Trop Anim Health Prod (2017) 49:1553–1556
DOI 10.1007/s11250-017-1354-0

SHORT COMMUNICATIONS

Spontaneous photosensitization by *Heterophyllaea pustulata* Hook. f. (Rubiaceae), in sheep from Northwestern Argentina

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Received: 2 April 2017 / Accepted: 3 July 2017 / Published online: 21 July 2017

Abstract *Heterophyllaea pustulata* Hook. f. (Rubiaceae) is a phototoxic plant. It grows in the Andean area of northwest of Argentina, and it causes significant economic losses in the livestock. This plant induces dermal lesions by photosensitization probably due to its content of photosensitizing anthraquinones. This paper describes an outbreak of poisoning in Corriedale sheepfold, which had an incidence of 49%. Ear skin biopsies and blood samples were collected of six affected animals. Liver enzymes remained within the reference limits. Histopathologically, a deep necrotizing dermatitis was identified in all samples. *H. pustulata* was identified in the areas of grazing. Anthraquinone concentration in leaves was 0.84% p/p, expressed as rubiadin. All findings allow us to conclude that the diagnosis is a primary photosensitization. Huge regional economic losses could be attributed to *H. pustulata* poisoning, although its toxicity has been little studied.

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Keywords Poisoning plants · Phototoxic · Primary photosensitization · Dermatitis

Heterophyllaea pustulata Hook. f. (Rubiaceae) is a wild bush 2–3 m high that grows in the Andean Northwest of Argentina, between 2500 and 3000 m above sea level. It is a phototoxic plant, popularly known in Spanish as "cegadera", which causes significant economic losses in the livestock of its habitat (Bacigalupo 1993). Experimental intoxication by "cegadera" has been reported for rabbits, sheep, and bovine individuals as dermatitis and blindness in severe cases, without death of the affected animals (Hansen and Martiarena 1967). More recent work concluded that the toxic effect of *H. pustulata* is due to the presence of anthraquinones (AQs), substances with photosensitizing action (Nuñez-Montoya et al. 2005, 2008). Thus, the phytochemical studies of *H. pustulata* conducted by Nuñez-Montoya et al. (2006) allowed isolating and identifying nine AQs aglycones and one biantraquinona (5,5′-bisoranjidiol) as main metabolites. Regionally, the poisoning by this plant is well known, although the records of natural cases are unusual. In this paper, we describe a natural case of poisoning by *H. pustulata* in a flock of sheep in the province of Salta.

Case report

The specialized diagnostic veterinary service (SDVE) INTA-Salta visited the place "Las Juntas" from the department "Guachipas" (25° 42′ 01.7″ S 65° 30′ 26.6″ W) in the province of Salta because of a history of skin lesions in Corriedale sheepfold. The flock consisted of 150 animals, from which 74 affected animals were observed. Six affected animals were selected, and blood samples and ear skin biopsies were

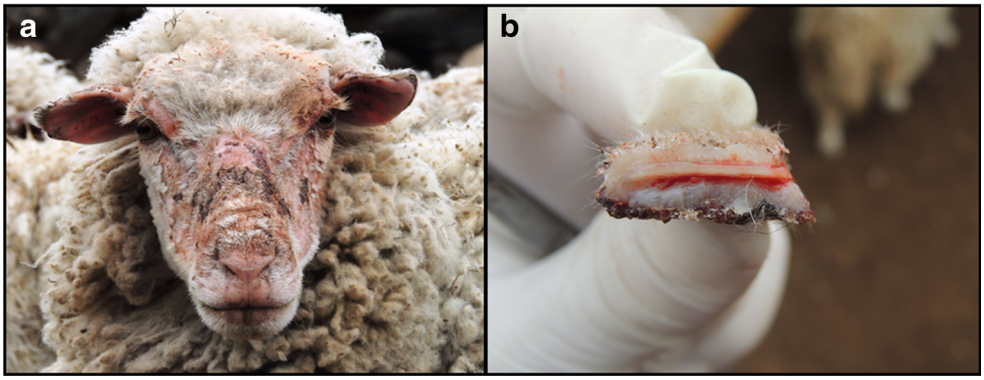

Fig. 1 **a** Affected sheep showed severe skin lesions in ears. **b** Ear biopsy, remarkable thickening of the epidermal layer

collected. Tissue samples were fixed in buffered formalin 10% and processed by routine histological techniques.

Serum samples were subjected to the determination of GGT, GPT, GOT, Alb, PT, DB, and TB, using previously described techniques (Schmid and von Forstner 1986) and commercial reagents. The following reagents were used: Wiener Lab®, Argentina. Grazing areas were observed, and plants samples were collected, herborized, and classified. Parts of the plant samples were destined for the determination of anthraquinones following the methods described by Nuñez-Montoya et al. (2006).

Results and discussion

The cumulative incidence was 49% in 45 days (counting from the appearance of the first animal). The lesions were affecting the skin devoid of wool and exposed to sunlight (face, ears, mammary gland, and perineum).Early signs corresponded to photophobia, itching, and restlessness. Later edema in the face and ears, followed by an intense tearing was observed. Finally, the affected skin areas suffered necrosis with crusting (Fig. 1). In some animals, cracks that deepened to the deep dermis were observed. In late stages, these areas were re-epithelialized, turning in alopecia regions lingered in wool cover again. In sheep, these changes are typically limited to non-pigmented, sparsely coated, or exposed skin. Although in severe cases, mild lesions occur in regions without wool, particularly on the head and ears (Tokarnia et al. 2012). Cutaneous lesions in ruminants include erythema, edema, fissuring, exudation, crust formation, alopecia, hypersalivation, skin necrosis and sloughing, secondary infection, and granulation areas (Glenn et al. 1964). Ocular pathology includes lacrimation, photophobia, periocular and corneal edema, keratitis, and blindness (Tokarnia et al. 2012). In this report, all affected individuals had eye injuries of varying severity, and several of them had unilateral (5%) or bilateral (20%) blindness. In photosensitization, early signs were anorexia, depression, recumbency, pruritus, and light avoidance (Ozmen et al. 2008). In cases observed here, these signs had ceased.

The affected skin showed diffuse necrosis of the entire epidermis and superficial dermis, even up to the depth of the apocrine sweat glands, whereas sebaceous glands still appeared viable (Fig. 2). There was thrombosis in dermal vessels and in sheets of fibrin of the hypodermis. Dilation of sweat glands and in some cases, atrophy of the sebaceous glands was observed. In addition, hialinosis and fibroplasia of the superficial dermis was identified. Thickening of the walls of the arteries due to the presence of aggregates of hyaline material was also observed. Inflammatory response of neutrophils and lymphocytes was in

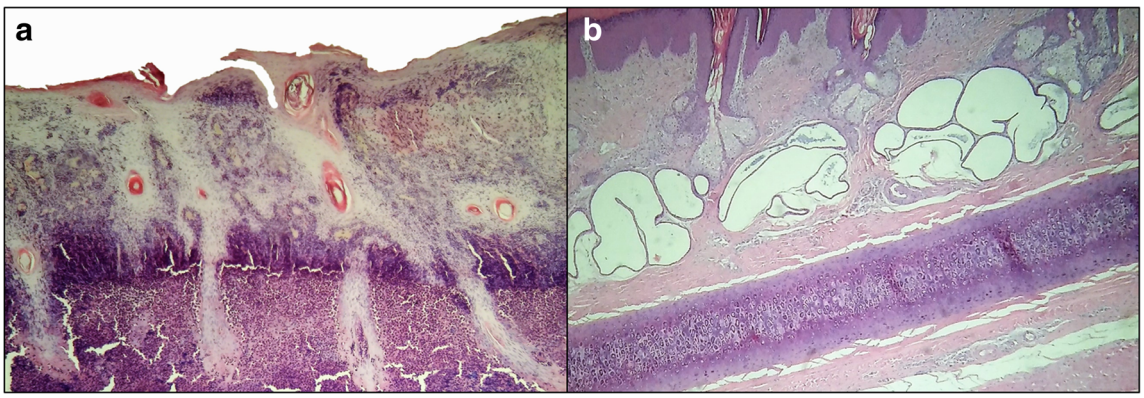

Fig. 2 Histopathology to skin. **a** Necrotizing exudative dermatitis with crusting formation [H&E 100×]. **b** Dilation of sweat glands [H&E 40×]

Table 1 Levels of liver enzymes, direct and indirect bilirubin, and total protein and albumin in affected sheep

Samples	GGT UI/l	GPT UI/l	GOT UI/l	BD (mg/dl)	BT (mg/dl)	Alb. g/dl	PT g/dl
1	123	24	228	0.07	0.12	2.4	5.8
2	145	30	284	0.06	0.12	3.2	6.3
3	54	36	363	0.06	0.08	3.1	5.8
4	72	21	233	0.06	0.09	3.2	6.1
5	106	24	169	0.07	0.11	2.8	5.7
6	110	20	164	0.04	0.07	2.1	5.6
Mean	101	26	240	0.06	0.10	2.8	5.9
SD	33	6	75	0.01	0.02	0.4	0.2

GGT gamma glutamyl transpeptidase, *GPT* glutamic-pyruvic transaminase, *GOT* glutamic oxaloacetic transaminase, *Alb* albumins, *PT* total protein, *DB* direct bilirubin, *TB* total bilirubin]

varying proportions. It was consistent with those reported for other cases of primary photosensitization (Scott et al. 1979). It should be noted that these studies are scarce, and most are based on cases of primary photosensitization due to congenital porphyria.

Biochemical results are presented in Table 1. Biochemical parameters found were within the limits indicated for sheep (Healy and Falk 1974). In secondary photosensitization, high levels of liver enzymes and total and direct bilirubin for a long time are normally observed (Glenn et al. 1964). Normally, in sheep, the GGT content in serum is specific for liver damage (Morris et al. 2004).

All the plant samples collected in grazing areas were identified as *H. pustulata* Hook. f. (Rubiaceae) (Fig. 3) and were recorded as specimen MCNS 13006 at the MCNS Herbarium of Universidad Nacional de Salta. Total anthraquinone concentration in the crushed samples, consisting in leaves and fine stems at early vegetative stage, was 0.84% p/p, expressed as rubiadin.

Finally, clinical, biochemical, and histopathological findings together with the discovery of *H. pustulata* in the grazing areas and the identification of photosensitizing anthraquinone allow us to conclude the diagnosis to primary photosensitization.

Photosensitization describes the pathogenesis and clinical syndrome resulting from the interaction of endogenous or exogenous photoactive chemicals with a specific wavelength of light and cutaneous tissues (Galitzer and Oehme 1978). Biochemical findings rule out liver damage and lead to the conclusion that it is a primary photosensitization as described by Núñez-Montoya et al. (2008).

Plants that cause primary photosensitization in animals include a small group of species, among which are reported: *Fagopyrum esculentum* (L) Moench (*Polygonaceae*) and *Hypericum perforatum* L. (*Hypericaceae*) containing photodynamic anthraquinones called fagopyrin and hypericin, respectively (Bourke and White 2004). *Froelichia humboldtiana* were mentioned with phototoxic effects; however, the toxin is unknown (Souza et al. 2012; Santos et al. 2017). Recently, the presence of anthraquinones in *Heterophyllaea lycioides*, another species of this genus, has been identified (Dimmer et al. 2017). However, the registry of their toxicity is scarce. In Northwestern Argentina, *H. pustulata* causes significant economic losses in the livestock. Both species are natives to South America (Taylor 2010). It is necessary to further investigate aspects of its toxicity and its effect on livestock.

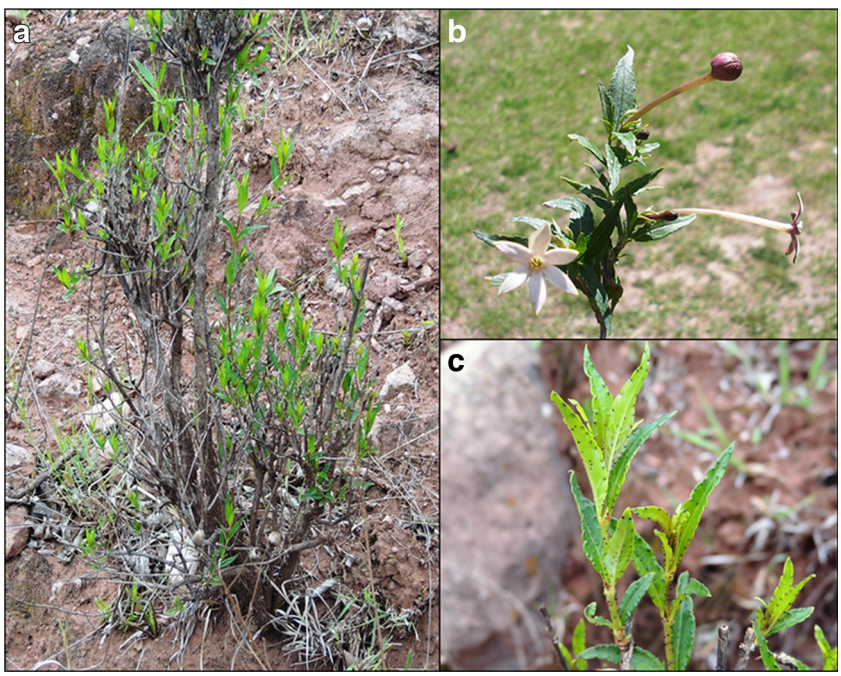

Fig. 3 Adult plant of *H. pustulata* present in the grazing area. Detail of the leaves and the inflorescence of the plant

Acknowledgements This study was supported by grants from INTA (Instituto Nacional de Tecnología Agropecuaria), Consejo de Investigación de la Universidad Católica de Salta, and PICT 2011/1379 (FONCyT). LRC, SCNM, JLC, and EJG are members of the Research Career of CONICET (Consejo Nacional e Investigaciones Científicas y Técnicas).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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