

Case report of fatal poisoning by *Nicotina tabacum* in cattle in Tunisia

T. BEN HASSINE^{1*}, A. BEN MANSOUR², S. HAMMAMI¹

¹National Center for Zoosanitary vigilance, 38 avenue Charles Nicolle 1082 Tunis, TUNISIA

²National Center for Nuclear sciences et technologies, Technopark, 2020 Sidi Thabet, TUNISIA

* Corresponding author: benhassinethameur@yahoo.fr

SUMMARY

Nicotina tabacum (Solanaceae) is a perennial herbaceous plant cultivated in the North and North West of Tunisia, which is highly toxic due to its nicotine alkaloid content. Because *Nicotina* species are unpalatable, the plant ingestion by ruminants will be observed only if animals are not well fed. This paper presents the first case report of *Nicotina tabacum* poisoning in cattle in Tunisia causing rapid death. Diagnosis was based on clinical neurologic signs, evidencing of numerous irregularly shaped leaves of tobacco plant in rumen at necropsy and nicotine dosage by HPLC/UV in rumen content samples.

Keywords: *Nicotina Tabacum*, poisoning, cattle, nicotine, tremor, Tunisia

RESUME

Nicotina tabacum (Solanaceae) est une plante herbacée cultivée au Nord et au Nord-Ouest de la Tunisie, hautement toxiques en raison de sa teneur en nicotine. Comme les plantes du genre *Nicotina* ne sont pas appétentes, leur ingestion par les ruminants n'est en général constatée que chez des animaux pas bien nourris. Cet article rapporte les premiers cas d'intoxication accidentelle et fatale par *Nicotina tabacum* chez les bovins en Tunisie. Le diagnostic a reposé sur la présence de signes cliniques neurologiques, la mise en évidence à l'autopsie de nombreuses feuilles de tabac de taille variée et le dosage de la nicotine par HPLC/UV dans le contenu ruminal.

Mots-clés : *Nicotina Tabacum*, intoxication, bovins, nicotine, tremblement, Tunisie.

Introduction

Nicotina tabacum is a plant native of tropical America, Mexico and the West Indies [30]. At present, it is cultivated in most parts of the world. Tobacco plant was introduced in the North of Tunisia in 1830 from Europe [26]. Three varieties of *N. tabacum* are currently grown in Tunisia mainly brown tobacco variety called "Arbi". It is a local variety grown in the North and the North West of Tunisia [26]. The Tunisia tobacco plant is annual and has a narcotic odour. It is cultivated during the summer. Leaves have air curing after undergoing fermentation to reduce the content of alkaloids naturally higher in brown tobacco than in blond tobacco [6].

Tobacco plants (*Nicotina spp.*) are uncommon causes of poisoning in many animal species [10, 23, 29]. Most cases of intoxication involving livestock result from the ingestion of limited quantities of this plant during the desiccation period [29]. Few cases of poisoned animals are reported in some countries, but no reports of animal intoxications by this plant in Tunisia were published. The following report is the first description of toxicity in a group of cattle caused by the ingestion of tobacco plant resulting in rapid death.

Case report

Three cows of local race, 6 to 8 years old, originating from a single farm in the region of Cap Bon (North East of Tunisia) died in a 24-hour period. Two of them were found dead and the third one had signs of ataxia and tremors before death. In addition to symptomatic therapy, treatment including

atropine sulfate (1mg/kg, 1/4 IV, 3/4 SC), was instituted. The therapeutic measures were unsuccessful because the cattle had already entered into the advanced paralytic phase of toxicosis. The two other cows showed the same clinical signs before death. As described by the owner, the first phase is characterized by generalized tremor. Then the second phase of coma is followed by death. The third animal found alive presented muscle tremors, hypersalivation and conjunctivitis. The animal quickly began to lose sensitivity. The sphincters are relaxed and the mucous membranes became very pale. At the final stage of disease, the animal falls into coma before death. The 3 animals have not exhibited any clinical signs the evening before. They have been tied throughout the day of intoxication to olive trees that were used to hang tobacco for drying. It is also reported that animals have not well fed during this day.

Two of them were autopsied. The lungs were severely oedematous and when cut, the surface showed a blood-tinged fluid. The pericardial sac contained a lot of a clear, blood tinged fluid. The rumen had a strong tobacco odour and contained numerous irregularly shaped leaves of tobacco and olive leaves mixed with straws. The liver and kidneys were slightly pale.

Toxicological analyses were performed from rumen contents, liver, kidney, brain, abdominal fat, urine and vitreous samples in the laboratory of pharmacy-toxicology at the Veterinary School at Sidi Thabet, Tunisia. No organophosphate or carbamate was found in the rumen contents and the tissues were examined using HPLC/UV.

For nicotine research, 10 mL of 1 N aqueous sodium hydroxide were added to 10 mL of rumen's contents. The mixture was homogenized for 1 minute with 100 mL of ethyl acetate and 50 g of sodium sulfate. After a 30 mL-aliquot extraction by 0.5 N aqueous hydrochloric acid (15 mL), the aqueous phase (10 mL) was adjusted to pH 12 with 10 N aqueous sodium hydroxide and homogenized for 1 minute with 100 mL of ethyl acetate and 50 g of sodium sulfate. The ethyl acetate fraction (40 mL) was evaporated. The sample was diluted for HPLC with acetonitrile/phosphate buffer pH 7.4. Chromatography was performed using a gradient of acetonitrile and ammonium bicarbonate buffer at pH 9.8. Nicotine is monitored at 260 nm [12]. Presence of nicotine was detected at 20 ppm. Thus, the suspicion of nicotine toxicosis was confirmed but it was difficult to determine the exact quantity of *Nicotina tabacum* that might have been consumed by each cattle. The simple observation indicated that the quantity ingested is not very important by each cattle (approximately 300 to 400 g). It is concluded also that the ingestion of olive leaves facilitated the absorption of nicotine and cause sudden death.

Discussion

A few animal intoxications by Tobacco plants were reported [5, 9, 20, 23]. Over 60 species of Tobacco plants contain one or more of the following alkaloids: nicotine, nornicotine, anatabine and anabasine [6]. Four of the *Nicotina* species (*N. acaulis*, *N. glauca*, *N. petunioides* and *N. solanifolia*) contain anabasine as the major alkaloid [6]. In *Nicotina tabacum*, nicotine is the major alkaloid found. Every part of the plant, except the seed, contains nicotine, but the concentration is related to different factors such as species, type of land, culture or weather conditions (Table I).

Most cases of intoxication involving livestock result from the accidental ingestion of limited quantities of Tobacco plants during maturation period. Poisoned animals are reported to have access to pasture or to be housed in a barn used to hang tobacco leaves for drying. Tobacco plants are generally unpalatable and are eaten only by hungry animals if other forage is not available [18]. Several cases of human poisoning by ingested cooked tobacco tree leaves in one family are reported. *Nicotina* plants can be confused with spinach [9]. Accidental ingestion of tobacco products like cigarette can be dangerous for dogs and cats [10]. But, toxicosis in small animals appears to be rare and not very dangerous [10, 31].

The concentration of nicotine increases with the plant's age. In the mature plant, a total of 64% of the total nicotine exists in the leaves [32]. Most intoxication involving livestock result from the ingestion of limited quantities of native plant [18]. Animals in poor condition are more susceptible than well-fed animals [25]. The oral toxic dose in cattle is estimated to be 300 to 2000 g of matured leaves. In sheep and goats, the oral toxic dose is about 30 to 100 g of matured tobacco leaves [24]. Doses of 0,2-0,3 g nicotine sulfate have been toxic

for lambs weighing 14-20 kg [24]. The DL50 is 1 mg/kg in dog and 200 to 300 mg in horse [27]. Probable oral lethal dose in humans is less than 5 mg/kg for a 70 kg person [13]. It may be assumed that ingestion of 40 mg to 60 mg of nicotine is lethal to humans [11].

The effects of nicotine alkaloid are a result of the summation of actions at ganglionic sites, motor end plates and smooth muscles. After ingestion, gastrointestinal signs include salivation, vomiting, and diarrhoea that are observed by parasympathetic stimulation [22, 28, 31]. In dogs, vomiting may occur soon after ingestion because of chemoreceptor trigger zone stimulation [22]. In poisoning case by *Nicotina tabacum*, the central nervous system is affected, initially by stimulation, progressing to depression [29]. High-dose intoxication stimulates the central nervous system, leading to excitement, tremors, incoordination, weakness, twitching, and possible convulsions [10]. The cardiovascular responses are generally due to stimulation of sympathetic ganglia and adrenal medulla combined with discharge of catecholamines [3]. In experimental preparations, nicotine in low doses causes ganglionic stimulation but in high doses it causes ganglionic blockade after brief stimulation [21]. At extremely high doses, nicotine produces hypotension and slowing of the heart rate, mediated by peripheral ganglionic blockade, vagal afferent nerve stimulation, or direct depressor effects mediated by action on the brain [21]. Death occurs as a result of respiratory failure [1]. Differential diagnosis includes intoxication with strychnine, methylxanthines, tremorgenic mycotoxins, organophosphates, carbamates, and depressants [19, 22].

Post-mortem findings in animals with nicotine toxicosis are usually nonspecific and include anoxia and internal organ congestion [19, 31]. There is no specific antidote. The treatment of acute nicotine poisoning has to begin as soon as possible after poisoning. Affected animals should be kept in a quiet, safe area and veterinary attention should be sought. For small animals, if the plant has been swallowed, the stomach should be emptied and a purgative administered. Convulsions should be controlled with diazepam, and airway ensured and respiration maintained. In dogs, the prognosis is grave to poor when large amounts of nicotine have been ingested [19, 31]. The prognosis is good if an animal survives the first four hours [2, 19, 31]. For ruminants, the gastrotomy is indicated if the amount ingested is large and if symptoms have not yet emerged. Because *Nicotina* species are unpalatable, provision of ample feed is the best way to prevent poisoning [7].

Nicotina species	Principal Toxic alkaloid	Intoxicated animals	Symptoms and circumstances
<i>N. glauca</i> (tree Tobacco)	- Anabasine (0.62%) [11]. - All parts of plant contain anabasine and are poisonous [8]. - Collections of <i>N. glauca</i> containing anabasine (0.45-1.14 mg/g dry weight of plant) [14]. - High amounts of anabasine were found in leaves [15].	Cattle [23], sheep, goats, swine [20], ostriches [4], horses [18] and humans [9].	<u>Clinical signs</u> : Weakness, weak pulse, staring eyes, stumbling, trembling, unsteadiness, salivation, frequent urination, colic, ataxia [23], irregular gait, wobbling while walking or standing, recumbency and death (nicotine-like effect) [14]. Amaurosis in horses in Australia [16]. <u>Teratogenic effect</u> : severe skeletal deformities in lambs and calves born to their mothers that consumed plant during the 30 th - 60 th of gestation [15]. Limb defects included fixed excessive carpal flexure with or without lateral or medial rotation of fore or rear limbs, lordosis, irregular shaped head or cleft palate [14]. <u>Toxicity</u> : In human, anabasine is considerably more toxic than nicotine [9]. In animal studies, anabasine was found to be less potent than nicotine. However, its lethality is three times greater than that of nicotine in rabbits and guinea pigs [17]. Severe toxic signs in pregnant ewes and teratogenic effects in their offspring with 1.66 - 3.42 mg/kg single daily doses in ewes [14].
<i>N. Tabacum</i> (tobacco)	- Nicotine (0.43%) [11]. - Every part of the plant, except the seed, contains nicotine [32]. - In the mature plant, a total of 64% of the total nicotine exists in the leaves [32].	Mules and cattle [29], dogs [10], cats [2] and humans [3].	<u>Clinical signs</u> : salivation, vomiting, and diarrhoea due to parasympathetic stimulation [29]; tremors, incoordination, weakness, twitching, and possible convulsions, progressing to depression [29]. <u>Toxicity</u> : The oral toxic doses are estimated to be 300 to 2000 g of matured leaves in cattle, about 30 to 100 g of matured tobacco leaves in sheep and goats [24]. The DL50 is 1mg/kg in dog and 200 to 300 mg in horse [27]. Probable oral lethal dose in humans is less than 5 mg/kg for a 70 kg person [13].
<i>N. trigonophylla</i> (desert tobacco)	- Nornicotine (0.20%) [11]. - Leaves, buds, flowers and stems seem to be toxic [16].	Cattle, sheep, horses and chickens [16].	<u>Clinical signs</u> (experimental work) agitation, ataxia, prostration, muscle trembling, weak pulse [16]. These clinical signs appeared very soon after feeding and continued for a considerable long period [16]. <u>Toxicity</u> [16]: minimal toxic doses are 0.5% bw in cattle, 1.42% bw in sheep and 0.68% bw in horses; minimal lethal doses are 2.0% bw in cattle and 3.25% bw in sheep.
<i>N. attenuata</i> (coyote tobacco)	- Nicotine (0.73%) [11]. - Stems are more toxic than leaves [16].	Cattle, sheep and horses [16].	<u>Clinical signs</u> : closely identical with those produced by <i>N. trigonophylla</i> ; most prominent ones were depression, irregular respiration, rapid and weak pulse, trembling, and muscular weakness resulting in a staggering gait. In some cases the symptoms included salivation and soft faeces [16]. <u>Toxicity</u> : the toxic and lethal dosages are slightly greater than those of <i>N. trigonophylla</i> [16]. The minimum toxic dose for sheep was 2.25% bw of green plant, and the lethal dose was 8.6% [16].

TABLE I: Principal species of *Nicotina* spp. incriminated in animal toxicity.

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