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Chromosomal Damage in Pigs from a Farm of Central Argentina

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Chromosomal aberrations can spontaneously occur in low proportion in pigs, therefore, high frequencies indicate a possible exposure to genotoxic agents. It seems that an increase in chromosomal mutation rate has taken place, taking into account the prevalence of such agents in the general environment of swine populations. For this reason, Gustavsson (1990) advocated the urgent necessity to identify environmental mutagens and to verify their effect on pig health.

Chromosomal damage induced by radiation and also by chemical mutagens in swine has already been reported by different authors. McFee *et al.* (1970a, 1970b, 1971, 1972, 1973, 1974) studied chromosomal mutation rate in lymphocytes caused by neutrons and gamma rays. Fries and Strazinger (1982) analyzed the mutagenic effect in pigs derived from X-irradiated semen, having as a main consequence a decrease of litter size due to an induction of chromosomal translocations and inversions. Forster and Butler (1978) demonstrated an *in vitro* adverse effect of halothane on pig lymphocyte chromosomes.

Pig chromosomal damage induced by virus was reported by Lodja and Rubes (1977) who found that the Swine Fever lapinized live vaccine induced structural chromosomal aberrations as well as lymphocyte polyploidy on vaccinated pigs.

Rubes (1987) suggested the routine examination of lymphocyte chromosome to assess the environmental quality of pig farms which are exposed to pollution from different substances such as aflatoxin B1, biphenols, polychlorinates, DDT, lindane, mercury and cadmium. Rubes *et al.* (1992) used this analysis not only in pigs but also in other farm animals as a pollution-level indicator due to agricultural and industrial activities in the Czech Republic. About 3.6% of pig lymphocytes showed chromosomal damage in highly contaminated farms of that country.

Several cases of chromosomal damage in pigs with reproductive problems from a farm located in Río Cuarto surroundings, an important swine production region of central Argentina, are reported in the present paper.

Material and methods

Cytogenetic analysis was performed in ten Duroc pigs from a farm located about 30 km away from Río Cuarto National University's campus, Córdoba Province, in central Argentina. Consultations resulted from a real necessity of farmers after having sudden reproductive problems: Last farrowings of sows which had previously given birth to normal size litters and then had begun to give birth to litters no bigger than three piglets each time. Two of those showed hindleg paralysis. One was sacrificed and sent to clinical observations. Adult animals had been recently vaccinated against Swine Fever virus; the region is endemic for Aujeszky disease.

In a first sampling, analyses in eight breeding pigs (7 sows and 1 boar) were performed; some

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months later, they were also done in a sow and its piglet. Since all animals were raised under similar farming management sharing the same type of environment, and therefore, they had been exposed to the same genotoxic agents, methodology control was made by using blood samples from three pigs of the same swine race which came from another farm. Those control animals presented a normal karyotype as it has been previously reported (Genghini *et al.* 1994). Lymphocyte cultures of both, affected and control animals, were done using the same batches of tissue culture materials when chromosome observation was being held.

Heparinized blood samples were obtained from auricular vein. Lymphocyte cultures were performed in TC199, since they give higher mitotic index than in RPMI medium. TC199 was supplemented with 10% calf serum plus bovine fetal serum, 1% glutamine, 1.5% cystine, 1% penicillin-streptomycin and phytohemagglutinin as mitogen. Cultures were incubated at 37°C for 72 hr and treated with colchicine (0.05 μg/ml) for 30 min before fixation. Air dried slides were made after hypotonic treatment and fixation with methanol-acetic acid 3 : 1.

Preparations were stained with Giemsa for aberration counting. Chromosome identification was made according to the Standard International Karyotype for pigs (Committee 1988) by means of GTG-banding using Seabright's technique (1971). About 100 metaphases per animal were scored blind.

Viral agents in macerated organs of an affected sow were searched. Samples of lungs, spleen, liver, tonsils, maxilar and suprahepatic ganglia were observed. Monolayers of Vero cells were infected with those organs looking for cytopathic effect of Aujeszky virus; the sow's serum was used to detect antiviral antibodies of Aujeszky disease by means of microserumneutralization.

Results

Cytogenetic results from the first sampling are shown in Table 1. The eight tested pigs from the first sampling showed a high incidence of chromosomal damage including chromatid and chromosome breaks, chromatid exchanges (triradial and quadriradial configurations) and even meta-

Table 1. Frequency of chromosome aberrations observed in hypoprolific and normal pigs

Animal N°	Number of cells scored	Abnormal metaphase per 100 cells	Chromosomal aberrations per 100 cells					
			AL	B′	B″	RB′	FRG	MA
Controls								
1	100	—	1.0	—	—	—	—	—
2	95	—	1.1	—	—	—	—	—
3	100	—	2.0	—	—	—	—	—
All controls	295	—	1.4	—	—	—	—	—
Hypoprolific pigs								
1	102	24	8.8	5.9	9.8	0.9	4.9	3.9
2	100	20	5.0	9.0	5.0	1.0	6.0	2.0
3	114	19	9.6	7.9	3.5	3.5	2.6	1.7
4	106	21	11.3	10.3	1.9	1.9	5.6	2.8
5	100	29	8.0	9.0	7.0	3.0	9.0	2.0
6	97	25	14.4	13.4	2.1	1.0	6.2	4.1
7	100	19	8.0	6.0	3.0	2.0	7.0	3.0
8	99	32	9.1	11.1	7.1	3.0	8.1	6.1
All hypoprolific pigs	818	23.5	9.0	9.0	4.9	2.1	6.1	3.2

Abnormal cells, cell with at least one chromosomal aberration. Cell which only had achromatic lesions were not scored as abnormal. AL, achromatic lesions (gaps); B′, chromatid break; B″, isochromatid or chromosome break; RB′, chromatid exchange; FRG, fragment; MA, multiple abnormalities.

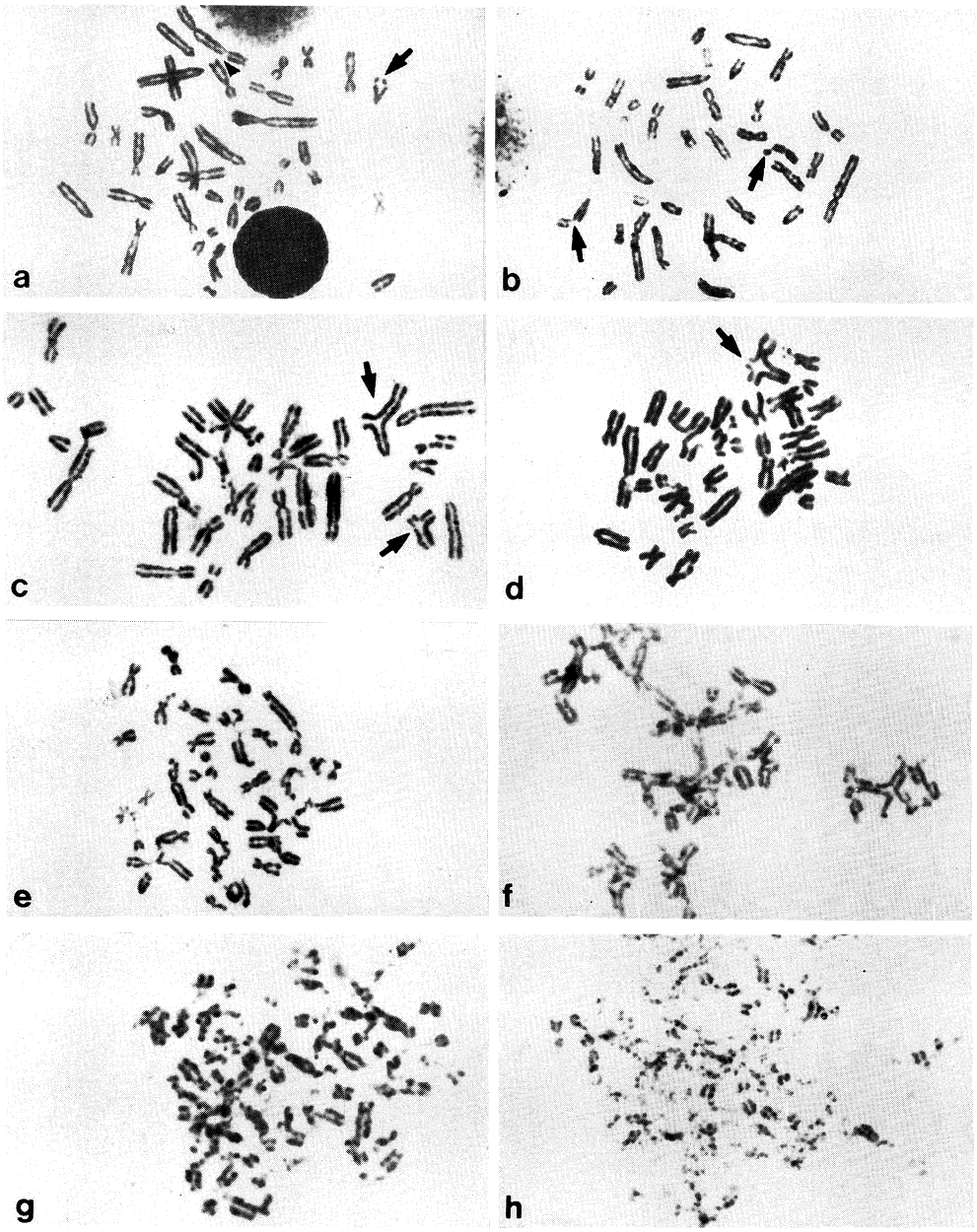

Fig. 1. Pig chromosomes with several types of aberrations. a) isochromatid gap (arrow), monochromatid break (arrow-head), b) fragments (arrows), c) trirradials (arrows), d) quadrirradial (arrows), e) and f) multiple abnormalities, g) and h) different expressions of chromosome pulverization.

phases with multiple aberrations (Figs. 1a–f). The frequency of abnormal cells (from one cromatid break to multiple abnormalities) ranged from 19 to 32%. The highest frequency was found in a sow with hindquarter paralysis. Blood samples taken from control animals gave normal karyotype, presenting monochromatid gaps in only 1.4% of the cells, that percentage is considered the basal damage value in this study.

Chromosomal pulverization, as defined by Savage (1976) was observed (Figs. 1g, h). This abnormality was not recorded individually for the first eight animals tested. Cells with pulverization

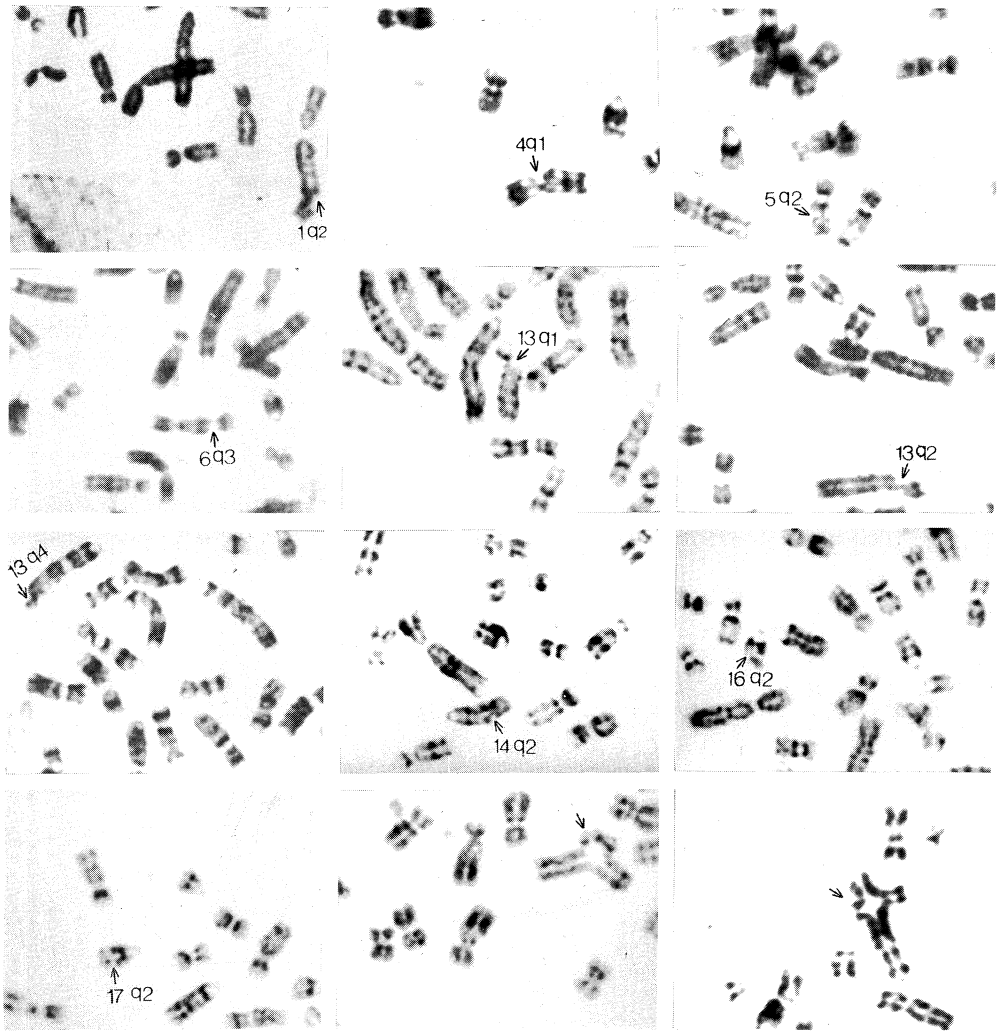

Fig. 2. Pig GTG-banded chromosome showing different breakage regions (arrows).

Table 2. Chromosome regions with breaking points revealed with GTG-banding

Chromosome regions	% Cells
1q2	20
4q1	6
5q2	3
6q3	6
13q1	6
13q2	23
13q4	13
14q2	6
16q2	6
17q2	3

reached a proportion of 15% when considering all these animals in bulk. An average of 80% of cells showed pulverized chromosomes in the sow and its only surviving piglet from the second sampling, as the only chromosomal aberration type recorded in these two animals.

The analysis by GTG-banding of cells with chromosome alterations evidenced that chromosome breakage mainly appeared in ten regions of eight chromosomes (Table 2, Fig. 2). Chromosome 13, the longest telocentric, and therefore easily identifiable without banding, showed the highest aberration frequency, with

breaks located mostly at q2 and q4 regions in 36.6% of cells. Breaks in 1q2 were detected in a lower proportion (20% of cells). Aberrations in other chromosomes happened in 3 to 6% of cells.

Clinical studies of the sacrificed sow as well as macroscopic analyses of several organs were within normal limits. There were no detectable effects for Aujeszky virus in macerated organs; although the hepatic suspension produced morphological alterations by modifying the Vero cell monolayers in a way non compatible with the typical cytopathic effect of Herpes Virus family to which Aujeszky virus belongs to.

Discussion

Pigs with reproductive impairment showed a high frequency of chromosomal damage in peripheral blood lymphocytes. However, as chromosomal aberrations were unstable and mainly of the chromatid type, it is not possible to assert that hypoprolificity was a direct consequence of the cytogenetic damage observed. A more reasonable interpretation could be given by inferring that both chromosomal damage and hypoprolificity are more likely to be originated by the same etiologic agent. This assumption is supported by the fact that hypoprolificity appeared in a sudden way in the farm.

The type and extent of chromosomal damage could be a virus infection indicator. It is well known that most virus assayed either *in vivo* or *in vitro* tests show clastogenic properties (Nichols *et al.* 1962, 1964, Stich *et al.* 1964, Stich and Yohn 1970, Krut 1974, Soldadovic *et al.* 1981, Dulout *et al.* 1983, 1985). Moreover, viral vaccines seem to have a similar effect, however, no conclusive results on this topic have been reported (Nichols 1963, Harnden 1964, Aula 1965, Lodja and Rubes 1977). The hypothesis of viral infection is supported by the occurrence of chromosome pulverization which is originated after cell fusion induced by certain virus promoting syncytium formation, according to Johnson and Rao (1970). When no syncytia induction occurs, this should be due to a degradation of condensed chromatin as a result of the virus infection, as observed by Stich *et al.* (1964) for Herpes and by Nichols *et al.* (1964, 1965) for Measles viruses.

The higher incidence of breaks found in chromosomes 13 and 1 was probably related to their inherent size. Breaking points, except for 13q1, are coincident with the well known reciprocal translocation points associated with fragile sites (Yang and Long 1993, Rigg *et al.* 1993).

The existence of two animals affected with hindleg paralysis would suggest the presence of some pollutant with genotoxic effects in the farm where samples were taken. This would support Long's observation (1991) that there are some chromosome damaging factors within the pig environment which would explain the origin of breaks producing reciprocal translocations. Such agents could be drugs, chemicals and pathogenic organisms which might be present in pig farms; although some kind of environmental stress cannot be excluded.

We believe that the chromosomal damage found in our material was induced by some genotoxic agent which affected the animals of the surveyed farm. Due to the prevalence of chromosome pulverization in the cytogenetically sampled animals, the most probable clastogenic agent might be a virus. However, further investigations are necessary before any definitive conclusion can be reached.

Summary

Cytogenetic analysis of peripheral blood lymphocytes was carried out in ten hypoprolific pigs from a farm located near Río Cuarto National University, where sows gave birth to no more than three piglets in their later farrows.

Blood samples from seven sows and one boar were obtained in a first instance. Animals showed a high frequency of chromosomal abnormalities (ranging from 19 to 32 per 100 cells

scored). Chromosomal aberrations were mainly chromatid breaks, chromatid exchanges (triradials, quadriradials) and chromosome fragments. In addition, 15% of the metaphases showed chromosomal pulverization. A sow and its piglet were studied in a second sampling detecting only chromosome pulverization aberration type (80% of the cells scored in average). Chromosomal localization of breaks was done with GTG banded metaphases. At least, eight chromosomes were involved, the highest frequency of breaks was located in the longest telocentric (chromosome nº 13). "Chromosome pulverization" has been associated to virus infection. All studied adult animals were vaccinated against Swine Fever, Aujeszky disease is endemic for the region. Consequently, chromosomal damage could be induced by subclinical viral infection. Chromosomal anomalies might be related to the reproductive impairment of pigs; however, further studies would be necessary to identify the clastogenic agent.

Key words : Pig cytogenetics, Chromosomal aberrations, Clastogenic agents.

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