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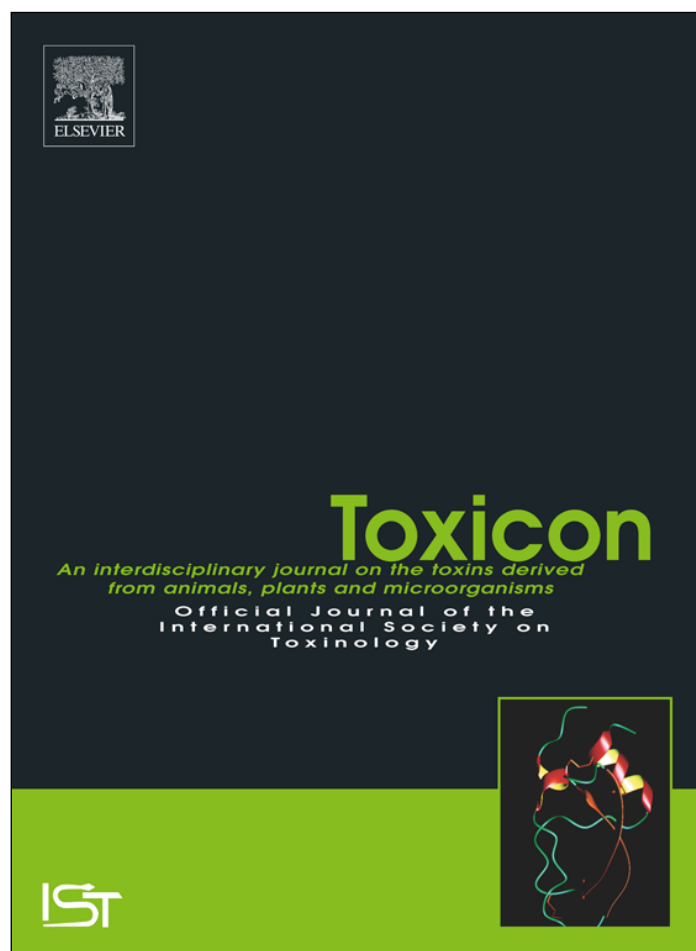


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Short communication

Bovine enzootic haematuria from consumption of *Pteris deflexa* and *Pteris plumula* in northwestern Argentina



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ABSTRACT

Bovine enzootic haematuria (BEH) is caused by prolonged ingestion of toxic principles of bracken fern, essentially by *Pteridium* spp. In northwestern Argentina, this disease has a great economic impact and it is attributed a chronic consumption to *Pteridium arachnoideum*. This paper describes two endemic areas for enzootic hematuria due to the consumption of *Pteris deflexa* and *Pteris plumula*. Two areas where *P. deflexa* and *P. plumula* are endemic, but free of *Pteridium* species, were devised and seven farms were visited. The disease was confirmed based on the presence of clinically affected animals. In four necropsies bleeding neoplastic lesions were observed in the mucosa of the urinary bladder. At phytochemical analysis, both ptaquiloside and pterisin B were found in *P. deflexa* and *P. plumula*. Thus, the consumption of *P. deflexa* and *P. plumula* can also cause BEH.

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Bovine enzootic haematuria (BEH) is a worldwide disease caused by prolonged ingestion of bracken fern of the species *Pteridium aquilinum*, *Pteridium arachnoideum*, *Pteridium caudatum*, *Pteridium esculentum*, and *Pteridium revolutum* (Smith, 1997; Vetter, 2009; Gil da Costa et al., 2012). Furthermore, this disease is supposed to be also caused by ingesting mulga fern or rock fern *Cheilanthes sieberi* in Australia (McKenzie, 1978) and by common fern *Onychium contiguum* in India (Dawra et al., 2001). BEH is characterized by the presence of bleeding inflammatory or neoplastic lesions in the urinary bladder (Perez-Alenza et al., 2006). The bracken contains the norsesquiterpene carcinogenic compound named ptaquiloside, that breaks into pterisin B (Yamada et al., 2007). This paper describes two endemic areas for enzootic hematuria due to the consumption of *Pteris deflexa* and *P. plumula*.

Two endemic areas for the disease in Tucuman-Bolivian Forest, Northwestern Argentina, were identified based on herds showing historical clinical cases of hematuria. Historic positive herds to BEH

were selected and visited, being three herds at Dpto. Orán, Los Naranjos, and four herds at Dpto. La Candelaria, El Jardín. Anamnestic data was investigated and clinically affected animals were inspected. Two necropsies were performed in each of the studied regions (four in total) to confirm the presence of lesions in urinary bladders. At each study site, grazing areas were examined to identify suspect species (Fig. 1). Relative abundance was considered. The specimens studied are deposited at Herbarium of the National University of Salta (MCNS) (Thiers, 2015). The registration numbers are listed in Table 1. Additionally part of the plant material was subjected to the determination of ptaquiloside and pterisin B levels by high pressure liquid chromatography method with UV detection (Alonso-Amelot et al., 1992). The presence of both ptaquiloside and pterisin B was also confirmed by mass spectrometry analysis (Waters Xevo™ G2-S QToF tandem mass spectrometer, Waters, Milford, USA) (Table 1).

In a total herd of 341 cattle, 31 cows showed clinical signs of BEH, and 24 from them died due to the disease. The clinically ill animals showed different degrees of anemia and hematuria. Some of the animals with the most advanced stages of the disease showed poor general condition, sallow mucous membrane and

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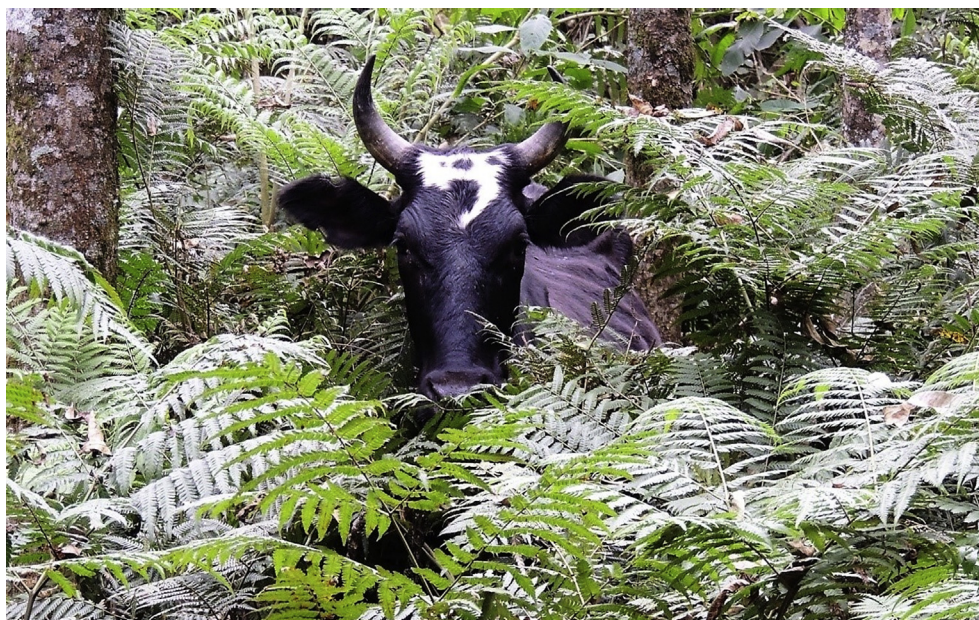


Fig. 1. Animal grazing in an area infected by *Pteris deflexa*.

Table 1
Epidemiological data in the farms visited in province of Salta (Argentina).

Herds	Location	N° animals/ herds	Animals showing clinic haematuria	Estimated annual mortality
1	Dpto. La Candelaria, El Jardín	22	3 (13.6%)	4 (18.2%)
2	Dpto. La Candelaria, El Jardín	21	5 (23.8%)	3 (14.3%)
3	Dpto. La Candelaria, El Jardín	80	7 (8.75%)	3 (3.75%)
4	Dpto. La Candelaria, El Jardín	50	5 (10.0%)	3 (6.0%)
5	Dpto. Orán, Los Naranjos	46	3 (6.52%)	5 (10.9%)
6	Dpto. Orán, Los Naranjos	80	3 (3.75%)	2 (2.50%)
7	Dpto. Orán, Los Naranjos	42	5 (11.9%)	4 (9.52%)
TOTAL		341	31 (9.09%)	24 (7.04%)

adynamia. These findings are similar to those reported in enzootic hematuria caused by *Pteridium* spp (Tokarnia et al., 2012). By urging animals to urinate by vulvar massage, urine could have a clear hemorrhage to show a pinkish color (Fig. 2A). The epidemiologic data was exposed in Table 2. No case of hemorrhagic syndrome or tumors in the upper digestive tract was observed.

At the four necropsies performed, bleeding neoplastic lesions were identified in the mucosa of the urinary bladder (Fig. 2B and C). Macroscopically one of the bladders presents a single, raised and bleeding lesion. In other animal had similar lesions, but they were multiple and smaller. The other two bladders had diffuse, yellowish lesions with irregular surface. Microscopically was identified: Cavernous hemangiosarcoma (2), haemangioma (1) and infiltrative transitional carcinoma (1). In addition to the mentioned lesions, bladder pre-neoplastic alterations were identified: hyperplasia, dysplasia, diffuse or focally lymphocytic infiltration. These lesions

in the animals from the present report are compatible with those described for HEB in the literature (Bringuier and Jean-Blain, 1987; Xu, 1992; Smith, 1997; Vetter, 2009; Gil da Costa et al., 2012). On the other hand, no tumour was found in tongue, pharynx/oropharynx, epiglottis, esophagus, cardia or rumen, as it may be induced by bracken fern ingestion (Smith, 1997; Vetter, 2009; Gil da Costa et al., 2012).

Enzootic hematuria is a disease of great importance in several countries of South America (Marrero et al., 2001; Calderón Tobar et al., 2011; Diaz, 2011; Tokarnia et al., 2012; Herencia et al., 2013). Plants of *Pteridium* genus, known as bracken fern, are reputed as the cause of BEH by different researchers in several regions of the world (Bringuier and Jean-Blain, 1987; Xu, 1992; Smith, 1997; Vetter, 2009; Gil da Costa et al., 2012). In Northwestern Argentina, this disease has a great economic impact in the region (Hansen, 1994; Marin, 2006). The first reports of this disease in the country date back to the 1980s and 1990s (Rosa Larriue, 1982; Idiart and Sanguinetti, 1986; Hansen, 1994); further studies characterized the disease, confirmed the consumption of *P. aquilinum* by cattle in the grazing areas and detected the presence of ptaquiloside in the plants (Marin, 2006).

The chemical compound responsible for the toxic effects of *Pteridium* spp. is ptaquiloside. This compound breaks into bracken dienone and then forming pterisin B or it may be decomposed directly into pterisin B. The bracken dienone is considered the active form of ptaquiloside, promoting the alkylation of amino acids, nucleosides, nucleotides and DNA (Yamada et al., 2007). BEH is characterized by formation of nodules in the bladder attributed to the carcinogenic effect of bracken dienone (Gil da Costa et al., 2012).

In the farms of present study no *Pteridium* was found, but cattle was seen grazing 2 species that we considered suspected for the poisoning, *Pteris deflexa* and *Pteris plumula*. In Northwestern Argentina *P. deflexa* grow in wet forests, whereas *P. plumula* is found in dry and wet forests. Both plants inhabit open spaces, roads, ravines, etc. They can be easily distinguished by their divided lamina with free venation and marginal sori (Martínez and Prado, 2016). The phytochemical analysis showed the presence of ptaquiloside and pterisin B in both plant species (Table 1). Thus, our results

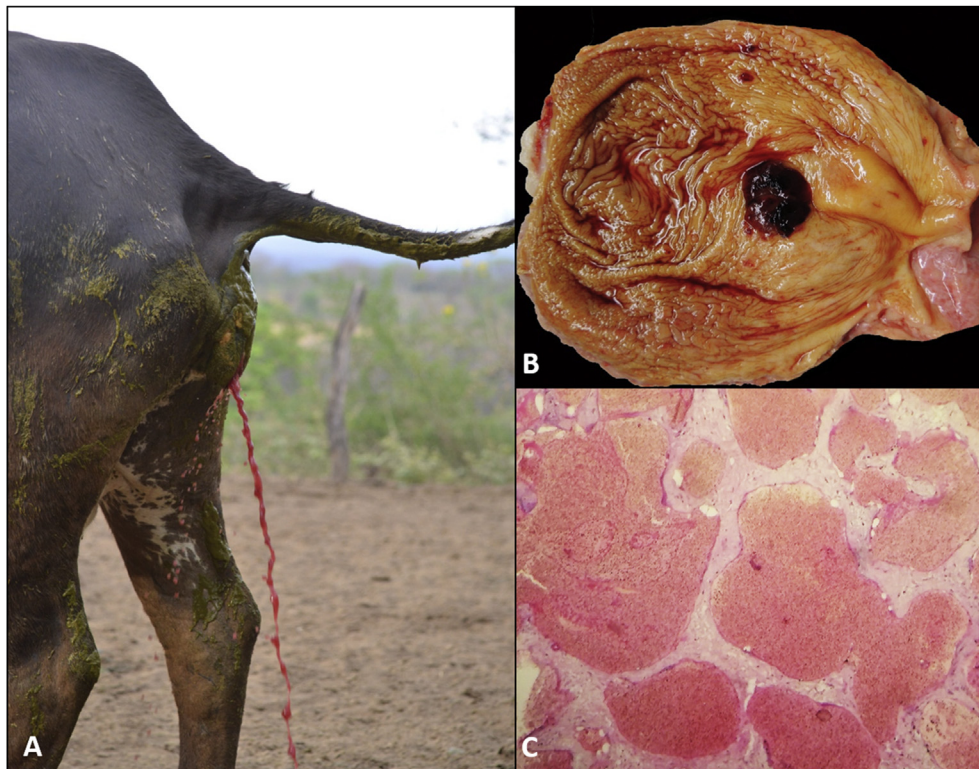


Fig. 2. Clinical signs. Affected animal with severe clinical hematuria (2-A), Neoplasia in the urinary bladder (2-B). Histological lesions. Cavernous hemangioma (2-C).

Table 2

Herbarium and toxicological data on the fern species identified in grazing areas in the two regions under study.

Species	Herbarium record (N°)	Location	Toxicological analysis (µg/g dry matter)
<i>Pteris deflexa</i>	(MCNS 4005)	26°03'39.0"S 65°24'14.9"W	Ptaquiloside: 3235 Pterisin B: 1543
<i>Pteris deflexa</i>	(MCNS 13195)	23°17'14.57"S 64°34'36.53"O	Ptaquiloside: 2424 Pterisin B: 740
<i>Pteris plumula</i>	(MCNS 13196)	23°17'14.57"S 64°34'36.53"O	Ptaquiloside: 2540 Pterisin B: 727

show that both *P. deflexa* and *P. plumula* may also be responsible for BEH in the evaluated area.

Pteris is a cosmopolitan fern genus with over 250 species (Chao et al., 2012). The presence of ptaquiloside was earlier reported in *Pteris quadriaurita* (Krishna and Dawa, 1994), *Pteris cretica*, *Pteris nipponica*, *Pteris oshimensis*, *Pteris tremula*, and *Pteris wallichiana* (Saito et al., 1989). On the other hand, no ptaquiloside was found in *Pteris stanophylla* (Somvanshi et al., 2006), *Pteris falriet*, *Pteris purpureorachis*, *Pteris ryukyuensis*, and *Pteris semipinnata* (Saito et al., 1989). However, this is the first study showing BEH in cows eating *Pteris* species. Plants other than *Pteridium* that were linked to BEH are mulga fern or rock fern *Cheilanthes sieberi* in Australia (McKenzie, 1978) and by common fern *Onychium contiguum* in India (Dawra et al., 2001). Further studies confirmed the presence of ptaquiloside was found in these two species (Potter and Baird, 2000; Somvanshi et al., 2006).

In conclusion, the clinical and pathological findings compatible with BEH and the presence of both ptaquiloside and pterisin B show that the consumption of *P. deflexa* and *P. plumula* may cause BEH.

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Transparency document

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References

- Alonso-Amelot, M.E., Pérez-Mena, M., Calcagno, M.P., Jaimes-Espinoza, R., 1992. Quantitation of pterosins A and B, and ptaquiloside, the main carcinogen of *Pteridium aquilinum* (L. Kuhn), by high pressure liquid chromatography. *Phytochem. Anal.* 3, 160–164.
- Bringuier, P.P., Jean-Blain, C., 1987. Hématurie chronique des bovins: étiologie et épidémiologie. *Point Vet.* 19 (107), 393–403.
- Calderón Tobar, A., Marrero, F., Murillo, V., Vega, V., 2011. Reporte de casos de hematuria enzoótica bovina por ingestión de *Pteridium arachnoideum* en la región ganadera de San Miguel de Bolívar, Provincia Bolívar. *Ecuad. Rev. Salud Anim.* 33, 197–202.
- Chao, Y.S., Liu, H.Y., Chiang, Y.C., Chiou, W.L., 2012. Polyploidy and speciation in *Pteris* (Pteridaceae). *J. Bot.* 2012, 817920.
- Dawra, R.K., Sharma, O.P., Somvanshi, R., 2001. A preliminary study on the carcinogenicity of the common fern *Onychium contiguum*. *Vet. Res. Comm.* 25, 413–420.
- Diaz, G.J., 2011. Toxic plants of veterinary and agricultural interest in Colombia. *Int. J.*

- Poison. *Plant Res.* 1, 1–19.
- Gil da Costa, R.M., Bastos, M.M.S.M., Oliveira, P.A., Lopes, C., 2012. Bracken-associated human and animal health hazards: chemical, biological and pathological evidence. *J. Hazard Mater* 203–204, 1–12.
- Hansen, E.W., 1994. Ganadería Bovina de la Raza Criolla en el Noroeste Argentino. Universidad Nacional de Jujuy, pp. 45–46.
- Herencia, K., Falcón, N., García, M., Chavera, A., Gonzáles, C., 2013. Prevalencia de hematuria vesical enzoótica bovina determinada mediante urianálisis en Oxapampa, pp. 50–57. *Perú. Rev. Investig. Vet. Perú* 24.
- Idiart, J.R., Sanguinetti, H., 1986. Bovine enzootic hematuria in Argentina. In: Proceedings SIDA (Swedish International Development Authority). Regional Follow-up Seminar on Veterinary Pathology University of La Plata, pp. 408–417.
- Krishna, L., Dawa, R.K., 1994. Bracken fern induced carcinoma in Guinea pigs. *Indian J. Vet. Pathol.* 18, 21–26.
- Marin, R.E., 2006. Caracterización clínica y patológica de la intoxicación con *Pteridium aquilinum* en bovinos en la provincia de Jujuy. Universidad Nacional de Mar del Plata. Balcarce, Buenos Aires, Argentina. Magister Scientie Thesis.
- Marrero, E., Bulnes, C., Sanchez, L.M., Palenzuela, I., Stuart, R., Jacobs, F., Romero, J., 2001. *Pteridium aquilinum* (bracken fern) toxicity in cattle in the humid Chaco of Tarija, Bolivia. *Vet. Hum. Toxicol.* 43, 156–158.
- Martínez, O.G., Prado, J., 2016. *Pteris* L. In: Zuloaga, F.O., Belgrano, M.J., Anton, A.M. (Eds.), Flora vascular de la República Argentina II, pp. 321–329.
- McKenzie, R.A., 1978. Bovine enzootic haematuria in Queensland. *Aust. Vet. J.* 54, 61–64.
- Perez-Alenza, M.D., Blanco, J., Sardon, D., Sanchez Moreiro, M.A., Rodriguez-Bertos, A., 2006. Clinico-pathological findings in cattle exposed to chronic bracken fern toxicity. *N. Z. Vet. J.* 54, 185–192.
- Potter, D.M., Baird, M.S., 2000. Carcinogenic effects of Ptaquiloside in bracken fern and related compounds. *Br. J. Cancer* 83, 914–920.
- Rosa Larrieu, E.H., 1982. Hematuria semipermanente bovina o cistitis hemorrágica crónica ó Meada rosada ó Meada roja ó Meada de sangre, vol. 44, p. 375. *Gac. Vet.* (Buenos Aires).
- Saito, K., Nagao, T., Matoba, M., Koyama, K., Natori, S., Murakami, T., Saiki, Y., 1989. Chemical assay of ptaquiloside, the carcinogen of *Pteridium aquilinum*, and the distribution of related compounds in the Pteridaceae. *Phytochemistry* 28, 1605–1611.
- Smith, B.L., 1997. The toxicity of bracken fern (genus *Pteridium*) to animals and its relevance to man. In: D'Mello, J.P.F. (Ed.), *Handbook of Plant and Fungal Toxicants*. CRC Press, London, pp. 63–76.
- Somvanshi, R., Lauren, D.R., Smith, B.L., Dawra, R.K., Sharma, O.P., Sharma, V.K., Singh, A.K., Gangwar, N.K., 2006. Estimation of the fern toxin, ptaquiloside, in certain Indian ferns other than bracken. *Curr. Sci.* 91, 1547–1525.
- Thiers, B., 2015. *Index Herbariorum: a Global Directory of Public Herbaria and Associated Staff*. New York Botanical Garden's Virtual Herbarium. Available from: <http://sweetgum.nybg.org/ih/>.
- Tokarnia, C.H., Farias-Britos, M., Barbosa, J.D., Vargas Peixoto, P., Döbereiner, J., 2012. *Plantas Tóxicas do Brasil para Animais de Produção*, 2th ed. Editora Helianthus, Rio de Janeiro, pp. 349–364.
- Vetter, J., 2009. A biological hazard of our age: bracken fern [*Pteridium aquilinum* (L.) Kuhn] - a review. *Acta Vet. Hung* 57, 183–196.
- Xu, L.R., 1992. Bracken poisoning and enzootic haematuria in cattle in China. *Res. Vet. Sci.* 53, 116–121.
- Yamada, K., Ojika, M., Kigoshi, H., 2007. Ptaquiloside, the major toxin of bracken, and related terpene glycosides: chemistry, biology and ecology. *Nat. Prod. Rep.* 24, 798–813.