

Leptospirosis Seroconversion During an Abortion Outbreak in a Mexican Hairless Swine Herd

M. A. Cisneros-Puebla, D. Mota-Rojas, L. P. Moles-Cervantes,
R. Ramírez-Necochea and M. Alonso-Spilsbury

Área de Investigación: Ecodesarrollo de la Producción Animal, Departamento de Producción Agrícola y Animal, Universidad Autónoma Metropolitana, Unidad Xochimilco. Calzada del Hueso 1100, Col. Villa Quietud, Coyoacán, México, D. F. 04960

Abstract: A herd of Mexican Hairless Swine, maintained in an agro-silvi-culture pasture in the village of Chapa de Mota, Mexico, underwent a serological study after one of the sows aborted a litter showing fever. Blood samples were drawn from thirty-four animals in reproductive stage, which then underwent a treatment, based on a streptomycin dose of 25 mg/kg live weight during five days. Twenty-eight samples were taken 15 days after the herd either farrowed or aborted. Seroconversion was found in five animals with a serovar of *hardjo* H 89 origin. One of the animals aborted after seventy-eight days of gestation; in all the cases there was a negative increase of between 1:200 to 1:800 titres, while the serovar *bratislava* presented titres in six animals. In two cases the seroconversion went from a negative to a 1:200, while one of the pigs miscarried on the 77th day of pregnancy, and two other animals went from a titre 1:100 to 1:200. Another sow lost a litter on day 84, and in the last two animals the titre was maintained at 1:100, though one of the pigs aborted on the 86th day. This paper constitutes the first report of an outbreak of abortions in the Mexican Hairless Swine caused by *Leptospira interrogans* serovars *bratislava* and *hardjo*.

Key words: Immunodiagnosis, seroconversion, abortion, leptospirosis, *L. bratislava*, *L. hardjo*, mexican hairless pigs, pigs

Introduction

Currently, the Mexican Hairless Swine (MHS) is an endangered genetic resource due to the constant introduction of better breeds (FAO, 1994; Lemus, 1999 and Lemus-Flores *et al.*, 2001). Traditionally the MHS has been bred in local backyards for family economics reasons, without veterinary advise. The importance of the MHS in the rural communities is double; because it improves the peasant or farmer's diet, and it also helps the family economy when the pigs are fattened up for sale (Conejo, 1993 and Becerril *et al.*, 2000). In order to increase the number of animals of this biotype, it is not only necessary to improve the productivity to an efficient level but there has to be action taken in terms of preserving its health through precise and opportune diagnosis.

In many cases the etiology of abortions in swine is little known. Nonetheless, what has been established is that one of the causing agents of *Leptospira* (Ellis *et al.*, 1986a) has been reported on farms practically worldwide (Faine, 1994 and Ellis, 1999).

Pigs act as maintenance hosts for serovars belonging to the Pomona, Australis and Tarassovi serogroups (Ellis, 1999); while strains belonging to the Canicola, Icterohaemorrhagiae, Grippotyphosa and Serjoe serogroups are among the more commonly identified incidental infections in swine (Faine, 1994 and Ellis, 1999). This has been confirmed in serological studies where samples of infections caused by serovars of the *serjoe* serogroup were present, and in which *hardjo* serovar were less frequent; while the infections caused by the *australis* serogroup were aply distributed (Ellis *et al.*, 1986a).

Porcupine, hedgehog and small rodents of the wild animal species (Ellis *et al.*, 1986c) play a very important role for *bratislava* serovar as maintenance carriers, as in the case of domestic animals such as the pig (Ellis, 1999). *Hardjo* serovar infection is maintained by cattle worldwide (Ellis *et al.*, 1986a), and where cattle and pigs come to close contact the opportunity arises for infection in pigs to occur (Ellis, 1999).

Serovar *bratislava* has taken on a great importance in the last decades. Its presence has been demonstrated by serological evidence in elevated frequencies, in different countries around the world, like Australia (Chappel *et al.*, 1992), the United States (Bolin and Cassells, 1990 and Bolin *et al.*, 1991), the United Kingdom (Hathaway and Little, 1981); Northern Ireland (Ellis *et al.*, 1986a), Brazil (Oliveira *et al.*, 1995), Zimbabwe (Mavenyengua *et al.*, 1999) and South Africa (Potts *et al.*, 1995); while countries like Argentina are not mentioned at all (Rosetti *et al.*, 1997 and Francois *et al.*, 1997).

For *bratislava* serovar, it has been stated that within the pig population there is a significant statistic correlation in the presentation of serum antibodies and abortions (Ellis *et al.*, 1986b). The infection by *Leptospira interrogans* of the *bratislava* serovar provokes reproductive failure. *Bratislava* and *hardjo* serovars have been isolated from litters of aborted and stillborn piglets (Ellis *et al.*, 1986a). The disease has been linked to early embryo deaths, birth of

weak piglets, stillbirth, abortion, and to infertility, as well as affecting kidneys and the genital tract of females and males that belong to herds which show reproductive problems (Ellis *et al.*, 1985 and Bolin *et al.*, 1991). It produces abortion in the last third of gestation (Ellis *et al.*, 1986b).

In Mexico, many serological surveys have been done; the presence of antibodies against 20 serovars of *Leptospira interrogans* has been reported by Moles *et al.* (1991). Until a few years ago, the serofrequency of *bratislava* leptospirosis was unknown (Moles *et al.*, 1998). One of the first reports emphasizes on an analysis conducted with the results of 4,534 pigs, which determined the presence of antibodies against *bratislava* serovar in 0.2% and *hardjo* serovar in 6.1% (Jiménez *et al.*, 1986). Afterwards, the results of 2,097 porcine serums were analyzed, and a frequency of 0.8% of *L. bratislava* was reported; 4% was found for *hardjo* serovar (Rojas *et al.*, 1994). Another mention of serological presence of *Leptospira* indicated that in the pig population of Central Mexico, findings demonstrated that in adult females the most frequent serovar was *bratislava* reporting 28% of seropositivity; while *hardjo* serovar appeared in fourth place with a frequency of 2.6% (Moles *et al.*, 1998). Recently, a serological study that included 1,970 swine serum of pigs from commercial farms from different parts of Mexico, stated that *bratislava* serovar occupied the first place of frequency with 22.5%, while *hardjo* showed 5.1% (Cisneros *et al.*, 2001).

The identification of seric titres with the microscopic agglutination test is very useful in diagnosing acute infections in a single animal test, and the rising antibodies titres together with paired serum samples are a good diagnostic tool (Ellis, 1999). A distinct serologic profile has been found in sows kept outdoors, the seroprevalences ($> = 1:100$) may be greater than 50% (Ellis, 1999). An increase of four fold dilutions or more, when we compare the samples or the conversion of a titre less than 1:50 to 1:100 or higher, when clinical data is present and is compatible with Leptospirosis, is considered an infection in course (Panamerican Health Organization, 1985).

The objective of this study was to carry out a serological diagnosis during an outbreak of abortions in a herd of Mexican Hairless Pigs, raised in agro-silvi-culture pastures in Chapa de Mota, in the State of Mexico.

Materials and Methods

During the outbreak of abortions, the herd was made up of 30 sows with different weeks of pregnancy, 8 replacement females and 5 boars. Twenty of the pregnant pigs were lodged during eighteen hours in a communal pen measuring 96 m² with a cement floor and straw bedding. The rest of the day they were put out to pasture in a forest of evergreen. The daily food intake during gestation was of 1.5 kg of concentrated feed per pig (12.5 MJ EM/kg and 15% of CP) as well as acorns and grass native to the area. During pasture the pigs shared space with 8 cows, 90 sheep and 68 goats.

The first clinical sign was detected when eighty per cent of the gestating sows (16) decreased their food intake up to 70% and resisted going out to pasture. The majority lay on the floor and began having bouts of fever (40.0 to 40.5°C). In the first 48 to 72 hours after the symptoms appeared, the first and second abortion occurred. The decision was made to take the first blood tests (34 pigs in reproductive stage were bled) and the gestating females were then treated with streptomycin (25 mg/kg of live weight) during five days. Two days after the blood samples were taken, two more abortions occurred. Twenty-eight samples were taken from the pigs that aborted 15 days later after the first sample had been taken. Twenty percent of the pregnant sows aborted.

Serological samples were analyzed using a microscopic agglutination test (OPS, 1985 and OIE, 1992), using a battery of nine serovars of *Leptospira interrogans*, of which six international references were used. These were: *icterohaemorrhagiae*, *grippotyphosa*, *pomona*, *hardjo*, *tarassovi*, *bratislava*, as well as the results of three Mexican isolated cases (Rojas *et al.*, 1994): *portland vere*, named Sinaloa ACR strain, (isolated during an outbreak of abortions in pigs); *hardjo* named H-89 strain, (isolated from a bovine fetus that was aborted); and *icterohaemorrhagiae*, named Palo Alto strain (isolated from a clinical case of canine leptospirosis). Those sera which titres were equal or higher to 1:100 were considered positive (OPS, 1985 and OIE, 1992).

Results and Discussion

The results indicated a seroconversion in five of the animals for serovar *hardjo* strain H-89, with one of the pigs aborting at 78 days of gestation. In all of the cases the negative factor changed to a titre between 1:200 and 1:800. *Bratislava* serovar on the other hand, presented titres in six animals; in two of them the seroconversion was reported as negative to a titre of 1:200. One of the females aborted after 77 days of pregnancy, while two other animals went from a titre of 1:100 to 1:200; another pig aborted on the 84th day. In the remaining two animals, the titre was maintained at 1:100, though one aborted on the 86th day. The sera of two bovines were tested as well and both showed seroconversion from negative to 1:200 and 1:400, respectively.

The diagnosis on the reproductive failure caused by leptospirosis is well founded worldwide by the detection of antibodies and through the isolation of the agent. The fact that seroconversion was found in the animals that were tested, is indicative of the bacteria spreading within the pigs and other domestic species commingling together. This factor indicates that two serovars: *bratislava* and *hardjo* caused abortion in pigs, *bratislava* being the most

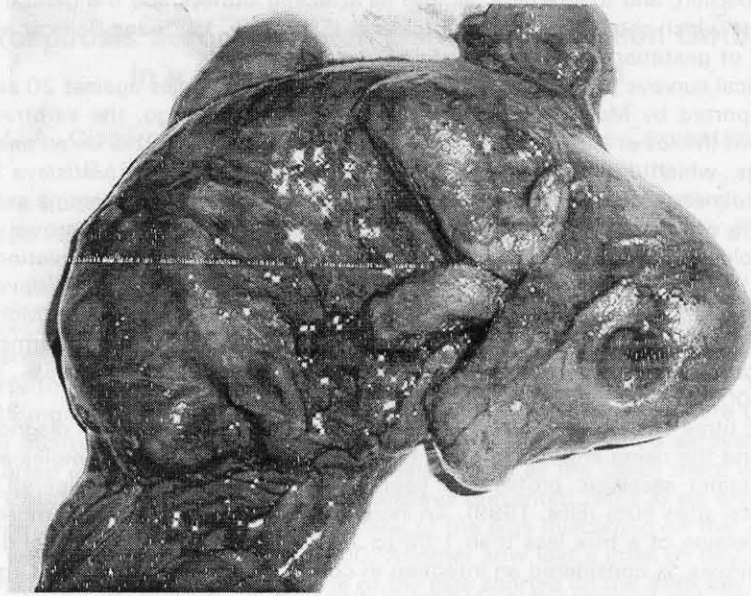


Fig. 1: Seventy-eight day aborted mummified Mexican hairless pig fetus

frequent serovar in the commercial swine production units, as reported by serological studies done in Mexico. The titres of microscopic agglutination antibodies against *bratislava* serovar are not high, usually ranging from 1:200 to 1:800 (Bolin and Cassells, 1990). On the other hand, studies demonstrated that the bovine is the maintenance carrier of *hardjo* serovar, of which antibodies with minor frequencies of 5.1% were detected. This determining factor indicates little activity in the porcine herds. In the United Kingdom it has been established that *hardjo* serovar may cause abortions in the last third of pregnancy and it has been isolated from a pregnant sow and aborted fetuses (Hathaway and Little, 1981 and Ellis *et al.*, 1986a), even though there have not been any national reports on the subject that would indicate outbreaks of abortions as a result of both serovars (Cisneros *et al.*, 2001 and Moles *et al.*, 1998).

The abortions occurred after the 77th day of gestation and it has been reported that *L. bratislava* is responsible of abortions mainly in the last third of the pregnancy (Ellis *et al.*, 1986a); the same pattern was observed in the herd of the present study.

It is important to reiterate that the type of pigs that were studied lived in agro-silvi-culture pastures, which allows for an intense coexistence with bovine herds. It was determined that pigs commingling with bovine herds were probably infected by the serovar *hardjo*, which has been reported as an incidental infection in the pig by Faine (1994) and Ellis (1999), so the source of infection by *L. hardjo* will probably be the bovine. Mean while the *L. bratislava* infection source is not clear due to the fact that there is no previous information of the serological status of the pig herd; although it has been suggested that swine can be a maintenance host of this serovar (Bolin and Cassells, 1990 and Bolin *et al.*, 1991).

The source of infection due to *L. bratislava* is uncertain too since there is no previous information about for how long the pigs have had antibodies against this serovar (Ellis *et al.*, 1986a). The animals in this herd had not received any type of vaccines; thus, the titres of the antibodies detected were exclusively caused by a field infection. Adequate and opportune treatment helped to avoid more abortions and at the same time decreased the elevation of antibodies during seroconversion. Reports have stated that pigs undergoing treatment can cause the titre of antibodies to diminish (Gerritsen *et al.*, 1993). Thus, this study constitutes the first report on an outbreak of abortions in the Mexican Hairless Pig as a result of the serovars of *Leptospira interrogans*, *bratislava* and *hardjo*.

References

- Becerril, H.M., R.D. Mota, S.M. Alonso, N.R. Ramírez, J.M. Berruecos, N.E. Dávalos, M.R.D. Méndez and L.M. Rubio, 2000. Production costs of Mexican hairless pigs raised in semi-intensive conditions. Proceedings of 16th. International Pig Veterinary Society Congress (IPVS). Melbourne, Australia, pp: 156.
- Bolin, C.A. and J.A. Cassells, 1990. Isolation of *Leptospira interrogans* serovar *bratislava* from stillborn and weak piglets in Iowa. JAVMA 196: 1601.

- Bolin, C.A., J.A. Cassells, H.T. Hill, J.C. Frantz and J.N. Nielsen, 1991. Reproductive failure associated with *Leptospira interrogans* serovar *bratislava* infection of swine. *J. Vet. Diagn. Invest.*, 3: 152.
- Chappel, R.J., W.A. Ellis, B. Adler, L. Amon, B.D. Millar, S.S. Zhu and R.W. Prime, 1992. Serological evidence for the presence of *Leptospira interrogans* serovar *bratislava* in Australian pigs. *Austr. Vet. J.*, 69: 119.
- Cisneros, P.M.A., C.L.P. Y. Moles and R.D. Gavaldón, 2001. Serovariedades de *Leptospira interrogans* en cerdos de México. *Cerdos-Swine* 4: 16.
- Conejo, N.J., 1993. La porcicultura rural de traspatio. *Nuestro Acontecer Porcin*, 1: 50-63.
- Ellis, W. A., 1999. Leptospirosis. In: *Diseases of Swine* (B.E. Straw; S. D'Allaire, W.L. Mengeling and D.J. Taylor Eds.) 8th ed., Iowa State University Press, Ames, Iowa, U.S.A. pp: 483-493.
- Ellis, W.A., P.J. McParland, D.G. Bryson and M.S. McNulty, 1985. Leptospire in pig urogenital tracts and fetuses. *Vet. Rec.*, 117: 66.
- Ellis, W.A., P.J. McParland, D.G. Bryson and J.A. Cassells, 1986a. Prevalence of *Leptospira* infection in aborted pigs in Northern Ireland. *Vet. Rec.*, 118: 63.
- Ellis, W.A., P.J. McParland and D.G. Bryson, A.B. Thiermann and J. Montgomery, 1986b. Isolation of leptospire from the genital tract and kidneys of aborted sows. *Vet. Rec.*, 118: 294.
- Ellis, W.A., P.J. McParland, D.G. Bryson and J.A. Cassells, 1986c. Boars as carriers of leptospire of the Australis serogroup on farms with an abortion problem. *Vet. Rec.*, 118: 563.
- Faine, S., 1994. *Leptospira and Leptospirosis*. CRC Press, Inc., Boca Ratón, Florida, U.S.A.
- FAO, 1994. Boletín de Información sobre Recursos Genéticos Animales. FAO-UNEP.
- Francois, S., N. Pereyra, E. Comba, B.J.C. Fain and L. Anthony, 1997. Leptospirosis porcina: resultados preliminares de un relevamiento serológico en el área sur de la pcia. de Santa Fe y sudeste de la pcia. de Córdoba. *Proceedings of VII Congreso Latinoamericano de Veterinarios Especialistas en Cerdos y V Congreso Nacional de Producción Porcina*. Río Cuarto, Córdoba, Argentina pp: 52.
- Gerritsen, M.J., M.J. Koopmans and T. Olyhoe, 1993. Effect of streptomycin treatment on the shedding of and the serologic response to *Leptospira interrogans* serovar subtype *hardjo bovis* in experimentally infected cows. *Vet. Microbiol.*, 38: 129.
- Hathaway, S.C. and T.W.A. Little, 1981. Prevalence and clinical significance of leptospiral antibodies in pigs in England. *Vet. Rec.*, 108: 224-228.
- Jiménez, G.E., R.C. Díaz and D.J.M. Doport, 1986. Detección de anticuerpos contra *Leptospira* de 4354 sueros porcinos. *Vet. Méx.*, 17: 35.
- Lemus, C., 1999. *Estudio Molecular de la Diversidad Genética del Cerdo Pelón Mexicano (Sus scrofa)*. Ph. D. Thesis. Universidad Autónoma de Nayarit, México.
- Lemus-Flores, C., R. Ulloa-Arvizu, M. Ramos-Kuri, F. J. Estrada and R. A. Alonso, 2001. Genetic analysis of Mexican hairless pig populations. *J. Anim. Sci.*, 79: 3021-3026.
- Mavenyengwa, M., E. Keller and T. Munyombwe, 1999. Seroprevalence of leptospiral antibodies in commercial pigs in the Mashonaland east province of Zimbabwe. *Zimbabwe Vet. J.*, 30: 85.
- Moles, C.L.P., R.D. Gavaldón, B.J. Torres, R.V.M. Banda, P.M.A. Cisneros, S.N. Rojas and A.M.A. Luna, 1991. Distribución de la leptospirosis porcina en México. *Memorias de la XXIII Reunión Anual de la Asociación Mexicana de Producción Animal*. Coahuila, México s/p.
- Moles, C.L.P., V.R.M. Urrutia, V.F. Diosdado and G.A. Morilla, 1998. Frecuencia de *Leptospira interrogans* en unidades de producción porcina del altiplano de México. *Vet. Méx.*, 29: 49.
- Oliveira, S.J., P.C.R. Lima, D.E.S.N. Barcellos and S.M. Borowski, 1995. Serological test for the diagnosis of leptospirosis in pigs in Rio Grande do Sul, Brazil: results obtained in farms with and without a history of reproductive disorders. *Pesquisa Agrop. Gaucha*, 1: 263.
- OIE (Office International des Epizooties), 1992. *Manual of Standards for Diagnostic Tests and Vaccines*. 2nd ed. Standards Commission Editor, France.
- OPS (Organización Panamericana de la Salud), 1985. *Manual de métodos para el diagnóstico de la leptospirosis*. OMS. Nota Técnica 30. Argentina.
- Potts, A.D., C. Lotter and J.T.R. Robinson, 1995. Serological prevalence of leptospiral antibodies in pigs in South Africa. *Onderstepoort J. of Vet. Res.*, 62: 281.
- Rojas, S.N., P.M.A. Cisneros, C.L.P. Moles, R.D.G. Gavaldón, A.M.A. Luna and B.J.I. Torres, 1994. Situación actual de la leptospirosis en México. *Proceedings of XIV Congreso Panamericano de Ciencias Veterinarias (PANVET), Simposium Leptospirosis*, Acapulco, México. pp: 531-532.
- Rossetti, C.A., G. Romero, C.D. Auteri, L. González and L.E. Samartino, 1997. Leptospirosis porcina: Consideraciones generales y encuesta serológica en la República Argentina. *Memorias del VII Congreso Latinoamericano de Veterinarios Especialistas en Cerdos y V Congreso Nacional de Producción Porcina*. Córdoba, Argentina, pp:53.