

Unexpected chromosomal alterations in *Tayassu tajacu* (Artiodactyla: Tayassuidae) in captivity

Alterações cromossômicas inesperadas em *Tayassu tajacu* (Artiodactyla: tayassuidae) de cativeiros

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Abstract

Wild animals have been used as bioindicators in situations in which the environment was exposed to chemical agents. In general, chemical agents may induce chromosomal aberrations, such as breaks and gaps. The peccary, *Tayassu tajacu* is a pig relative that exhibits a very stable karyotype with the only described alterations being of the form of the X chromosome. Chromosomal gaps and breaks were observed at high frequencies during cytogenetics analyses. These alterations were observed in the chromosomes autosomically. Reviews of the literature and of the data described herein suggests that an vermifuge, the ivermectin base, was the most likely cause of these chromosomal alterations.

Key-words:

Tayassu.
Karyotype.
Chromosome.
Chromosomal alterations.

Introduction

Wild animals are used to study genotoxicity and mutagenicity of chemical agents to understand the consequences of these chemical agents in wild populations.^{1,2} These chemical, stress-causing, agents usually enter the environment because of accidental spills, from indiscriminant use of chemicals in agriculture and mining, and from industrial wastes and by-products. Organisms may suffer in its set of chromosomal alterations during irregular nuclear division, or accidents can happen (due to radiation or to chemicals). The occurrence of chromosomal alterations in the form of breaks are linked genic mutations, due to most mutagens studied to date induce these kinds of chromosomal

alterations and genic mutations.³ These mutagens also are often carcinogenic.

In the last years, interest has increased in the occurrence, importance and consequences of potential genotoxic activity of a variety of drugs and chemicals. Concurrently, interest has also increased in the potential to cause genic mutation or chromosomal aberration of these chemicals. At the same time, to combat parasite infections in a variety of animals, large doses of therapeutic agents are required^{4,5}, and these same antiparasitics chemicals may also have mutagenic effects on the organisms they are designed to protect. To date, for example, no antihelminthic agents have been shown to be risk-free.⁶

Two species of wild pigs are known to occur in nature in Brazil: the collared

peccary (*Tayassu tajacu*) and the white-lipped peccary (*T. pecari*).⁷ While they are within the same Order as true pigs (Artiodactyla) they are placed in their own Family, Tayassuidae, while true pigs are in the Family Suidae. Peccaries are increasingly being raised in captivity for economics ends. Cytogenetic studies have demonstrated that the peccaries have a very stable $2n$.^{8,9,10,11,12,13,14}

Data presented here were gathered as part of a study in which genetic, cytogenetic and molecular variability are being studied in captive peccary herds in southern Brazil. Our objective here is to report on unexpected genetic alterations that were observed in *T. tajacu* taken from these herds.

Materials and Methods

Four males and six females of the collared peccary from the ranch, *Fazenda da Praia* (BR 376, Km 454), in the Ponta Grossa municipality, in Paraná State, were analyzed cytogenetically. Peripheral blood was collected in the field and lymphocytes were obtained from the sample by culture following Moorhead et al.¹⁵, with modifications. Slides were colored using conventional procedures (Giemsa at 10%), then analyzed, with the best metaphases being photographed. When chromosomal alterations were observed in this sample, a second sample was taken five months after the first from the same individuals.

Chromosomal alterations types were identified and the frequency of chromosomal alterations were obtained according to Beiguelman^{16,17}, respectively. Examples from nine of the individuals studied were used, in order to obtain samples of the best quality. Thirty cells per individual were examined, sketched and analyzed. Results from this study were compared to the same data taken from two ranch employees and two horses. This group had taken no vermifuge, used the same water source as the peccaries. Additionally, the horses ate the same feed as the peccaries.

To attempt to determine the possible

agents of the chromosomal alterations, a questionnaire was developed and given to the employees responsible for the animals. This questionnaire was given in the form of an interview, enclosing information as: types of medicines, therapeutics indications, data of medicine, dosage, composition, frequency of use, feeding type, origin of the feeding, storage of the feeding and localization of the captivity.

Results, Discussion and Conclusions

The karyotype of the Collared Peccary is $2n=30$ and $NA=46$. The X chromosome is acrocentric median and the Y is acrocentric, smaller chromosome of the set, the same as that found in other studies.^{8,10,13} Chromosomal alterations were in the form of gaps and breaks of the chromatids and isochromatids¹⁶, which varied from 20-53% of the cells observed (Figura 1). The alterations occurred in the larger chromosomes of the complement, in the short arms of metacentric of the pair 1 chromosomes (Figura 2a) and in the long arms of submetacentric of the pair 2 chromosomes (Figuras 2b,d). These same alterations also occurred in the metacentric chromosome pairs 3, 4 or 5 (Figura 2c) and in the acrocentric pairs 8 or 9 (Figura 2d). Three metaphases showed multiple breaks and gaps (Figura 2e).

No alterations of any kind were observed in the chromosomes taken from ranch employees and horses cells. Prior to this study, chromosomal variation in *T. tajacu* had only been in the form of the X chromosome (metacentric or submetacentric in North America^{9,12} and telocentric in Brazil^{8,10,13}) and one translocation chromosomal polymorphism was detected in single specimen of sample from São Paulo State, before chromosomes X and of pair 8¹³.

During the interviews with questionnaires, it was discovered that an antihelmintic had been applied, which have as solution basic the ivermectin. This application had taken place eight months and

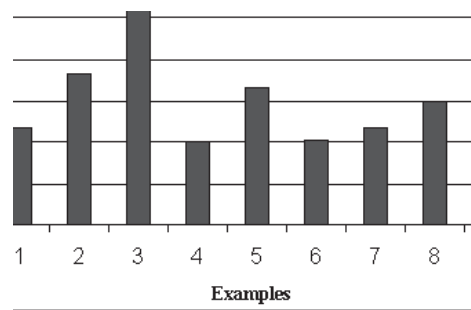


Figure 1
Frequency of chromosome alterations in the studied sample

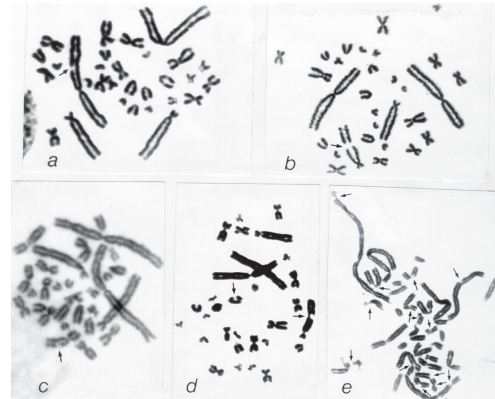


Figure 2
Chromosomal alterations (arrows), in females metaphases cells: a) Chromatid gap in the short arm of the pair 1 chromosome; b) isochromatids breaks in the long arm of the pair 2 chromosome; c) Chromatid break in the pair 3 chromosome; d) Chromatids gaps in the pairs 2 and 8 chromosomes; e) Metaphase showing several breaks and gaps

again 26 days prior to the first sample collection. Ivermectin is a potent monocyclic lactone which pertains to the class of compounds known as avermectin⁴. Ivermectin has high activity levels and an ample vermicide spectrum, for both, endo and ectoparasites, and is used for parasite control in cattle, pigs and sheep.

In the literature it is reported that antihelmintics in general have high toxicities with clastogenic, teratogenic and possibly mutagenic activities.^{4,5,6,18,19} More specific studies of antihelmintic compounds have

shown mutagenic activities in various bacterial assays and in animal cells, including those of humans.^{5,19} The following drugs have also shown mutagenic activity: pyrinium pamoate and pyrantel pamoate, with effects in various lineages of *Escherichia coli* and *Salmonella typhimurium*; niridazole, which is mutagenic and carcinogenic in bacteria, *Neurospora crassa* and in mammal tissue cultures; derivatives of benzimidazole, with effects in *S. typhimurium*; albendazole, with possible teratogenic effects during early stages of pregnancy in some domestic animals; mebendazole and thiabendazole, which induce the formation of abnormal sperm cells in rats.

Studies in humans and pigs show that praziquantel can induce high frequencies of polyploid lymphocytes as well as structural aberrations.¹⁹ Albendazole, mebendazole, praziquantel and ivermectin, while very useful for parasite control, are at least potentially embryotoxic, fetotoxic, mutagenic and teratogenic.⁶ Ivermectin has also been associated with palatal fissures and inexplicable maternal deaths in mice⁸. The size of the gene *mdr-3* (from the retrovirus LTR) transcript for mutant rats sensitive to ivermectin is different than that for the transcript of wild rats.²⁰

Despite the fact that our study includes captive animals, we believe that our data are valid and are in agreement with Fox.² When affirm that “investigation of Chemically induced in population wildlife may identify the potential risk to sub population humans. Evidence evaluated using the epidemiologic criteria may assist environmental managers to determine whether a substantive case can be made to initiate preventive or remedial action”.

Altered chromosome structure in peccaries is here demonstrated for the first time in any populations, captive or otherwise. Also, the potential agent inducing such alterations appears to be action of the ivermectin. Further controlled studies should be carried out to test the potentiality read of the drug.

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Resumo

Os animais silvestres têm sido utilizados como bioindicadores quando o ambiente é exposto a estressores químicos. Em geral, os agentes químicos podem induzir às alterações cromossômicas dos tipos falhas e quebras. *Tayassu tajacu*, é uma espécie aparentada dos porcos verdadeiro e apresenta uma grande estabilidade cariotípica. As únicas alterações descritas são em relação a forma do cromossomo X. Foram observadas falhas e quebras cromossômicas durante as análises citogenéticas. Estas alterações foram detectadas em cromossomos autossômicos. Levantamentos realizados na literatura associados aos dados observados nos exemplares estudados, indicam um vermífugo, a base de ivermectina, como o possível causador dessas alterações cromossômicas.

Palavras-chave:

Tayassu.
Cariótipo.
Cromossomo.
Alterações cromossômicas.

References

1. BUENO, A. M. S. **Utilização de roedores silvestres no biomonitoramento citogenético *in situ***. 1997. 165 p. Tese (Doutorado) - Instituto de Biociências, Universidade de São Paulo, São Paulo, 1997.
2. FOX, G. A. Practical causal inference for ecopidemiologists. **J. Toxicol. Environ. Health.**, v. 33, n. 4, p. 359-373, 1991.
3. THERMAN, E.; SUSMAN, M. **Cromosomas humanos: estructura, comportamiento y efectos**. 3ª ed. Ribeirão Preto: Revista Brasileira de Genética, 1996. 383 p.
4. De SILVA, N.; GUYATT, H.; BUNDY, D. Anthelmintics. A Comparative Review of Their Clinical Pharmacology. **Drugs**, v. 53, n. 5, p. 769-788, 1997.
5. OTUBANJO, O. A.; MOSURO, A. A. An in vivo of induction of abnormal sperm morphology by some anthelmintic drugs in mice. **Mutation Research**, v. 497, n. 1-2, p. 131-138, 2001.
6. BIALEK, R.; KNOBLOCH, J. Parasitare Inektionen in der Schwangerschaft und konnatale Parasitosen II. Teil: Helmintheninfektionen. **Z. Geburtsh Neonatol**, v. 203, n.3, p.128-133, 1999.
7. MMONS, L. E.; FEER, F. **Neotropical rainforest mammals**. A field guide. Chicago: University of Chicago, 1997. 307 p.
8. ANDREA, M. V.; OLIVEIRA, C.; ROCHA, G. T.; FORESTI, F. Cytogenetical and histological studies in testis of *Tayassu tajacu* (Cateto), *Tayassu pecari* (Queixada) and a natural interspecific hybrid. **J. Anim. Breed. Genet.**, v. 118, n. 2, p. 125-133, 2001.
9. BENIRSCHKE, K.; KUMAMOTO, A. T. Further studies on the chromosomes of three species of peccary. **Advances in Neotropical Mammalogy**, p. 309-316, 1989.
10. GIANNONI, M. A.; FERRARI, I. Padrões de formação de G-bandas dos cromossomos da espécie *Tayassu tajacu*. I- Análise dos pares cromossômicos números 1 a 7. **Científica**, v. 4, n. 1, p. 72-77, 1976.
11. GIANNONI, M. A.; FERRARI, I. Padrões de formação de G-bandas dos cromossomos da espécie *Tayassu tajacu*. I- Análise dos pares cromossômicos números 8 a 15. **Científica**, v. 4, n. 1, p. 78-81, 1976.
12. HUFTY, M. P.; SEDGWICK; BENIRSCHKE. The karyotypes of the white-lipped and collared peccaries. Aspects of their chromosomal evolution. **Genen Phaenen**, v. 16, n. 3, p. 81-86, 1973.
13. ROCHA, G. T. **Aplicações da citogenética na preservação de animais silvestres**. 1993. 204 p. Tese (Doutorado) - Instituto de Biociências, Universidade Estadual Paulista, Botucatu, 1993.
14. VASSART, M.; PINTON, A.; SÉGUÉLA, A.; DUTERTRE, C. New data on chromosomes of peccaries. **Mammalia**, v. 58, n. 3, p. 500-507, 1994.
15. MOORHEAD, P. S.; NORWELL, P. C.; MELMAN, W. J.; BATTISP, D. M.; HUNGERFORD, D. A. Chromosome preparation of leukocytes cultured from human peripheral blood. **Exp. Cell. Res.**, v. 20, p. 603-216, 1960.
16. BEIGUELMAN, B. **Citogenética humana**. Rio de Janeiro, Guanabara Koogan, 1982. 328 p.
17. BEIGUELMAN, B. **Curso prático de bioestatística**. Ribeirão Preto, Revista Brasileira de Genética, 1994. 231 p.
18. ASTEINZA, J.; CAMACHO-CARRANZA, R.; REYES-REYES, E.; DORADO-GONZÁLEZ, V.; ESPINOSA-AGUIRRE, J. J. Induction of cytochrome P450 enzymes by albendazole treatment in rat. **Environmental Toxicology and Pharmacology**, v. 9, n. 1-2, p. 31-37, 2000.
19. MONTERO, R.; OSTROSKY, P. Genotoxic activity of Praziquantel. **Mutation Research**, v. 387, n. 3, p. 123-139, 1997.
20. JUN, K.; LEE, S. B.; SHIN, H. S. Insertion of a retroviral solo long terminal repeat in *mdr-3* locus disrupts mRNA splicing in mice. **Mammalian Genome**, v. 11, n. 10, p. 843-848, 2000.