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External validation by correlation of the evaluation of animal welfare of canines during training

Validación externa por correlación de la evaluación del bienestar animal de caninos en entrenamiento

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ABSTRACT

Studies have demonstrated the ability of the canine sense of smell to detect people with infectious diseases. Animal Welfare (AW) care should be a priority when working with animals. The objective was to validate the animal welfare assessment of canines in training by correlation analysis of the human and animal components to determine if it is reliable. Observation, behavior logs, and 5-month medical reports were used for data collection. Both components were categorized and evaluated. AW per territory was classified as "low" if it obtained from 0 to 67 points, "medium" if it obtained from 68 to 135 and "high" if it obtained from 136 to 200 points. AW per territory was "medium", with 133.6/200 points. The Spearman correlation of 0.6 confirmed that there is a statistically significant association ($P < 0.5$) between both components of AW. The methodology for the AW evaluation applied to canines in scent training determined a reliable and adequate category for the animals evaluated due to the correlation that exists between the variables of the human and animal components.

Keywords: validation, animal welfare, animal component, human component, canine training.

RESUMEN

Estudios han demostrado la habilidad del olfato canino para detectar personas con enfermedades infecciosas. El cuidado del Bienestar Animal (BA) debe ser prioridad al trabajar con animales. El objetivo fue validar la evaluación del bienestar animal de caninos en entrenamiento por medio del análisis de correlación de los componentes humano y animal para determinar si es confiable. Para la recolección de datos se utilizó la observación, bitácoras de comportamientos, y reportes médicos de 5 meses. Se categorizaron y evaluaron ambos componentes. El BA por territorio se clasificó como “bajo” si obtuvo de 0 a 67 puntos, “medio” si obtuvo de 68 a 135 y “alto” si obtuvo de 136 a 200. Se obtuvo categoría de bienestar

animal individual “alta” en 5/9 caninos y el resto categoría “media”. El BA por territorio fue “medio”, con 133.6/200 puntos. La correlación de Spearman de 0.6 confirmó que existe asociación estadísticamente significativa (P < 0.5) entre ambos componentes de BA. La metodología para la evaluación del BA aplicada a caninos en entrenamiento por olfato determinó una categoría confiable y adecuada a los animales evaluados debido a la correlación que existe entre las variables de los componentes humano y animal.
Palabras clave: validación, bienestar animal, componente animal, componente humano, entrenamiento canino.

INTRODUCTION

Animal welfare (AW) can be defined as a state of complete physical and mental health, where the animal is in harmony with the surrounding environment (Broom, 2011; Hemsworth *et al*., 2015; Salas & Manteca, 2016). To ensure AW, access to the five freedoms indicated by the Farm Animal Welfare Council must be ensured: 1) the animal must be free from hunger, thirst and malnutrition; 2) free from physical and thermal stress; 3) free from fear and distress; 4) free from pain, injury and/or disease; and 5) free to manifest normal behavioral patterns (Temple, 2021; Salas & Manteca, 2016).

Thus, AW integrates much more than just physical health. It is quantified by assessing how the animal copes with the environment it is in. This response can be classified from poor to good AW (Sejian *et al*., 2011). The measurement must be objective, and then consider ethical conditions in order to improve the animal's quality of life. It can be evaluated per individual or per group of animals, and per given time interval; it is suggested to take into account physiological, behavioral and environmental indicators, since AW is determined by the balance of these factors (Polgár *et al*., 2016; Van der Harst & Spruijt, 2007). In the evaluation of physiological indicators, diseases and injuries that animals may present should be considered; while for behavioral indicators, social interactions should be evaluated; and environmental indicators include management and housing conditions. All the mentioned indicators have important effects that influence the AW (Broom, 2011; Dawkins, 2003).

Different methods have been proposed to evaluate AW, such as avoidance and positive preference tests, measurement of the ability to have a normal behavior (Broom, 2011), evaluation of cognitive measures, where mention is made of cognitive bias, when the animal makes decisions based on past experiences and the ability to learn (Polgár *et al*., 2016), and of behaviors related to fear and anxiety (Sejian *et al*., 2011), to mention a few. Other authors propose using direct and indirect indicators, where direct indicators are based on the animal, and include physiological and behavioral components; and indirect indicators focus on the animal's resources, such as access to food and water, environment and health care (Chavez *et al*., 2020).

Lack of control over interactions with the environment and difficulty adapting are indicative of poor AW; therefore, when using working animals in a human environment, AW care should be a priority and it is necessary to consider the animal's abilities to adapt to its environment on an ongoing basis. In the case of canines in scent training that will be used for work, trainers must follow up on the behavioral changes of the animals, these changes can occur for different causes such as pain, stress, social dynamics, and anything that may represent a problem in the performance of the animal's zootechnical purpose.

Canines are able to identify molecules even in small concentrations, the sensitivity of canine olfaction has been used as a detection tool in medical, scientific, police, military and social areas (Edwards *et al*., 2017; Else, 2020; Wackermannová *et al*., 2016). Several studies have demonstrated the ability and acuity of canine olfaction to detect people with infectious and non-infectious diseases, such as different types of cancer, diabetes, cirrhosis, malaria, viral and bacterial infections, with high sensitivity and specificity (Leroy *et al*., 2020). Canines identify with their sense of smell these components generated during the disease, from samples and/or directly from infected people (Vesga *et al*., 2021).

Finally, it is recommended that the tools to collect information for the evaluation of animal welfare comply with two fundamental elements: validity and reliability, to match the gold standard instrument. Validity is defined as the degree to which an instrument measures what it should measure and reliability is the degree of congruence with which an instrument measures the variable (López-Fernández *et al*., 2019). The validity of the instruments responds to an interest in the search for contextualization and homogeneity in the results obtained.

Although it is true that canines have been used for the detection of different diseases by scent, few studies have been developed to evaluate their welfare during the training process, which must be ensured for ethical and health reasons. Monitoring the animal and human components to evaluate the AW of canines in training process, related to physical and mental health indicators, as well as elements of the environment and animal care allow maintaining an adequate quality of life of these, in the understanding that the increase of animal welfare would not affect their performance during training. The objective of the present study was to validate the evaluation of animal welfare of canines in training by means of the correlation analysis of the human and animal components to determine if it is reliable.

MATERIAL AND METHODS

The study population was 9 whole canines: 4/9 German Shepherd, 4/9 Belgian Shepherd Malinois and 1/9 Labrador Golden Retriever, 6/9 males and 3/9 females, with an average age of 1.5 years and 25 kg of which 6/9 canines had previous training for odor detection, obtaining a toy as a reward. All in training for SARS-CoV-2 (COVID-19) detection of

positive human samples by sniffing, in a canine training center located in Hermosillo, Sonora, Mexico. This project was approved by the research ethics committee of the University of Sonora Oficio No. CEI-UNISON 016/2020.

Canines were divided into two groups. The first group of 4/9 canines was trained for identification of saliva samples, and the second group of 5/9 canines, for identification of sweat samples, both samples came from COVID-19 positive individuals confirmed by Polymerase Chain Reaction (PCR).

The training lasted 5 months and was divided into three phases. The first phase consisted of presenting the detection process by identifying the prize (toy) inside stainless steel bases. Once the reward was identified, the canines were allowed playing with it for 2 minutes as positive reinforcement. The second phase was based on teaching the association that exists between the positive sample to COVID-19 and the reward (toy or food), so that they would learn to correctly identify the odor with the understanding that they would be rewarded. In the third phase, the double-blind method (Rodríguez-Martín & Casado-Collado, 2002) was applied and the correct and incorrect markings were recorded in individual logs. At the end of the three stages of training, the specificity and sensitivity of each dog was calculated by distinguishing between positive and negative samples to measure its performance.

Canines were kept in individual wooden cages and steel grids, approximately 1 m^2 in size, in a refrigerated area maintained at 25 °C at all times, from which they were allowed leavinge for defecation and training.

The management from Monday to Saturday from 6:00 to 7:00 a.m. was directed to spend energy playing with the ball with the same trainer. Subsequently, from 7:00 to 9:00 a.m., sniffing training exercises with positive reinforcement were performed for all animals, with rest on Sundays. Canines were fed with Premium® brand commercial kibble once a day; the daily grams of food were calculated using Atwater's caloric intake calculations (metabolizable energy), according to their age, weight, sex and physical activity (Muñoz-Rascón *et al*., 2021).

Measurement of Animal Welfare. The animal welfare measurement instrument of Castillo-Cuenca *et al*., 2012, with slight modifications, was used, which consisted of the evaluation of the animal and human components. The results were based on the observation of the animals and the human-animal relationship with their trainers in person and by means of cameras, carried out by two zootechnical veterinarians during training 3 times a week during the 5 months of training; in addition, individual and group handling and behavior logs were used, which include the variables that measure both components (Table 1). Scores were given to the variables of each animal and human component, which included indicators of physical and mental health, as well as elements of the

environment and animal care. The canines were under the care of a small-species clinical zootechnician veterinarian responsible for safeguarding their health, so information from weekly medical reports was also used. To obtain the categories of individual animal welfare, the sum of the animal and human components was considered, since the way in which humans treat animals influences their degree of development (Castillo-Cuenca *et al*., 2012).

Table 1. Human and animal component variables for animal welfare assessment of canines in training

Human component variables	Score	Animal component variables	Score
Animal selection	Yes = 20 points No= 0 points	Behavioral disorders	0 = 60 points 1 = 20 points 2 or more = 10 points
Socialization	Yes = 20 points No= 0 points	Unmet social needs	None = 10 points 1 = 3 points 2 or more = 2 points
Training	Yes = 20 points No= 0 points	Unmet developmental needs	None = 10 points 1 = 3 points 2 or more = 2 points
Care and welfare	Yes = 20 points No= 0 points	Unmet learning needs	None = 10 points 1 = 3 points 2 or more = 2 points
Good coexistence	Yes = 20 points No= 0 points	Unmet physiological needs	None = 10 points 1 = 3 points 2 or more = 2 points
Totals: 0/100 points per component Animal welfare level per component: 0-32 points = low 33-65 points= medium 66-100 points= high			

Human Component. It was quantified based on 100 points, which contains five variables to be analyzed. The first variable was animal selection, which perceived the physical and behavioral characteristics of the animal for use in the zootechnical purpose. The second variable was socialization, which assessed their relationship with trainers and other people. The third training variable assessed its behavior and development in training. The fourth variable established care and welfare, where the animal's health, physical, emotional and environmental characteristics were calculated; and the fifth variable was good coexistence, which evaluated its behavior with its trainers and other people. These variables were dichotomous, a "Yes" corresponded to 20 points, a "No" to 2 points.

To quantify animal selection, socialization and good coexistence, direct observation of the animals was carried out; for the training variable, the training time logs were also used;

and for the care and welfare variable, information from the cleaning and medication logs and individual health medical reports were integrated.

Animal Component. It was also evaluated with a base score of 100 and five other variables were analyzed. The first variable was behavioral disorders, which quantified the presence of abnormal behaviors of the species, such as stereotyped behaviors. The second variable measured unsatisfied social needs in an interspecific social structure, including their relationship with other animals, and was calculated with demonstrations of aggression or closeness with other animals. The third variable evaluated the unmet developmental needs, perceiving the elements that limited the animal to perform its normal behavior and development. The fourth variable assessed unmet learning needs, determining the presence of obstacles in the development of learning, observing its progress during training. The fifth and last variable measured the unsatisfied individual physiological needs, which evaluated the presence of physical elements that influenced the animal and limited the performance of physiological processes.

With respect to scoring, the first variable of behavioral disorders was assigned from 0 to 60 points. The score corresponded to a quantitative analysis where there could be one of three answers: 10 points when there were two or more disorders, 20 points when there was one disorder, and 60 points when there were no disorders. In the following four variables, needs were evaluated with a value of 2 points when there were two or more, 3 points when there was one, and 10 points when there was none, as recommended by Castillo-Cuenca *et al*., 2012, modified.

To quantify these variables, the daily time, feeding and cleaning logs were used, as well as the direct observation method during training.

Data collection. Individual logs were designed to include the basic daily needs of feeding, bathing and brushing, cage cleaning, training and liberty times, and considering the needs, medication logs were also included. All logs contained the date, handling schedules, name and signature of the responsible personnel.

The "cleaning" log included variables of date and time (continuous variables), cage cleaning, if the animal had a complete bath that day (Yes/No), if brushing was applied (Yes/No) (dichotomous variables), and if any special soap was applied (nominal variable). In the "time" log, date and time variables were used (continuous variables), recording the time in minutes in each area (liberty place, defecation place and play place; continuous variables). In the "feeding" log, variables of date and time (continuous variables), amount of food (continuous variable) and type of food (nominal variable) were measured. Finally, the "medication" log was used to control the doses of medications administered to the

canines after consultation with their veterinarian. The variables used were date and time (continuous variables), diagnosis, signology, medication administered (nominal variables), dose and route of administration (continuous variable).

Data analysis. Once the data from the logs were obtained, the averages of the daily frequencies per animal were calculated to determine the score for each variable that integrates the human and animal components. In addition, the following formula was applied: AW = AC+HC, where AW represents Animal Welfare, AC the Animal Component, and HC the Human Component. The results obtained for each component (AC and HC) were classified as: low (0 to 32 points), medium (33 to 65 points) and high (66 to 100 points).

According to Castillo-Cuenca *et al*., 2012, to evaluate the AW in a given territory (group average), the same criteria are used and the following formula is applied: E AW t= Σ AW/n. Where: EAW t is the estimate of animal welfare per territory, ΣAW is the summation of the animal welfare values of each individual, and n is the number of individuals evaluated. The result obtained was classified as low (0 to 67 points), medium (68 to 135 points) or high (136 to 200 points) animal welfare per territory. Finally, the Spearman correlation coefficient formula was applied: rs = å x2 + åy2 - åd2 y 2 √ (å x2 åy2), to determine whether or not there is an association between the human and animal components.

RESULTS

Direct observation and the use of logs during follow-up visits made it possible to concretely evaluate the different variables of the human and animal components in order to calculate the animal welfare of the canines during scent training.

Human component. The evaluation in the selection of animals, training and good coexistence was positive, the maximum value of 20/20 points was granted. Since all the canines included in the study had desirable anatomical and behavioral traits for the zootechnical purpose of odor detection. It was reported in the logs that, prior to training, the animals had a range of play time between 6 and 60 minutes with a mean of 56.2 minutes, they trained with a punctual routine and the training area and the exercises applied were adequate. The animals did not show aversion towards their trainers, nor did they show aggression towards the people around them. However, in the socialization variable they were assigned only 2/20 points with the exception of one of the females, since, when evaluating the relationship between the canines, rivalry and marked aggression was observed between the males and two of the females.

In the last variable of care and welfare, the minimum value of 2/20 points was assigned to all animals. Although the cleaning log showed that the individual cages were washed with water, soap and bleach every day at 7:00 hours and the individual baths were reported every 2 to 28 days, the medication logs and medical reports indicated in October

2020 that 7/9 canines presented diarrhea and lack of appetite, which were diagnosed with *Giardia spp*. parasitosis by means of coproparasitoscopy, hemogram and blood chemistries. In addition, 1/9 canine presented a mild degree of dehydration. The canines received integral therapy, with systemic treatment, constant cleaning and disinfection of the areas according to current literature with favorable results.

Four months later, it was identified that 4/9 canines presented thrombocytopenia, leukocytosis and presence of morulae in the cell cytoplasm in the blood smear, in addition to decreased body condition, lymphadenomegaly and fever. The presumptive diagnosis was infection by vector transmission such as *Erlichia canis* or rickettsial infections. On this second occasion, the animals were rested for 21 days and were given the integral treatment for vector-borne diseases, recommended by their responsible veterinarian, also with favorable results.

Finally, other health problems were observed, such as wounds on the limbs, slight dehydration, decrease in feed intake and body condition of 2/5 during the periods of both diseases. For disease control, the cages were disinfected daily and the animals were bathed every 10 days for 3 times. In addition to the application of preventive measures such as not exchanging spaces and not allowing the entry of new animals, the animals' health was adequately stabilized.

When integrating the information in the evaluation of the human component, the majority of the canines, 8/9, obtained a value of 64/100 points, classifying their welfare as "medium", only 1/9 obtained the category "high" (Table 2).

Table 2. Scores and individual category per variable of the human component

Canine identification number	AS	S	T	C & W	GC	Individual score HC 1/100	Individual category HC
1	20	2	20	2	20	64	Medium
2	20	2	20	2	20	64	Medium
3	20	20	20	2	20	82	High
4	20	2	20	2	20	64	Medium
5	20	2	20	2	20	64	Medium
6	20	2	20	2	20	64	Medium
7	20	2	20	2	20	64	Medium
8	20	2	20	2	20	64	Medium
9	20	2	20	2	20	64	Medium

AS: Animal Selection; S: Socialization; T: Training; C & W: Care and Welfare; GC: Good Coexistence; AW: Animal Welfare; HC: Human Component

Animal component. In the behavioral disorders variable, a value of 10/60 points was assigned. It was identified that 4/9 canines presented two or more stereotypies such as: excessive licking of forelimbs, tail flicking, circling incessantly inside the cage and chewing on water cans, to mention a few.

Regarding the evaluation of unmet social needs, males (5/6) were assigned 3/10 points since socialization time was restricted. In the case of the three females and one of the males, they were assigned 10/10 points, as they were allowed socializing with a little more freedom. It was identified that the average time of freedom for all animals according to the logs was 28.6 minutes per day.

In the developmental and learning needs variable, they were assigned 10/10 points. The average time to complete the first two phases of training was 7 weeks. In the third phase of training, a similar learning curve was observed in all animals with a tendency to increase the percentages of sensitivity and specificity for the correct identification of COVID-19 positive samples.

With respect to physiological needs, 8/9 canines were assigned a value of 3/10 in this category. Access to water was allowed before and after training because they presented the stereotype of biting the water cans in their individual cages. The canine identified with number 3, being more docile and not presenting this stereotypy, was allowed free access to water, for which he was assigned 10/10 points. The average daily amount of feed per animal was 681.5 grams per day and the average time for all animals to defecate was 35.4 minutes per day.

In the results of the animal component evaluation, the minimum value obtained was 36/100 points, while 1/9 of the canines obtained the maximum value of 100/100. More than half of the canines, 5/9 obtained the "high" category, the other 4/9 obtained the "medium" category (Table 3).

Estimation of animal welfare. In the final category of "medium" animal welfare, 4/9 canines were classified, obtaining a "medium" category in the human and animal components. In the final category of "high" animal welfare, 4/9 canines were classified and obtained a "high" category in the animal component and a "medium" category in the human component. Only 1/9 canines obtained a "high" category in both components. In the sum of the animal component and the human component, a range of 100 to 182 points was obtained, with an average of 133.6/200 points, which is classified as "medium" animal welfare by territory (Table 4).

The value of Spearman's rank was 0.6, which indicates that the relationship of the variables is statistically significant ($P < 0.5$), because the value was equal to the critical value of Spearman's correlation table (0.60) (Table 5).

DISCUSSION

The collection of the variables to be evaluated was carried out by the same certified person who was in charge of the training and care of the animals, since there are different approaches for the collection of information for the evaluation of animal welfare. The assessment tool was quick, practical and easy to use, using parameters based on animal and environmental variables, agreeing with the suggestions of (Whitham & Wielebnowski, 2013), who point out that the tools should be used by experienced animal care professionals, as they should detect subtle changes in behavior and individual conditions.

Table 3. Scores and individual category per animal component variable

Number identification number of the canines	BD	SN	DN	LN	PN	Individual score AC 0/100	Individual category AC
1	60	3	10	10	3	86	High
2	60	10	10	10	3	93	High
3	60	10	10	10	10	100	High
4	60	10	10	10	3	93	High
5	10	3	10	10	3	36	Medium
6	60	10	10	10	3	93	High
7	10	3	10	10	3	36	Medium
8	10	3	10	10	3	36	Medium
9	10	3	10	10	3	36	Medium

BD: Behavioral Disorder; SN: Social Needs; DN: Developmental Needs; LN: Learning Needs; PN: Physiological Needs; AC: Animal Component

Records allowed proposing changes to improve the areas of opportunity detected individually and collectively. In this research, 4/9 canines with stereotypies were registered, where the origin and reasons for this behavior were considered, which could be associated to what was reflected in the time log, where it was observed that the time of freedom and training was restricted. Reaffirming what is pointed out by (Whitham & Wielebnowski, 2013), who indicate that individuals of the same species can express different behaviors when facing the elements of the environment, and the origin and motives of the behavior should be considered.

Table 4. Final animal welfare assessment of canines in training using the sum of human and animal component points

Canine identification number	HC (0/100 points)	AC (0/100 points)	ΣAW (0/200 points)	AW final category
1	64	86	150	Alto
2	64	93	157	Alto
3	82	100	182	Alto
4	64	93	157	Alto
5	64	36	100	Medio
6	64	93	157	Alto
7	64	36	100	Medio
8	64	36	100	Medio
9	64	36	100	Medio
Mean	66	67.6	133.6	

AC: Animal Component, HC: Human Component; ΣAW: Animal Welfare Additive; AW: Animal Welfare

In the results obtained, negative elements were identified, such as the need for more socialization time, as well as areas of opportunity in health and welfare. In the same way, positive elements were also identified, such as the needs for learning and effective training, due to the precise canine training regimen, which facilitated their learning and maintained their daily cleaning routine. In order to make the identification of the factors that affected the animal welfare of the canines more efficient, individual, environmental and disciplinary elements were combined. Coinciding with (Polgár *et al*., 2019; Whitham & Wielebnowski, 2013), who recommend combining and integrating diverse environmental and individual elements, as this made it easier for them to recognize positive and negative elements, to detect areas for improvement. By using the methodology for calculating animal welfare (Castillo-Cuenca *et al*., 2012) and the application of individual logbooks.

Table 5. Spearman's correlation between the human and animal components for animal welfare assessment of canines in training process

Canine identification number	HB (x)	AB (y)	Range x	Range y	Difference (D)	D2
9	64	36	2.5	4.5	-2	4
8	64	36	2.5	4.5	-2	4
7	64	36	2.5	4.5	-2	4
5	64	36	2.5	4.5	-2	4
1	64	86	5	4.5	0.5	0.25
6	64	93	7	4.5	2.5	6.25
4	64	93	7	4.5	2.5	6.25
2	64	93	7	4.5	2.5	6.25
3	82	100	9	9	0	0
						40

Relation of variables 0.6; Significance 0.05; Confidence 95 %, Critical Value 0.6; HB: Human Behavior, AB: Animal Behavior

Human component. The canines were apt for the odor discrimination activities; due to the fact that in the selection of animals, training and good coexistence a positive result was obtained with the maximum value of 20/20 points, since the behavioral traits of good attention (response capacity), responsiveness (degree of interest with which it responds), initiative (resolution capacity), persistence or determination (effort and vigor used in the development of a task) were considered. As well as intensity (response to the stimulus), competitiveness (desire to perform the task against the opposition of a peer or person) and energy (level of physical power), according to what was suggested by (Paramio-Miranda, 2010); so they entered the training process for olfactory detection of SARS-CoV-2 positive people (COVID-19), agreeing with (Prada-Tiedemann *et al*., 2019; (Mancilla-Tapia et al., 2022), who pointed out that the results are reliable when the canines are selected and handled by a certified trainer.

In socialization, a low value of 2/20 points was obtained, except for one female, observing rivalry and marked aggression in males and two females, which was influenced by the quantity and quality of interactions, as well as by the training methods; these results agree with (Arhant & Troxler, 2014); who point out that humans have influence in the animal's

environment, where negative reactions are an indication of their poor welfare, factors that should be considered in the evaluation of good coexistence and socialization. About the evaluation of the training variable, some researchers expose that training more than 4 hours per week is positively related to the training term in canines (Cobb *et al*., 2015; Troisi *et al*., 2019), which agrees with the results of the training of the animals included in the present project.

About care and welfare, giardiasis gastroenteritis is characterized as difficult to treat in multi-animal settings (Tangtrongsup & Scorza, 2010). The diagnosis of *Giardia spp*. in 7/9 canines was performed with the support of corresponding laboratory tests, and its elimination was possible by the timely integral therapy of systemic treatment, cleaning and disinfection of areas; avoiding with this what was pointed out by (Dantas-Torres *et al*., 2020; Ruiz *et al*., 2019; Yazdani *et al*., 2017), who refer that re-infestation is common, when integral therapy is not performed. Likewise, in 4 months later, a new presumptive diagnosis of vector transmission such as *Erlichia canis* or rickettsial infections was performed in 4/9 canines, based on the fact that diseases originated by vectors, such as those produced by: *R. conorii*, *R. belli*, *R. riphicephali* and *R. montanensis* are asymptomatic in canines, being *R. rickettsii* the only species causing clinical pictures naturally and experimentally (López Del P *et al*., 2007). Considering also that serological studies have found anti-*R. rickettsii* antibodies in 5 to 15 % of canines in the United States of America and 4 to 31 % in Brazil. In addition, there are reports of simultaneous canine and human infection in one household. For *R. conorii*, seropositivity has been found in 14 % of canines studied in Spain and 15 to 35 % in Italy, countries where spotted fever is endemic in humans (López Del P *et al*., 2007; Martínez *et al*., 2016).

It is important to emphasize that any disease is a negative indicator of well-being, as expressed by (Salas & Manteca, 2016). And it was considered that health problems could be associated to the physical location; since the training site before starting the project was attended by external animals, being possible the contamination of the area by some kind of vector that transmitted diseases. However, it was possible to maintain the health of the animals by implementing the appropriate preventive measures indicated in the results.

Animal component. The results were low 10/60 points in the behavioral disorders variable, where 4/9 canines showed stereotypies as abnormal behavior, such as those that (Salas & Manteca, 2016) define as repetitive behaviors caused by repeated attempts to adapt to the environment, which are indicators of poor welfare. It is possible that the disorders observed in the animals are associated with the need for larger cages and the time allocated in them, where it confirms what was pointed out by (Polgár *et al*., 2019), and contradicts the Mexican Official Standard NOM-062-ZOO-1999 (SAGARPA, 1999), the measurements of cages for research canines should be 1.11 m^2 floor area/animal in

animals up to 30 kg and 2.23 m^2 floor area/animal in animals over 30 kg, and at a height that the animal can stand. And the size of the experimental cages was smaller than indicated, which limited the movement of the animals, as was observed in the stereotypies, when some of the animals moved in circles on their own axis. In canines, the most commonly observed abnormal repetitive behaviors are circling on their own axis, repeatedly walking around the perimeter of their kennel. While less common behaviors include biting on their cage (bars or walls), excessive licking of their environment, and self-mutilating behaviors such as foot chewing and excessive self-licking (Polgár *et al*., 2019); all of these abnormal behaviors were observed in animals. To counteract this abnormal behavior, it was recommended to increase freedom times and specific recommendations were offered for the construction of cages adequate to the needs of the animals according to current regulations.

With respect to unmet social needs, males were assigned a low score 3/10, due to the fact that they needed more socialization time, which caused them a little more difficulty to concentrate during training, so it was agreed with (Troisi *et al*., 2019; Polgár *et al*., 2019; Wells, 2009), which show evidence that stressful housing conditions decrease the efficiency in odor detection of canines; as well as confinement, lack of predictability, control and choice, originate stress, anxiety, frustration or fear in canines. For this reason, it w.as recommended to increase the time of freedom, as well as to improve the environmental enrichment by placing toys and offering fleshings.

There is a traditional idea that canines are pack animals that have a linear hierarchy and that their behavior is driven by the desire to be the "alpha" or "dominant" of the pack (Guilherme-Fernandes *et al*., 2017). Because of this idea, trainers chose to limit interactions between canines and thus keep the animals as "alpha". However, a stable hierarchy decreases conflicts and aggression; ranks are mostly linear, but triangular hierarchies can occur (Schilder *et al*., 2014). Assignment of ranks in the herd can be used for behavioral description and not necessarily to describe social organization (Schilder *et al*., 2014). It is possible that the lack of a stable hierarchy among the canines evaluated may have negatively affected the assessment of social needs in males. And that there is evidence that canine interaction with humans can reduce stress (Arhant & Troxler, 2014). Therefore, it was generally recommended to increase the play time of all animals with the trainer and caretakers, in addition to adding more time to socialize with other animals to improve coexistence and socialization.

In the variable of developmental and learning needs, it was identified that the animals trained with saliva samples obtained a sensitivity between 70 and 78 % and specificity between 53 and 69 %. Animals trained with sweat samples obtained a sensitivity between 58 and 80 % and specificity between 64 and 88 % (P<.05) (Mancilla-Tapia *et al.*, 2022). It is worth mentioning that the non-randomized results obtained by the canines were due

to the fact that in the first training trials to detect COVID-19 positive individuals in Mexico, they were low during the first weeks and continuously increased until reaching a score of 10/10, so it was considered that the training procedure was successful. All animals were offered the conditions for optimal behavior and development during training, which included good facilities, stable routine and training with positive reinforcement, equivalent to those recommended by (Fattah & Hamid, 2020).

With respect to physiological needs, a low value of 3/10 points was reached, since they did not have free access to water. This contradicts the Mexican Official Norm NOM-062-ZOO-1999 (SAGARPA, 1999), which states that canine cages must have watering and feeding troughs. In addition, Article 21 of the Federal Animal Health Law (SENASICA, 2012) mentions that owners or holders of domestic animals must provide them with food and water in adequate quantity and quality according to their species and productive stage. This indicates that the canines could have suffered thirst, being affected the animal freedom that refers to this aspect, as referred by (Salas & Manteca, 2016; Temple, 2021).

Finally, regarding the validity of the study, the direct observation application and the use of logbooks has been determined in the literature and high levels of concordance have been found, making it a method with high reliability and feasibility, as demonstrated by (Whitham & Wielebnowski, 2013). Also, there is evidence of the application of direct observation to assess the animal welfare of 45 canines at the reception of private veterinary clinics, using videos and surveys to owners and canine ethologists (Mariti *et al*., 2015). Other researchers evaluated the reliability of the second version Shelter Quality Protocol (SPQ) by calculating interobserver agreement between two independent assessors and demonstrated consistent interobserver agreement in assessing canine animal welfare (Berteselli *et al*., 2019). The execution of the logs should be performed by multidisciplinary professionals, this facilitates the objective assessment of animal-based parameters and environmental variables, since, according to the literature, animal welfare is determined by the balance of these factors (Broom, 2011; Dawkins, 2003; Polgár *et al*., 2019; Van der Harst & Spruijt, 2007). This is reaffirmed by the analysis of the evaluation tools included in the protocol applied in the present project, where the Spearman correlation of 0.06, confirmed that there is an association between both components.

The result obtained for animal welfare by "medium" territory identified in the canines in training, allowed identifying areas of opportunity focused on the improvement of the evaluated components, so that the different specialists in animal welfare and health made individual and group recommendations, avoiding factors that interfere with their performance. The latter was corroborated by observing the learning curve of the animals in spite of the identified stereotypes, the presence of diseases and the deficits found in the cages. The individual and territory animal welfare categories could be improved if the recommendations of the experts are heeded.

CONCLUSION

The methodology for the evaluation of animal welfare applied to canines in scent training determined a reliable and adequate category for the animals evaluated due to the relationship that exists between the variables of the human and animal components, which was confirmed by the application of Spearman's correlation with statistically significant results. It is recommended that the observation and implementation of the logs be carried out by multidisciplinary professionals focused on animal care.

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Conflict of Interest

None of the authors have a financial or personal relationship with other individuals or organizations that could inappropriately influence or bias the content of this article.

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Errata Erratum

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