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Effects of orthochlorobenzalmalononitrile on horses used in Public Security

[*Efeitos do ortoclorobenzalmalonitrilo sobre equinos empregados na segurança pública*]

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ABSTRACT

The effects on human beings of tear gas (CS) used by police forces during the control of civil disturbances are widely known and amply described in numerous scientific papers. However, the advent of the concepts of animal welfare raises the question of whether animals exposed to CS in such events, specifically horses, would suffer the same effects as those described for humans. The purpose of this study was to determine whether mounted police horses exposed to CS exhibit the same symptoms as humans. In this study, 12 horses of the Military Police of Paraná, healthy and with no history of respiratory tract disorders, were led through a gas cloud caused by the detonation of six teargas grenades. The horses' physiological parameters of respiratory rate, heart rate, rectal temperature, and color of the eye mucosa were evaluated 24 hours before exposure, 30 min, and 24 hours after exposure. Blood samples were collected for complete blood count (CBC) and blood gas analysis and samples of eye mucosa were obtained using sterile swabs. The analysis of these parameters did not reveal effects analogous to those described in humans.

Keywords: horse, mounted police, orthochlorobenzalmalononitrile, tear gas

RESUMO

Os efeitos sobre seres humanos do gás lacrimogêneo (CS) utilizado pelas forças policiais no controle de distúrbios civis são amplamente conhecidos e largamente descritos em numerosos artigos científicos. No entanto, com o advento dos conceitos de bem-estar animal, levanta-se a questão se animais expostos ao CS em tais eventos, especificamente cavalos, sofreriam os mesmos efeitos descritos em seres humanos. O objetivo deste estudo foi determinar se cavalos da polícia montada expostos ao CS apresentam os mesmos sintomas descritos em seres humanos. Neste estudo, 12 cavalos da Polícia Militar do Paraná, saudáveis e sem histórico de doença do trato respiratório, foram conduzidos por uma nuvem de gás causada pela detonação de seis granadas de gás lacrimogêneo. Os parâmetros fisiológicos de frequência respiratória, frequência cardíaca, temperatura retal e coloração das mucosas foram avaliados 24 horas antes da exposição, 30 min e 24 horas após a exposição. Amostras de sangue foram coletadas para realização de hemograma completo e hemogasometria, e amostras da mucosa ocular foram obtidas por meio de swabs *estéreis. A análise desses parâmetros não revelou efeitos análogos aos descritos em seres humanos.*

Palavras-chave: cavalo, polícia montada, ortoclorobenzalmalonitrilo, gás lacrimogêneo

INTRODUCTION

Horses were domesticated by prehistoric humans, and are second among the animals used by humans, after dogs. Since their domestication, horses have been man's inseparable friend throughout history (Bondaruk, 2005).

The use of horses is still very important to public safety, for the ostensible policing of urban centers, parks, agricultural exhibitions, shows, sporting events, in guarding the perimeters of prisons and in searching for people (Bondaruk, 2005). Therefore, there is a growing need for actions aimed at keeping these animals in perfect health. Healthy horses are more attentive and

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willing to obey the commands of the public security professional, responding efficiently and safely vis-à-vis its rider and the population. Indeed, the latter's safety is the main purpose for using horses, which are effective members of the troop.

One of the tasks these horses are used in is during situations of serious public order disturbance, such as the public demonstrations that took place major Brazilian cities in June and July 2013, which evolved into civil unrest with depredations of public and private property, and clashes between protesters and police.

To curb acts of vandalism while ensuring the population's right to free peaceful protests and demonstrations, Brazil's public security forces, specifically the Military Police in Brazil, use various instruments. These include the use of horses, which are sometimes exposed to another instrument of civil disturbance control, i.e., tear gas, a non-lethal chemical agent known technically as orthochlorobenzalmalononitrile (CS). The purpose of this investigation was three-fold: 1) to ascertain if CS has harmful effects on horses; 2) to provide guidelines for the correct use of this chemical agent; and 3) to create protocols for the emergency care of horses whose health status may change in response to exposure to CS.

The effects of chemical artifacts on humans are well known, specifically on their ocular and oral mucous membranes and on the upper airways (Sidell,, 2008).

It should be borne in mind that the responsibility for the welfare of these animals rests first and foremost on the ethical stance expected of those responsible for them in the military police companies. These responsibilities range from the minimum possible exposure to chemical agents in situations of disturbance of public order to providing proper emergency veterinary care using specific protocols. Ethics is the science of human conduct, which, in practical terms, involves acting honorably, i.e., doing the right thing for its own sake (Valla, 1998).

Within this concept, the public agent, in this case the military police officer responsible for the welfare of these animals should, as a matter of ethics, preserve the integrity of the animals employed in policing as much as possible, in line with today's concept of animal welfare. An article published in the journal Archives of Veterinary Science and Medicine, entitled "Animal welfare: concept and related issues," offers a clear definition that underpins the main points of this research project:

"In any welfare assessment, individual variations in facing adversity and their effects on animals must be taken into account" (Broom and Molento, 2004).

Given the lack of specific theoretical material about the effects of CS on horses, a literature review of its effects on humans was conducted to determine if horses exhibit the same effects, as well as others that might be identified.

This chemical agent, which vaporizes in the air by special processes, is a substance that causes several instantaneous and transitory psychophysiological effects. These effects include chest pain, difficulty in breathing, coughing, diarrhea, irritation of the eyes, skin, the mucous membranes of the respiratory tract, and a sensation of discomfort and distress (Viala *et al*., 2005).

The aforementioned effects were also manifested by French police officers exposed to CS tear gas, who immediately began coughing and reported a sensation of suffocation. Exposure to CS also resulted in significant excessive lacrimation, conjunctival irritation, reflex eyelid occlusion and intense photophobia (Viala *et al*., 2005). Other experiments on humans have also revealed the manifestation of asthma, hypertension, or cardiovascular disease (Bradbury, 1999).

The aim of this study was to determine whether mounted police horses exposed to CS present the same symptoms as humans.

MATERIAL AND METHODS

The project was approved by the Comissão de Ética no Uso de Animais (CEUA) of Evangelical College of Paraná, under protocol number 004476/2012.

This experiment involved 12 horses, approximately eight years old, of the following breeds: Brazilian Sport Horse (BSH), Lusitano

(L), and Mixed Breed Horses (MBH), trained to work in ostensive policing and restoration of public order. The clinical parameters of these horses were evaluated 24 hours before exposure, when blood samples were drawn for complete blood count and blood gas analysis, and swabs of the ocular mucosa were collected for evaluation.

The six tear gas grenades were of the brand (GL-309/CS, orthochlorobenzalmalononitrile, Condor, Brazil), containing the active ingredient orthochlorobenzalmalononitrile. The grenades were fired within a stipulated area of 50 m^2 at the training ground of the Coronel Dulcidio Mounted Police Regiment of the Paraná Military Police. The six grenades were fired in this space, where a 10-meter strip was stipulated between the start and finish lines, to form a cloud of CS gas. The twelve mounted horses were ridden at a walking gait, passing through the site twice, simulating a real situation in which horses are exposed to the tear gas. The horses were then taken to the Veterinary Center, where new CBC and blood gas analysis were performed, ocular mucosa swab samples were collected, and clinical exams were performed 30 minutes and 24 hours after exposure, according to the protocol described in Table 1.

Table 1. Data collection protocol for the experiment

Time	Method	Material
24 hours prior to exposure	Clinical exam	Stethoscope, clipboard and stopwatch
	Blood sampling for CBC	5ml needle and syringe
	Blood sampling for blood gas analysis	5ml needle and heparinized syringe
	Eye swabbing	Swab moistened with physiological solution
30 minutes after exposure	Clinical exam	Stethoscope and stopwatch
	Blood sampling for CBC	5ml needle and syringe
	Blood sampling for blood gas analysis	5ml needle and heparinized syringe
	Eye swabbing	Swab moistened with physiological solution
24 hours after exposure	Clinical exam	Stethoscope and stopwatch
	Blood sampling for CBC	5ml needle and syringe
	Blood sampling for blood gas analysis	5ml needle and heparinized syringe
	Eye swabbing	Swab moistened with physiological solution

The clinical exam consisted of measuring heart rate, respiratory rate, capillary filling time, rectal temperature, and skin turgor, and evaluating the abdominal quadrants and ocular and oral mucosa coloration. In addition, venous blood samples were collected for complete blood count (CBC) using a hematology analyzer (CELL DYN 1400, Cell Dyn, USA). Arterial blood samples were drawn into heparinized blood gas syringes on the premises of the Veterinary Center of the Military Police of Paraná, using a blood gas analyzer (Rapid Lab 348, LAFT, Brazil).

Ocular mucosa samples were collected using sterile swabs moistened with physiological solution. These samples were smeared onto slides and allowed to dry at room temperature, fixed with methanol and stained using the May-Grunwald-Giemsa (MGG) staining method. The slides were then subjected to microscopic analysis to identify allergic effects (presence of eosinophils) resulting from exposure to CS gas. This involved scanning more than 10 homogeneous fields without cell overlap, in addition to other findings.

The findings consisted of mucus filaments, keratinocytes, calciform cells, neutrophils, and cellular debris. No microorganisms were found in the few eosinophils.

The microscopy data were classified according to grade, i.e., grade 0: absent or rare; grade 1 (+):one to five elements; grade 2 (+ +) six to ten elements; grade 3 (+ + +) eleven to fifteen elements, and grade 4 (+ + + +) more than 15 elements, as described in Table 2.

Table 2. Cross pattern of elements found

Number of elements (findings)	Representation in crosses
One to five	+
Six to ten	+ +
Eleven to fifteen	+ + +
More than fifteen	+ + + +

The data of clinical exams, CBC, blood gas and ocular mucosa were subjected to a statistical analysis using GraphPad Prism v. 7. The multi-level comparison of Tukey's was used to compare the moments.

RESULTS

The results of the clinical exam, CBC (Table 3), blood gas analysis (Table 4) and ocular mucosa (Table 5) showed no statistically significant changes 30 minutes and 24 hours after exposure to the gas. In other words, the horses did not exhibit the effects observed in humans exposed to tear gas.

Table 3. Mean and standard deviation of the complete blood count of horses exposed to CS tear gas. Where different letters represent statistical differences, $p<0.05$

Element	Control	After 30 Min	After 24 Hours
Erythrocytes	7.6 ± 0.57[a]	8.50±0.53[b]	7.37±0.85[a]
Hematocrit	35.95±2.85[a]	39.85±2.14[b]	35.28±4.19[a]
Hemoglobin	14.13±1.14[a]	16.52±1.15[b]	13.90±1.36[a]
MCV	47.3 ±2.25[a]	48.27±2.29[a]	47.83±1.41[b]
MCH	18.6±0.37[a]	19.53±0.62[b]	18.87±0.59[a]
MCHC	39.3±1.37[a]	40.03±1.19[a]	39.50±1.12[a]
Leucocytes	8316.7±1527.63[a]	10183.33±2057.59[a]	8650.00±1674.22[a]
Segmented neutrophils	55.5±3.56[a]	57.83±8.45[a]	62.33±12.12[a]
Lymphocytes	39.0±3.46[a]	36.00±7.04[a]	33.00±13.30[a]
Monocytes	2.0±2.10[a]	3.67±2.34[a]	2.00±3.29[a]
Eosinophils	1.7±1.21[a]	2.17±2.23[a]	2.33±2.19[a]
Basophils	0.7±1.03[a]	0.33±0.82[a]	0.33±0.89[a]
Platelets	251833.3±81184.70[a]	257500±257500.00[a]	219000±92979.57[a]

Table 4. Mean and standard deviation of the blood gases of horses exposed to CS tear gas. Where different letters represent statistical differences, $p<0.05$

Parameter	Control	30 Min	24 Hours
pH	7.44±0.01[a]	7.46±0.05[a]	7.34±0.18[a]
pCO_2 (mmHg)	42.88±1.29[a]	37.80±3.59[a]	41.23±14.84[a]
pO_2 (mmHg)	78.50±20.96[a]	96.78±12.63[a]	92.28±13.45[a]
HCO_3 (mmol/L)	28.75±1.14[a]	26.63±2.65[a]	30.00±8.54[a]
O_2%	93.65±6.95[a]	97.53±0.74[a]	96.35±1.11[a]
CO_2CT (mmol/L)	30.08±1.17[a]	27.73±2.75[a]	31.72±8.80[a]

Table 5. Comparison of effects of CS tear gas on humans and on horses

Area	Humans	Horses
Skin	Irritation Allergic dermatitis Blisters Erythema Burns	No effect
Eyes	Blepharospasm Conjunctivitis Temporary loss of eyesight Burning sensation of the eyes Tearing	No effect
Nose	Sneezing Coughing Chest pain and chest tightness	No effect
Attention	mental confusion, disorientation	No effect
Breathing	Coughing Gagging and chest pain Pulmonary irritation	No effect
Mouth	Salivation	No effect

DISCUSSION

The clinical parameters remained within normal limits differently from the signs analogous to those observed in humans, strong sense of burning in the eyes, accompanied by intense tearing, suffocation, difficulty breathing and constriction of the chest, involuntary closure of eyes, burning sensation of moist skin, runny nose, and vertigo or
stun. (Curso..., 2009).

The complete blood count (CBC) carried out 24 hours prior to exposure, and 30 minutes and 24 hours after exposure showed no variation regarding the presence of eosinophils (Ivester, 2013), thus indicating the absence of any allergic response to exposure to CS. It should also be noted that the blood count did not indicate an increase in neutrophils and/or lymphocytes, thus demonstrating that exposure to this chemical agent elicited no response.

Another fact confirming that the horses did not develop any allergic reactions was the low levels of mast cells and basophils found at the different times evaluated (Wagner, 2016). An increase in the presence of mast cells and basophils would have indicated an allergic reaction.

Blood gas analysis is the exam of choice for the assessment of pulmonary function and respiratory disorders (Day, 2002). The horses exposed to CS showed no clinical signs of transient acidemia, such as tachycardia and hypoventilation due to a change in pCO^2 (Kowal, *et al*., 2008). Moreover, pH levels also remained unaltered (Silva, 2013) at the exposure times analyzed here.

An evaluation of the presence of dermatological lesions revealed no alterations, such as dermal reactions, hyperpigmentation, lichenification (thickened skin), erythema (redness of the skin caused by capillary congestion) and urticaria (Irving, 2016). In addition, the evaluation of ocular mucosa cellularity showed no changes in mast cells and basophils, thus demonstrating that the dermatological effects observed in humans do not occur in horses exposed to this tear gas.

Chemical agents may cause ocular allergic reactions in horses (Scarabelli, *et al*., 2008). However, the horses exposed to orthochlorobenzalmalononitrile (CS) in this experiment showed no clinical signs analogous to those observed in humans (Sebastião *et al*., 1988; Viala *et al*., 2005). Other clinical signs,

e.g., eye injuries such as epiphora, blepharospasm, photophobia, corneal edema, conjunctival hyperemia and myosis were also absent.

An analysis of the results obtained allow us to state that the horses exposed to CS gas in open environments, as recommended by the manufacturer, did not present the effects found in humans, nor did this exposure represent a stress factor for horses trained for activities aimed at public safety.

Lastly, it should be noted that the CS in tear gas grenades has a vapor density 6.5 times greater than air and a vapor pressure of 0.00034 mmHg at 20ºC, aimed at eliciting a temporary disabling effect on humans.

CONCLUSIONS

Based on the data obtained in this study, we conclude that the horses exposed to orthochlorobenzalmalononitrile (CS gas) did not exhibit any clinical symptoms or signs of discomfort such as those suffered by humans in response to exposure to tear gas.

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