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Short Communication

Outbreaks of Aujeszky's Disease in Pigs from Panama

N. Obaldía III[1,2]
[1]*Gorgas Memorial Institute, Panamá,* [2]*Biotecnologia Veterinaria S.A., (Biovet S.A.). Panamá, Republic of Panamá*
**Correspondence: Apdo 6-197, El Dorado, Panamá, Republic of Panamá*
E-mail: nobaldia@biovetsa.com

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Abbreviations: CNS, central nervous system signs; ELISA, enzyme-linked immunosorbent assay; FA, immunofluorescence analysis; FCS, fetal calf serum; PR, pseudorabies

INTRODUCTION

Pseudorabies (PR), Aujeszky's disease, is an important endemic disease of swine that causes extensive losses in Latin America (Martell *et al.*, 1976; Echeverria *et al.*, 1992; Obaldía and Rodriguez, 1994; Rodriguez-Buenfill *et al.*, 2002). PR affects pigs of all ages but mainly neonatal pigs, which present neurological signs and nearly 100% mortality. Although older pigs can develop nervous signs and die, they are affected primarily with respiratory signs (Kluge *et al.*, 1999). This DNA virus belonging to the suis herpesvirus type 1, was first isolated in Panama from neonatal pigs in 1994 during an outbreak of PR on a single farm (Obaldía and Rodriguez, 1994). The aim of this report is to describe the first epizootic of PR in Panama.

MATERIALS AND METHODS

In July 2001, farm 1, located in the province of Panama, 40 km to the west of Panama City, reported cases of neonatal pigs dying with central nervous system signs (CNS). Losses on this farm were estimated to be around 400 neonatal pigs during the outbreak, although exact numbers were not available. Paired sera collected in this farm in September tested positive by ELISA and showed active seroconversion to PR. One year later, in July 2002, farm 2, located in the province of Los Santos, 250 km to the south-west of Panama City, reported deaths of neonatal pigs with CNS, with estimated losses of more than 300 animals. Sera taken from pigs on this farm tested

positive to PR. Similarly, pigs on farm 3, located in the province of Veraguas 250 km to the south-west of Panama City and 100 km to the north-west of farm 2, tested positive to PR. This farm reported losses of 398 neonatal pigs with CNS during the outbreak. Finally, in October 2002, PR virus was isolated from the brains of two neonatal pigs on this farm. Farms 4 to 7 belonged to the owner of farm 1 and were located in the same district in the Province of Panama 40–80 km west of Panama City (Figure 1).

Histopathology

Brain tissue from two neonatal pigs from farm 3 was fixed in 10% formalin, embedded in paraffin, sectioned at 5 µm and stained with haematoxylin and eosin.

Viral isolation

A 20% suspension of brain from two neonatal pigs received from farm 3 was made using a TenBroeck grinder with 2 g of brain tissue and 3 ml of PBS–gelatin, pH 7.2, containing penicillin and streptomycin. The suspension was centrifuged at 800g for 30 min and the supernatant was diluted further 1:3 and 1:5 in cell culture medium M-199 (Gibco, USA) containg 2% FCS, penicillin and streptomycin. Confluent Vero cells monolayers in 25 cm^3 flasks were inoculated with 0.2 ml of the 1:3, 1:5 and undiluted brain suspensions and one flask was left uninoculated as control (Cottral, 1978). Infected Vero cells were harvested 72 h post inoculation and cell spots were made in 12-well slides for direct immunofluorescence analysis (FA). A PR conjugate diluted 1:30, obtained from the National Veterinary Diagnostic Laboratory, USDA, Ames, IA, USA, was used to detect viral antigen in cold acetone fixed slides. Non inoculated Vero cells spot slides were used as negative controls.

Serology

Sera were analysed using a PR virus ELISA screening kit (IDEXX, Westbrooke, ME, USA). The plates were read using a 650 nm filter in a Dynatech MR5000 ELISA reader. S/P ratios over 0.2 were considered positive. Paired serum samples were collected 12 days apart on farm 1 from 50 pigs (33 sows and 17 boars) in order to demonstrate active seroconversion against PR. A seroprevalence study was carried out in farms 1–7 between September 2001 and September 2002. In total, 410 serum samples were analysed from the farms as follows: farm 1 (50); farm 2 (106); farm 3 (43); farm 4 (45); farm 5 (96); farm 6 (45) and farm 7 (25). A cross-sectional prevalence study was carried out in September 2002 on farm 3 that included groups of 20 pigs per cohort as follows: weaned-finishing pigs 22–60 days, 61–120 days and > 121 days old, gilts and sows.

A questionnaire (results shown in Table I) was sent to the three PR positive farms (farms 1 to 3) in order to obtain data on mortality.

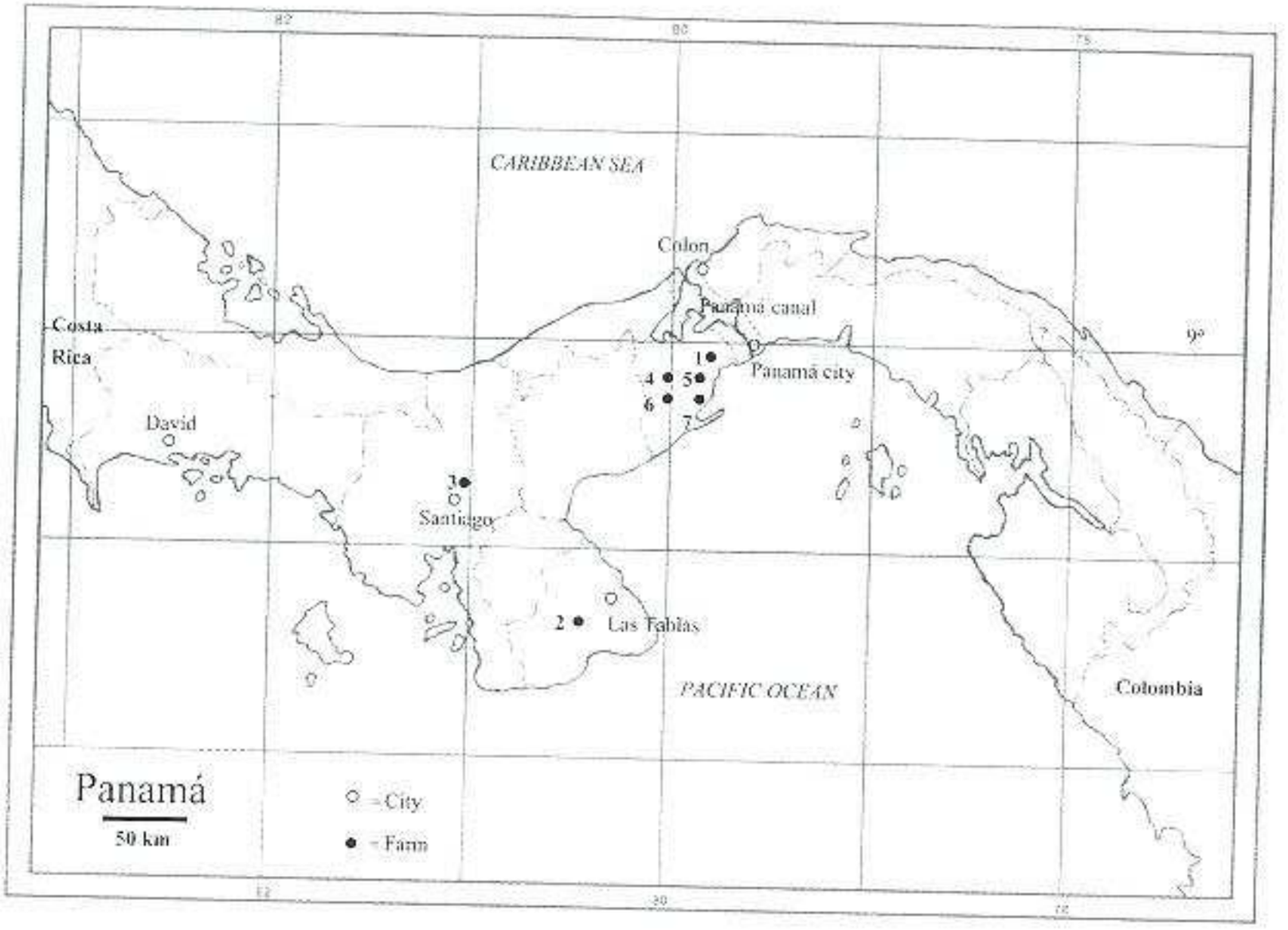

Figure 1. Map of Panamá showing location of pig farms 1–7 (black dots) during outbreaks of pseudorabies, 2001–2002

TABLE I
Mortality and production parameters of farm 3 during an outbreak of Aujeszky's disease

		No.	%
Date of introduction of new breeding stock	06/02/2002		
Date of index case of CNS	10/05/2002		
Total sows		353	
Total litters		227	
Total live births		2266	
Average litter size		10.8	
Average age days initiation of CNS		5.5	
Deaths total		475	20.9
Neonatal deaths attributed to Aujeszky's		398	17.5
Neonatal deaths other causes		77	3.4
Mummified pigs		27	1.2
Litters affected by Aujeszky's		71	31.3
Abortions		4	1.8
Sows death CNS		2	0.6

CNS, central nervous system signs

RESULTS

Clinical signs in neonatal pigs from farm 1, 2 and 3 were similar and included, vomiting, fever, anorexia, trembling, hypersalivation, incoordination, ataxia, ophistotonos and death.

Histopathology

Focal gliosis, perivascular cuffing with mononuclear cell infiltration as well as a non-suppurative meningitis was evident in tissue sections from the brain of both neonatal pigs from farm 3.

Viral isolation

A cytopathic viral agent isolated from the brain of two neonatal pigs from farm 3 using Vero tissue culture cells was confirmed by FA. Cytophatic effect and syncytium formation was observed 24 h after inoculation in all three flasks of Vero cells, inoculated with 1:3 and 1:5 and with undiluted 20% brain suspension. The infected cells were positive for PR specific antigen by FA.

Serology

As shown in Table II, paired sera collected on farm 1 all tested positive to PR and demonstrated active seroconversion (sample 1, $n_1 = 40$, S/P value $\bar{x} = 2.70$, $s_1 = \pm 0.37$; sample 2, $n_2 = 50$, S/P value $\bar{x} = 3.54$, $s_2 = \pm 0.47$; *t*-test, $p = 1.68576 \times 10^{-13}$). On farm 2, 92/106 (87%) pigs tested positive to PR. Farm 3 reported clinical cases in September 2002 and 14/43 (33%) pigs (22 gilts and 21 sows) tested positive to PR. In the farms reporting PR cases (farms 1–3) the antibody prevalence varied between 14% and 84% in gilts, between 52% and 100% in sows, and between 60% and 100% in boars (Table II). No PR antibodies were detected in farms 4–7. Table III shows the prevalence rate observed in a cross-sectional study done in weaned-finishing pigs, gilts and sows from farm 3. In total, 43% (3/7) of all farms and 38% (156/410) of the pigs studied tested positive to PR with 89% (139/156) having ELISA S/P values >2.51.

TABLE II
Seroprevalence of antibodies for Aujeszky's disease on farms during outbreaks

	Gilts +/total (%)	Sows +/total (%)	Boars +/total (%)	Total +/total (%)
Farm 1	ND	33/33 (100)	17/17 (100)	50/50 (100)
Farm 2	52/62 (84)	37/39 (95)	3/5 (60)	92/106 (87)
Farm 3	3/22 (14)	11/21 (52)	ND	14/43 (33)
Total	55/84 (65)	81/93 (87)	20/22 (91)	156/199 (78)

+, number positive

ND, not determined

TABLE III
Cross-sectional ELISA Aujeszky's antibodies seroprevalence study in pigs from farm 3

Weaned-finishing pigs				
Age (days)				
22–60 +/total (%)	61–120 +/total (%)	>121 +?total (%)	Gilts +/total (%)	Sows +/total (%)
21/21 (100)	14/16 (87.5)	13/20 (65)	3/22 (14)	11/21 (52)

+, number positive

Only farm 3 answered the questionnaire. Losses from this farm were estimated to be on the order of US$75 000, based on a 10% mortality to finishing and an average weight of 82 kg live weight per pig to market including veterinary care and diagnostics.

DISCUSSION

It is postulated that the outbreak in susceptible animals introduced onto the premises on farm 1 originated from PR virus that had remained circulating latently within the premises since 1994, when PR was isolated for the first time by Obaldía and Rodriguez (1994). However, this hypothesis remains to be tested using molecular biology techniques to compare both isolates (Echeverria *et al.*, 1994; Nosetto *et al.*, 1997). In the case of farms 2 and 3, an outside source of transmission, such as newly introduced breeding stock seems the most likely source of infection. The movement of actively shedding or latently infected swine is the most frequent single source of transmission from one farm to another (Kluge *et al.*, 1999). It is also known that, after a primary outbreak of PR has been resolved, transmission becomes effectively limited to the breeding herd and, depending on their access to exposure, the finishing swine (Kluge *et al.*, 1999). This seem not to be the case for farm 3, where 14% of gilts and 52% of sows had a low seroconversion rate in a cross-sectional study when compared to the high prevalence (100%) found in 22- to 60-day-old piglets, indicating a recent introduction of the disease on this farm. This is supported by the fact that 89% of the pigs tested had high ELISA antibody S/P values > 2.51, indicating seroconversion at less than 60 days of exposure. Losses attributed to Aujezsky's disease during these outbreaks in farm 3 were similar to those reported previously by Obaldía and Rodriguez (1994) during an outbreak in farm 1, where 23.8% of neonatal pigs and 31.4% of litters were affected with PR. Following these outbreaks, the Department of Animal Health of the Ministry of Agriculture and Livestock Development (MIDA) started an intensive serological campaign to detect infected farms and develop a national control strategy against the disease. These outbreaks are considered to be the first reported epizootic of PR disease in Panama.

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