepared with cuts of approximately 1 cm².
2. It is crushed with 5cc of diluting liquid and then it is submitted to centrifugation at 6.000 revolutions per minute for 30 minutes. The floating matter is withdrawn.
3. The culture medium is eliminated from the Leighton tube and it is washed with PBS.
4. 1cc of the floating matter is inoculated and then placed to the heater at 37°C for one hour.
5. A virus control is made with a well known virus.
6. A maintenance medium is added to the cell control.

7. After one hour of heat the virus previously inoculated is eliminated and 2 ml of maintenance medium added.
8. It is taken to the heater for 48 to 72 hours. (5% of CO₂)
9. The maintenance medium is eliminated and the tubes are washed with phosphate tampon.
10. The lamina is withdrawn and introduced in a tube with cold acetone and placed 10 minutes to the refrigerator.
11. The lamina is withdrawn and left to dry at room temperature.
12. The lamina is totally covered with PPC conjunction and taken to the heater at 37°C for 30 minutes in a humid chamber.
13. The laminas are withdrawn and placed in tubes with carbonate-bicarbonate tampon during 10 minutes at room temperature.
14. The tampon is eliminated and passed to tubes with distilled water for 10 minutes, at room temperature.
15. The laminas are withdrawn and placed on the slides with a drop of glycerin.
16. The presence or absence of fluorescence is observed at an ultra-violet light microscope.

Note: This point has been observed and verified in situ by the U.S. mission.

FACTOR No. 11: Legal norms and infrastructure presently existing for the control of the disease in the country.

- How long does disease detection take?

The shortest time to detect clinical signs compatible with PPC and perform the diagnosis is 3 days, although 5 days is the most probable period.

- How long did it take to notify commercial partners about PPC focus occurrence in the past 10 years?

Once disease was confirmed, notification was immediate.

Once the diagnosis is confirmed, it is possible to suspend certification in the export of live swine and by-products within 24 hours.

- Is there an Animal Emergency System?

Yes, a Master Plan is available.

Additionally, two sanitary emergencies have been simulated in the regions VII and IX during years 1999 and 2000.

Complementing the above, the risk of the Foot and Mouth Disease introduction from Argentine during the years 1999 to 2002 has resulted in alerting both the private and the SAG systems of animal health.

Note: The English versions of the Master Plan and the Contingency Plan have been handed to the U.S. Mission.

Note: English versions of the documents related with Sanitary Requirements for the process of importation: Regulations related with the zoo-sanitary barriers and SAG Organizational Law were also delivered to the U.S. Mission.
