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# Respiratory distress in Holstein cows fed corn silage: case report

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**Abstract**
An outbreak of respiratory distress was observed in Holstein cows immediately after consuming a new batch of corn-silage, presumably due to the presence of pneumotoxic gases. Signs were observed in 10% of the cows, and characterized as tachypnea, orthopneic position, cough and notable anxiety a few minutes after offering the silage. No deaths were recorded. Mild leucocytosis was observed in the hemogram. Nutritional analysis and nitrate determination of the corn-silage revealed usual values. Nitrous oxide and methane concentration were evaluated by gas chromatography and nitrogen oxides concentration directly with a portable device, resulting in acceptable values. Although high concentrations of toxic gases could not be evidenced in the air sampled on farm, the clinical signs observed coincide with those reported in cases of exposure to gases formed during corn silage processes. A greater emphasis is required in the evaluation of the corn to be ensiled, given the possible existence of factors that can generate the accumulation of potentially toxic gases for cattle that affect negatively their health and productivity.

**Key words:** ruminant, dairy, dyspnea, maize, toxic gases.

# Enfermedad respiratoria en vacas Holstein alimentadas con silaje de maíz: reporte de caso

**Resumen.** Se describe un brote de enfermedad respiratoria en vacas Holando Argentino, inmediatamente luego del consumo de un nuevo silaje de planta entera de maíz, presuntamente con presencia de gases neumotóxicos. Se observaron signos en el 10% de las vacas, caracterizados como taquipnea, posición ortopnéica, episodios de tos y ansiedad, unos minutos luego del ofrecimiento del silaje. No se registraron muertes. En el hemograma se observó leucocitosis leve. El análisis de calidad nutricional del alimento y la concentración de nitratos en el silaje de maíz resultó con los valores habituales. Se evaluó la concentración de óxido nitroso y metano en una muestra de silaje de maíz recolectada herméticamente por cromatografía gaseosa, así como la concentración de óxidos de nitrógeno *in situ* con un instrumento portátil, resultando en valores aceptables. Aunque no se pudieron evidenciar concentraciones de gases elevadas en el aire del silaje muestreado en el tambo, los signos clínicos observados coinciden con los casos reportados de exposición a gases que se forman durante la fermentación del ensilado. Se debería tener en cuenta la evaluación del maíz a ser ensilado, dadas las posibilidades de que se genere la acumulación de gases potencialmente tóxicos para el ganado, que puedan afectar negativamente su salud y productividad.

**Palabras clave:** rumiantes, tambo, disnea, maíz, gases tóxicos.

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## INTRODUCTION

Inhalation of pneumotoxic gases has been sporadically associated with respiratory distress in cattle. These gases can be of a very varied nature, with cases reported in dairy cows due to inhalation of nitrogen gases present in corn silage, mainly nitrogen dioxide ($NO_2$) (Verhoeff et al. 2007, Bolsen 2018). Corn, under certain conditions of humidity and temperature, can accumulate nitrate ($NO_3$) (Spoelstra 1985). During silage fermentation, $NO_3$ breaks down into water and nitrogen oxides: nitrogen dioxide ($NO_2$), nitric oxide (NO), nitrous oxide ($N_2O$), amongst others (Grayson 1956, Verhoeff et al. 2007, Driehuis et al. 2018). Some of these gases have been characterized as pneumotoxic after inhalation due to mucosal irritation (Cutlip 1966, Bolsen 2018). After the exposure to pneumotoxic gases, respiratory clinical signs appear abruptly characterized by cough, tachypnea, dyspnea, nasal secretion, and loss of appetite (O'Kiely et al. 1999).

This work reports a clinical outbreak of respiratory disease in Holstein cows in a commercial dairy farm in Buenos Aires province (35°43'05.7"S 58°36'03.2"W), Argentina, immediately after the exposure of cows to a new batch of recently made corn silage.

The dairy farm was visited in three opportunities after the initial onset of respiratory distress. Information regarding the nutritional husbandry of the dairy herd was collected. During the visits, clinical information was collected from the affected cattle.

## MATERIALS AND METHODS

The dairy herd comprised 280 Holstein lactating cows, with an average milk production of 27 L $day^{-1}$. Cows strip-grazed an alfalfa-based pasture with a stocking rate of 280 heads $ha^{-1}$. Cows were milked twice-daily at 4:00 and 15:10. After milking, they received 10 kg of corn-silage per animal on the ground. Two silages were identified in the farm, the "normal silage" was made on 2019-2020 and the "problem silage" was made in 2020-2021, which suffered a water stress during the summer (two months before silage was made). Both were made using the same simple hybrid corn (ACA VG 48 RR2, Buenos Aires, Argentina), characterized by being an intermediate cycle, semi-dented grain and medium drying speed. Both were sawn at the beginning of October with a density of 65,000 plants per hectare, and fertilized at sawing with 100 kg $ha^{-1}$ of diammonium phosphate, and with 196 L $ha^{-1}$ of urea/ammonium nitrate solution (UAN) at stage V4/V5. Corn was harvested and chopped at the beginning of February (4 months after sawing) when the corn grain had a pasty consistency. The silage was preserved in plastic sealed bag of 9-foot diameter. Subsequently, the silage was mechanically extracted into a mixer and offered to the animals on the ground.

Samples were collected from two corn-silages, the "problem silage" and another silage that offered to other herd of lactating cows did not produce clinical signs. Samples were obtained from the central part of the silos and kept at 4ºC in sealed plastic bags for further chemical composition and gas analysis. For the analyses of gas concentration inside the silos, two methods were applied. Firstly, gas samples were taken from the internal air contained inside the silage sealed bags using 25 ml plastic syringes, at a depth of 10 cm and sent for gas chromatography analysis to determine $N_2O$ and $CH_4$. Samples were analyzed with an Agilent 7890A gas chromatograph equipped with an electron capture detector to determine $N_2O$ concentration and a flame ionization detector to determine methane ($CH_4$). Secondly, concentrations of NO, $NO_2$, carbon monoxide (CO), carbon dioxide ($CO_2$) and oxygen ($O_2$) were evaluated *in situ* using a portable and direct reading detector (Testo 360, Testo SE & Co, Titisee-Neustadt, Germany), during a monitoring period of 30 minutes at an average ambient temperature of 12.9°C. Blood samples were taken from six affected cows.

## RESULTS AND DISCUSSION

On March 2021, 20 days after making, a newly made batch of the "problem silage" was offered. Five to ten minutes after its consumption, 10% of the 280 exposed cows showed respiratory distress. Thirty minutes after the silage consumption, all signs concluded and animals returned to a state of normality. No other sings or deaths were observed. The same silage was offered the next day and similar signs were observed. Clinical signs were evaluated during each episode. The respiratory signs observed were tachypnea (60 to 72 respiratory movements per minute), dyspnea (Figure 1), cough, and anxiety. No increase in rectal temperature was recorded. A mild leucocytosis was registered, but other biochemical parameters were within normal values (data not shown).

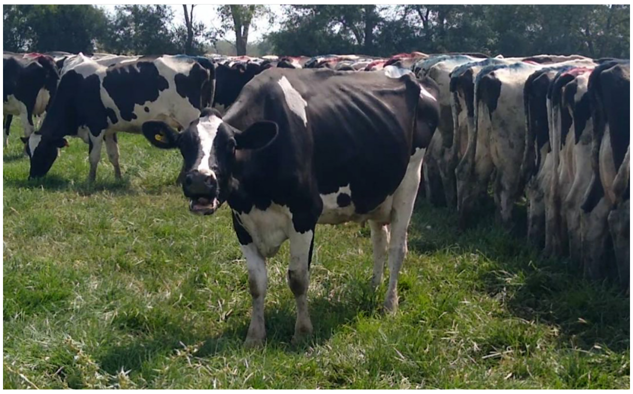

**Figure 1.** Holstein cow with marked dyspnea.

Chemical composition of both corn-silages ("problem silage" and "control silage") revealed expected values (Table 1). Ten samples were collected from different areas of the silo and pooled. For gas measurement, individual samples were taken in sealed plastic bags. Concentration of $NO_3$ ranged between 171.7- 242.5 ppm for the "problem" and 162.8 – 269 ppm for the "control" silages, representing between 0.049 and 0.086% of the DM, respectively (Table 1). The $NO_3$ concentration were within the range of expected values for the same type of feed (0.05 and 0.1%) (Verhoeff et al. 2007).

$N_2O$ and $CH_4$ concentrations in silage samples are shown in Table 1, moderately above ambient air concentrations (data not shown). Concentrations of NO, $NO_2$, CO, $CO_2$ and $O_2$ *in situ* were <2.0 ppm, <1.0 ppm, 15.5 ppm, 2.0% v/v and 18.3% v/v, respectively, within the range of values found in the literature (Epler 1989, Verhoeff et al. 2007).

**Table 1:** Nutritional and gas analysis in corn silage samples.

| Samples | DM[a] % | OM[b] % | IVDMD[c] % | ME[d] Mcal $kg^{-1}$ | NDF[e] % | CP[f] % | TP[g] % | pH[h] | $NO_3$[i] ppm | $N_2O$[j] ppm | $CH_4$[k] ppm |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Silage "problem" | 30.6 | 93.6 | 70.3 | 2.54 | 38.1 | 5.6 | 20.7 | 3.73 | 242.5 | 0.894 | 13.581 |
| Silage "control" | 31.0 | 94.3 | 75.1 | 2.71 | 31.7 | 4.1 | 25.9 | 3.55 | 269.0 | 0.677 | 15.094 |

[a] dry matter: silage was dried at 60°C for 48 h. [b] organic matter: dried silage was calcined in muffles at 600°C for 4 h. [c] *in vitro* dry matter digestibility: using an Ankom Daisy II incubator, placing the samples in filter bags of standard porosity and incubating them in flasks containing a mixture of buffer solution and ruminal fluid for 48 h., at a temperature of 39°C and constant rotation. [d] metabolizable energy: calculation using dry matter content. [e] neutral detergent fiber: evaluated using a Daisy II Ankom fiber analyser. [f] crude protein: determined using a LECO FP-528 nitrogen autoanalyzer that uses the technique of Dumas modified. [g] true protein: determined on crude protein using a LECO FP-528 nitrogen autoanalyzer that uses the technique of Dumas modified. [h] using pH meter. [i] nitrates: determined using the phenol disulfonic colorimetric technique as described by Bremner (1965). [j] nitrous oxide: determined by Agilent 7890A gas chromatograph equipped with an electron capture detector. [k] methane: determined by Agilent 7890A gas cromatograph equipped with a flame ionization detector.

Respiratory disease in cattle exposed to toxic gases is a poorly documented in the literature. Due to the mild respiratory distress observed in the affected animals it would be probably underdiagnosed, unless cattle are exposed to very high concentrations. Similar clinical signs to the ones described here were previously reported by O'Kiely et al. (1999) and Verhoeff et al. (2007) were a strong bleach-like odour without coloration was described. Similarly, in this report the personnel working at the farm described the same type of odour. Unlike previous reports where episodes were recorded in closed environments (O'Kiely et al. 1999, Verhoeff et al. 2007), this case describes the presence of respiratory distress in cows fed outdoors, which could lead to less severe symptoms.

High concentrations of gases commonly associated with this type of clinical syndrome could not be determined *in situ*. Even though, after *in situ* readings, clinical signs were observed in cattle exposed to the ensiled material, confirming that probably low concentrations of nitrogen gases or the presence of other gases may be involved in the presentation.

In this case, the "problem" silage was made with maize exposed to water stress, which is in agreement with reported cases of nitrous poisoning gas in cattle and humans due to exposure to ensiled fodder after a severe drought (Grayson 1956, Driehuis et al. 2018).

Acceptable $NO_3$ concentration was detected in silages samples, although it is possible that denitrification process of the silage may have reduced the initial maize $NO_3$ concentration. Furthermore, $N_2O$ levels were above ambient values, they could be an indirect indicator of the presence of high $NO_3$ in the sampled silos (Peterson et al. 1958). During anaerobic fermentation, the $NO_3$ of the silage is transformed into nitrite ($NO_2^-$) and oxygen. Under favourable pH conditions, $NO_2^-$ can be associated with organic acids forming nitrous acids, which due to the effect of temperature can be transformed into water and nitrogen oxides (Maw et al. 2002). On the other hand, if strong aerobic conditions occur nitrogen dioxides, trioxides and tetroxides can be generated, which can be visible with different colour depending on their concentration in the air (Grayson 1956, Peterson et al. 1958, Verhoeff et al. 2007). In this report, the pH of the "problem silage" was 3.7 and the $CH_4$ concentration was lower in the "problem" than the "control" silage.

This report demonstrates the potential risk that could be associated with the use of maize silages that may have accumulated $NO_3$ during its preparation, i.e. after a water stress period. Although the clinical signs observed in this case were similar to those described in the literature, the measured gases were in lower concentration than previously reported in the literature. Further studies are needed in order to better characterized this type of ensiled material and the occurrence of other gases probably provoking similar clinical signs.

When respiratory distress is observed after the offer of the ensiled material, the withdrawal of this problematic food is recommended. Empirical evidence observed in the present case shows that pre-ventilation of the silage is an alternative to reduce the clinical signs of the exposed cattle (data not showed), probably confirming the presence of pneumotoxic gases. More detailed studies should be made in order to evaluate the effect of such exposure on production losses and possible deaths.

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