CVJ
CLUJ VETERINARY JOURNAL

DOI: https://doi.org/10.52331/cvj.v28i2
ISSN: 2066-9399
Cluj Vet J 2023, vol. 28, issue 3
http://clujveterinaryjournal.ro

Societatea Romana Veterinara de Neurologie, Neurochirurgie si Medicina comportamentala
NEUROVET

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Article

Welfare assessment of a dog shelter using the Shelter Quality Protocol

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Abstract: The purpose of this study was to practically apply this protocol in a private shelter in Cluj county, Romania. The welfare of the sheltered dogs was carried out on three levels: the management of the shelter, measures at pen level and the individual evaluation of the dogs. The main problems identified in the studied shelter were: the lack of indoor enclosures to protect the dogs in cold or hot periods; permanently deficient bedding; the diet rich in carbohydrates and low in fats and proteins; showing fear towards the evaluator; lack of a socialization program; the absence of a coherent adoption strategy; unqualified staff in animal behavior and welfare. Applying the shelter quality protocol is useful because it identifies the shelter's critical control points regarding the welfare of the housed dogs. Their prompt remediation will lead to an adequate animal welfare.

Keywords: dog; shelter; protocol; score

Received: 26.10.2023
Accepted: 02.11.2023
Published: 30.12.2023

DOI: 10.52331/cvj.v28i3.54

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1. Introduction

Stray dogs are a huge animal welfare issue in the European Union, and Romania, has one of the largest stray animal populations on the continent, up to 500,000 dogs. This category of animals represents a social and economic problem closely related to the costs of population control programs, as well as zoonotic risks. The major causes of the huge number of stray dogs are: the total or partial lack of sterilization programs for dogs with or without owners, the abandonment of adult dogs (mainly represented by geriatric dogs or those prone to genetic or incurable diseases) and the abandonment of puppies. Of course, we cannot exclude one of the most important factors, the cultural one, often dogs are killed with unprecedented cruelty or simply tied up in the forest and subjected to starvation and water deprivation [1]. Annually in Romania, thousands of medical interventions take place that lead to the euthanasia of stray animals that have been captured and kept for more than 6 months in private or state shelters [1]. According to the World Organization for Animal Health (International Office of Epizootics - OIE), prevention is the most effective method applied to stray dogs, euthanasia, on the other hand, has been proven by studies to be ineffective and unethical [2].

The dog shelter is a space that receives and cares for a certain number of animals, most of them collected from the streets. That's why meeting the needs of the sheltered animals is a rather difficult task that involves exact planning of all activities and special involvement of the staff. In addition to these tasks, physical and behavioral evaluations of the sheltered animals should also be mentioned [3].

The management of a shelter requires many other aspects worth taking into account, such as: obtaining an authorization letter, meeting the minimum conditions stipulated by

law, as well as staff training programs. One thing worth considering is that the shelter is not always the best solution to improve the well-being of the housed animals.

In many countries such as Italy, the euthanasia of stray dogs from shelters is prohibited by law. However, despite many positive ethical aspects, the "no-kill policy" has only prolonged the stay of dogs in state or private shelters, their number growing rapidly along with the public costs regarding the maintenance of these facilities [4]. In Romania, the euthanasia of dogs that have spent more than 14 days in the shelter is allowed according to law 155/2001, being among the few countries in Europe that allows this aspect [5].

The European regulatory framework does not have a standardized measure of minimum requirements for the facilities of dog shelters, so this makes the welfare standards of dogs in these facilities a challenge. However, there are several indicators of welfare that must be appreciated: the management of the shelter, the type of accommodation, the environmental conditions and the possibility of enriching it, the health of the animals, the human-animal relationship, veterinary care [3, 6]. In conclusion, it is vital to have a tool that evaluates the real condition of the dogs in the shelter.The Shelter Quality (SQ) protocol was developed to provide a valid, reliable, and practical tool for assessing shelter dog welfare [7]. The aim of this study was to test the Shelter Quality protocol in a private shelter in Cluj county, Romania.

2. Materials and Methods

The research was carried out in a private dog shelter in Cluj county, Romania. In order to complete this survey, 40 dogs were selected, their number being in accordance with the protocol recommendations. As reported to the shelter's register the animals did not appear with health problems at the beginning of the study.

The Shelter Quality (SQ) protocol [8] is based on the four welfare principles that include twelve criteria defined within the Welfare Quality® protocols of livestock species [9].

In order to make the assessment of animal welfare more efficient, this protocol was divided into two parts: the first part referred to the dogs welfare assessment in the shelter by SQ protocol, and the second part was oriented towards the adaptation of the protocol indicators and the addition of new findings/measurements suitable for the field conditions in Romania.

The information regarding the shelter management was included in the form of a questionnaire and referred to the total population of dogs at the time of the visit, housing conditions, their condition, mortality, morbidity as well as other aspects mentioned in table 1.

The resource-based indicators were evaluated at the level of each individual pen and basically looked at the housing conditions of the dogs: the space for each animal; the presence or absence of indoor and outdoor spaces; the bedding used and its type; the presence of sharp edges in the pen; type and functionality of the watering system; water cleannes; number of animals shivering or huddling; the number of animals barking in the presence of the evaluator; the number of dogs presenting one or more stereotypes (repetitive or compulsive behaviour); the number of animals showing pain; the presence of fecal samples with diarrhea in the pens [10].

Animal-based indicators were represented by: the dog's condition; the number of animals with wounds, swellings; presence of ectoparasites; the presence of lameness and cough (kennel); behavioral indicators [10].

3. Results and discussion

In total 19 pens were evaluated and a total of 40 dogs were individually assessed using the SQ protocol.

Table 1 presents the management (administrative) questionnaire. Within the shelter, the dogs are kept in boxes with outside space that facilitates their movement. No surgical interventions are performed in the shelter, and all therapy protocols are performed in the USAMV Cluj-Napoca Emergency Hospital.

Table 1. Management questionnaire (administrative)

General information about the shelter	
Number of dogs in the shelter the day of the visit	**75**
Number of hospitalised dogs the day of the visit	**0**
Temperature (°C) and humidity (%) the day of the visit	**10°C** **77%**
Housing	
No. single pens: **5**	No. pens housing a pair: **17**
No. pens housing a group of animals (≤ 5): **11**	No. pens housing a group of animals (> 5): **0**
Total no. of pens:	**34**
Exercise	
Dogs are left in a fenced outdoor area:	daily (30 minutes or more) **x**
	weekly
	no/not regular
Dogs are walked on leash by shelter staff or volunteers:	daily
	weekly
	no/not regular **x**
Surgeries / pain control	
Presence of hospital pens:	yes/**no**
Presence of operating procedures for post-surgical monitoring:	yes/**no**
Presence of protocol of analgesia:	yes/**no**
Mortality	
No. euthanasia due to health problems: **3**	No. of deaths (other than euthanasia):**0**
No. euthanasia due to behavioral problems: **0**	Population of dogs in the shelter (average number of animals):**75**
Morbidity	
Costs for clinical treatments (12 months):	**Very high costs with variable amount**
Feeding	
Type of diet: • **dry pellets** • **cooked** • **wet/canned**	Feeding regime: • **once/day** • twice/day • *ad libitum*
Special diets for puppies: **yes**/no	Special diets for hospitalised: yes/**no**
Special diets for geriatrics: **yes**/no	
Notes: One empty pen. Feeding takes place every day around 14:00 - 16:00	
The assessment started at: **11:30**	The assessment ended at: **13:30**

Regarding animal-based idicators at pen level out of 40 evaluated dogs, five (12.5%) exhibited panting on remote inspection, and 32.5% (n=13) barked at the sight of the evaluator. As the temperature dropped, a percentage of 32.5% of the dogs (n=13) showed signs of diarrhea. Seven dogs (17.5%) showed repetitive behavior, which is often associated with pathological conditions, while 12.5% (n=5) of subjects had compulsive behavior.

The assessment of dog welfare was based on measurements of physiological parameters related to stress or dog behaviors that were previously associated with stressful situations such as: panting, paw-lifting, repeated licking and avoidance behaviors. Even so, identifying poor welfare indicators only partially addresses the concept of animal welfare [11].

A small proportion of dogs may exhibit aggressive/compulsive behavior in response to the stress of the shelter environment. Other dogs begin to perform behaviors of a repetitive nature, the frequency of barking and vocalizations increases, begin to develop destructive behavior on surrounding materials, and start to urinate or defecate more frequently in the box [12].

Diarrhea is a common phenomenon present in kennels and can evolve from occasional pathologies with a low level of risk to outbreaks with high mortality. Along with acute diarrhea, chronic diarrhea is equally common in shelters. Many cases are stress-related, but if the animal is clinically healthy and gaining weight, the diarrhea may resolve on its own at the time of adoption. Weight loss is often associated with persistent severe diarrhea and the animal's inability to gain weight. The condition can be complicated with other clinical signs such as vomiting, and it is therefore recommended to perform additional diagnostic tests [13].

Intraspecific communication in canids is achieved by barking, growling, howling and whining [14]. Barking is the most used acoustic method of communication between dogs, and a high-pitched bark indicates fear, agitation, as opposed to a low-pitched bark that indicates aggression [15, 16].

Stereotypy is a problem often reported in animals in captivity and is nothing more than repetitive behavioral sequences without any defined functionality [17]. Another study also included behavioral disorders in this category: repeated jumping, self-mutilation, spinning in a circle or insistent licking of a body region [18].

Shelters are usually designed in such a way that hygiene can be maintained as well as housing multiple dogs in a limited space [19]. In our study, the housing spaces (n=19) mostly corresponded to the animal requirements (table 2).

Almost half of the pens (n=9, 47.37%) had sharp edges inside which could lead to injury to the animals. There must be no sharp point or rough material that could injure the dogs [20]. Drinking water was provided manually in bowls or buckets by the shelter staff. Regarding the water quality, only 10.53% were not considered safe (they had sharp edges). The water was clean in 89.47% of the examined pens, the rest showing traces of mud inside.

At the individual evaluation, most dogs were friendly, playful, curious, relaxed and self-assured, wanting attention from the assessor. Barnard et al 2014 included the term "playful" in the emotional state profile of dogs as an indicator of a positive emotional state. At the same time, it should be noted that a decrease in the negative behavior of people towards dogs does not necessarily lead to a positive state of the dog [21]. Negative states can prevent animals from playing, so the willingness to play cannot be considered as a definitive parameter denoting good animal welfare [22].

Table 2. **Resources-based indicators at pen level**

Indicators			No. of pens
Housing	inside	**yes**/no	19
	outside	yes/no	0
Housing type	kennel	**yes**/no	19
	basket	yes/no	0
	wooden pellets	yes/no	0
Bedding	adequate	**yes**/no	19
	inadequate	yes/no	0
	absent	yes/no	0
Sharp edges		-	9
Type of drinkers	bowl/bucket	**yes**/no	19
	others	yes/no	0
Functioning		**yes**	19
		no	0
Safe		**yes**	17
		no	2
Water quality	dirty		2
	clean		17

The age of the dogs was established through a questionnaire addressed to the shelter staff. Thus, most of the dogs were adults aged between 1 and 6 years (85%, n= 34), 10% were geriatric, aged over 6 years and 5% were young. In our research, the body condition score revealed that 95% of the subjects had an adequate body condition and only two animals showed obesity.

The dogs showed no signs of lameness, ectoparasites or swelling. A number of 16 dogs (40%) were classified as "dirty", and 5% of them had injuries on their bodies following an altercation with other dogs. The presence of cough was reported for 7.5% of the dogs and alopecia conditions were obbserved in a small proportions (10%, n=4). These results are presented in table 3.

A dirty and wet body surface negatively affects the well-being of dogs. In our study, the condition of alopecia was associated with the quality and type of material used to cover the area around the pen. The same observation was described in other study [10].

During the fear test, characterized by the dogs' indifference towards the assessor or by the presence of fear towards him, 12.5% of the dogs showed fear or signs of aggression towards the evaluator. In a study by Gácsi et al 2001, it was shown that animals housed in shelters for a longer period of time are able to attach to new people in a relatively short time [23]. The difference from 12.5% to 100% is represented by the absence of any sign of fear or aggression.

All shelters must provide decent conditions with sufficient space for the animals to walk freely and stand up, turn around, lie down and as far as possible avoid harmful stimuli from the environment. Shelters must have separate rest areas, food in sufficient quantity and quality, acceptable water intake, sufficient and regular exercise for dogs, and space for excretion [24].

Table 3. Individual assessment of dogs

Indicators		No. animals	%
Age category	young	2	5
	adult	34	85
	geriatric	4	10
Body condition	adequate	38	95
	too thin	0	0
	too heavy	2	5
Body cleanliness	clean	24	60
	dirty/wet	16	40
Skin tissue condition	wounds	2	5
	alopecia	4	10
	swelling	0	0
	ectoparasites	0	0
Lameness	present	0	0
	absent	40	100
Cough	yes	3	7.5
	no	37	92.5
Fear test	no signs	35	87.5
	fear/aggression	5	12.5

Conclusions. Applying the shelter quality protocol is useful because it identifies the shelter's critical control points regarding the welfare of the housed dogs. Their prompt remediation will lead to an adequate animal welfare. For a better understanding of the well-being of shelter dogs, a coherent strategy is needed at the national level to promote animal protection policies, but also to finance these programs of major importance.

Author Contributions: Conceptualization, A.B.P., S.P.; methodology, A.B.P., S.P., A.S.C.; software, S.P.; validation, A.B.P., S.P. and A.S.C.; formal analysis, S.P.; investigation, A.B.P. and A.S.C.; writing—original draft preparation, A.B.P. and S.P.; writing—review and editing, A.B.P., S.P. and A.S.C.; supervision, S.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

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Article

Prevalence of bovine schistosomosis in fogera district

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Abstract: A cross-sectional study was conducted from February 2019 to April 2020 in Fogera district to determine the prevalence of *bovine Schistosomosis.* Schistosomosis in cattle is one of the well known parasitic diseases locally referred to as ''Yeweha till'' meaning water-borne worm infection. From the total of 430 cattle examined using coproscopical examination in the field survey 27.9% (n= 120) were found to be positive for *schistosoma bovis.* Of the total 80 cattle examined in the abattoir, 12.5% (n=10) were positive for shistosoma adult female and male worms during postmortem finding but only 6.25% (n=5) of them were found positive schistosoma eggs using coproscopical examination. The prevalence of schistosomosis was found also higher in local cattle (23.49%) than that of Fogera (6.04%) and cross-bred cattle (Local X Fogera) (4.42%). The prevalence of the disease was higher in age group of cattle above 5 years of age (15.8%) than that of age groups between 1.5 to 5 years (10%) and below 1.5 years (2.09%). The prevalence of bovine Schistosomosis in female cattle (14.90 %) was found greater than that of male (13.02%). The present study was carried out on bovine schistosomosis in Fogera district has the objectives of to provide detailed information of cattle Schistosomosis, to determine the prevalence of bovine schistosomosis according to sex, age and breed of cattle and to determine the prevalence of bovine schistosomosis in slaughtered animals.

Keywords: Prevalence, *Schistosoma bovis*, Fogera, Ethiopia

Received: 27.10.2023
Accepted: 27.11.2023
Published: 30.12.2023

DOI: 10.52331/cvj.v28i3.55

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1. Introduction

Schistosomosis (blood fluke disease or bilharzosis) is an infection due to the genus schistsoma.

Although this parasite occur in many tropical and subtropical areas, the disease is important in livestock mainly in Easter Asia, Africa and India [1, 2]. Adult Schistosomes are obligate parasite of the blood vascular system of vertebrates. Schistosomes are dioecious (unisexual) worms, which is an exception among the trematodes [3, 4].

Visceral Schistosomes mature in the hepatic portal veins, mate and migrate to the mesenteric veins where egg production starts [3]. The female in the mesenteric vein insert her tail in to the venule. The eggs penetrate the venule endothelium aided by their spines and by proteolytic enzymes secreted by the unhatched miracidia [5, 7]. Egg lay by the female worm penetrate the wall of the veins and migrate to the intestinal lumen or the nasal cavity. (*S.nasale*) of the host are retained inside the body and it is the retained eggs and their products that responsible for most morbidity from Schistosomosis [8].

In addition to the high prevalence rate and outbreak of the disease, it has an economic impact like production losses due to *S.bovis* result from mortality, delayed growth, partial liver condemnation and poor future reproduction performance and sub clinical infections cause significant losses due to long term effects on animal growth and productive capacity or milk yield, draft power and increase susceptibility to other parasitic or bacterial disease [9, 10]. In humans' economic losses in terms of working hours has been shown [11].

A form of cutanous larva migrants often called "swimmers itch" (cercarial dermatitis) occurs in man and Schistosomes which have a limited migration in human skin [7, 12]. Migratory water fowl frequently harber schistosomes (blood flukes) in their blood vasculature. These schistosomes produce eggs that pass in the bird's feces to the water environment. The eggs hatch, producing miracidia, which turn penetrate a aquatic snails with the snail, the miracidium undergo asexual reproduction and produce thousands of cercariae these cercariae exit the snail hope fully to penetrate the definitive host, the migratory water fowl [6, 12]. Humans serve as incidental hosts for these avian schistosomes. During the swimmer months, people swim or wade in the lakes, ponds, rivers and even ocean waters frequented by the wild birds.

In Ethiopia, reports on animal schistosomes are very scanty and until recently it has been considered as an occasional finding in slaughter house and postmortem examinations [13]. It has been reported that *S.bovis* is the only species reported with localized distribution in ten out of fourteen administrative regions in the country [14, 15]. Detailed information on prevalence and intensity of infection of *S.bovis* in Ethiopia and various factors, which influence the host parasite relationship, are generally lacking. The present study was carried out on bovine schistosomosis in Fogera district with the following objectives;

- To provide detailed information of cattle Schistosomosis.
- To determine the prevalence of bovine schistosomosis according to sex, age and breed of cattle.
- To determine the prevalence of bovine schistosomosis in slaughtered animals in abattoirs.

2. Materials and methods

2.1 The Study Area

The study was conducted from February 2019 to April 2020 in Fogera area. The study area is located 1800 to 2000 m a.s.l with elevation of the area is feature predominantly moderate Woynadega temperate high land Dega climates. The land scope is marked by the present of Lake Tana in adjacent side which drains of water shed about 3000km^2 and surrounded by High Mountain with plains slopping patches of land near the shore line. The area has a summer rain fall with mean annual rain fall and mean annual temperature of 1600 mm and 20^{0}C respectively.

The rich agricultural land of the area supports a large livestock population, water and grazing pastures being abundant for months of the year, but due to periodical flooding during rainy season, cattle have to move to the hill side.

2.2 Animals and management

The dominant cattle breed in this region is local indigenous Fogera cattle. In the study area both traditional and modern (semi-intensive) livestock farming are practiced. In the traditional management system animals are often kept out-doors and grazed all day near the vicinity of the Lake Tana. These grazing areas

are potential sources of schistosome infection due to the frequent contact of animals to the water bodies. In the semi-intensive management system, cattle are kept in-doors and partly out-door. While indoors, they are supplemented with adequate qualities of feed and clean water. Their management of outdoors is similar to the traditional extensive farming type.

2.3 Study population

The sampling units of the study were local and cross breed cattle. A total of 430 cattle were considered in this study for coproscopical examination and were registered according to their breed, sex and age. The age of the study animals was determined by dental eruption formula which involves counting number of permanent incisors [3, 6, 16]. The group of age of animals are Group I:0≤X< 1.5 years, Group II: 1.5≤X ≤5 years and Group III >5 years.

2.4 Study Design

A cross sectional study was conducted to determine the prevalence of *bovine Schistosomosis*. The desired sample size was calculated using the formula given by Thrusfied [17]. 263 cattle were selected using random systematic sampling method to estimated prevalence of the disease. However, due to low number of positive animals at the beginning of the study, the sample size was increased to 430 cattle.

2.5 Study Methodology

2.5.1 Coproscopical Examination

The purpose of coproscopical examination was to determine the presence or absence of schistosoma egg in the feces. Fresh fecal samples were directly collected form rectum of 516 animals and preserved with 10% formalin in a universal bottle to prevent hatching of miracidia then after sedimentation procedure was done till the sediment of the fecal sample become clear. Following these all procedures the prepared sample observed under low power microscope in the laboratory [18].

2.5.2 Post -mortem Examination

The fecal sample were collected during antemortem examination with universal bottles and labeled to examine the same animals during post mortem time. At post mortem examination the liver, portal vein, mesenteric vein where observed and incised to find the adult schistosomes and also the whole root of intestine were examined superficially to appreciate the presence of lesions and dead parasites at the junction of the tip of the vein and the wall, serosa and subserosa of the intestine [7].

2.6. Statistical Analysis

The sample size of the field survey were classified in to three parameters breed, sex, and age then the data was managed by using SPSS (17 version) program and the prevalence between the parameters was analyzed using chi-square (χ^2) test and binary logistic analysis. While the comparison between post mortem finding and fecal examination was handled by using simple prevalence rate.

3. Results

3.1. Overall Prevalence

From the total of 430 cattle examined using coproscopical examination in the field survey 27.9% (n= 120) were found to be positive for *schistosoma bovis.* Of the total 80 cattle examined in the abattoir, 12.5% (n=10) were positive for shistosoma adult female and male worms during postmortem finding but only 6.25% (n=5) of them were found positive schistosoma eggs using coproscopical examination.

Table 1 Infection prevalence of bovine Schistosomosis in different breeds, sex and age groups of Fogera district.

	Animals	Total number of Animals examined	Number of positive Animals	Prevalence (%)
Breed	Local	211	75	17.44
	Fogera	145	26	6.04
	Cross	74	19	4.42
Age group	I	52	9	2.09
	II	154	43	10
	III	224	68	15.8
Sex	Male	182	56	13.02
	Female	248	64	14.9
	Total	**430**	**120**	**27.9**

The prevalence of schistosomosis was found also higher in local cattle (23.49%) than that of Fogera (6.04%) and cross-bred cattle (Local X Fogera) (4.42%). The prevalence of the disease was higher in age group of cattle above 5 years of age (15.8%) than that of age groups between 1.5 to 5 years (10%) and below 1.5 years (2.09%). The prevalence of bovine Schistosomosis in female cattle (14.90 %) was found greater than that of male (13.02%).was found greater than that of male (13.02%).

3.2. Abattoir survey

From the total of 80 male cattle slaughtered at Wereta Municipal Abattoir, the purpose of this survey was to compare the prevalence difference between post mortem finding and coproscopic examination. During post mortem *Schistosoma bovis* was found in the mesenteric, portal veins were examined and incised. 12.5% (n= 10) were found to be positive but only 6.25% (n= 5) of the 80 cattle were positive in coproscopic examination.

4. Discussion

Schistosomosis in cattle is one of the well known parasitic diseases locally referred to as ''Yeweha till'' meaning water-borne worm infection. As well as most of the slaughtering practice took place in backyard slaughtering system so that the dumping of the stomach and intestinal contents, including the blood and

washed material nearby water bodies (rivers, irrigation canals, ponds e.t.c) can create an easy access to the snail intermediate to the egg of schistosoma from such materials. This practice together with contamination of water bodies with manure and defecates, as in case in some areas where there is poor watering facilities, could highly contribute to the spread of the disease in surrounding at large, in addition to the above problem the koladiba municipal abattoir itself has got hygienic problem that will contribute for the occurrence of the disease.

The overall prevalence of *S .bovis* infection 27.9% in the study area was found lower than the previous studies in which prevalence rate around Bahir Dar were 33.8% [19] and 34% [20]. The lower prevalence of schistosomosis recorded in this study may be due to the fact that trematodes are intermittent egg layers so that the chance of detecting eggs by fecal examination may be minimal. In addition to this not all schistosoma eggs are excreted in the faeces, many of them may be trapped tissue [21]. Moreover, the number of adult parasite established in the mesenteric veins and the stages of infection may determine fecal egg output thus, postmortem examination is more specific to detected schistosoma infection than coproscopical examination.

The prevalence of *Bovine Schistosomosis* was found higher in local cattle (17.44%) that of Fogera (6.04%) and cross-bred cattle (Local X Fogera) (4.42%). This finding is not in line with other reports in which the prevalence of bovine schistosomosis higher in crosses cattle than local cattle [15, 20]. The reason for this difference in prevalence rate between breeds may be due to the cross breeds are mostly indoored for fatting or dairy purpose by supplementing good feed and clean water so that they cannot get access to the miracidium; while the local once are mostly released extensively to graze freely and then they will get acquired immunity through long time exposure the above statement also related with research paper that was done in Sudan which suggests that Sudanese cattle were apparently acquired immunity to *S. bovis* as a result of repeated exposure [22].

The prevalence of schistosomosis in this study which was higher in age group of cattle greater than 5 years than that of 1.5 to 5 years and below 1.5 years of age. This finding is not in line with other reports [15, 20] around Bahir Dar .The lower prevalence (2.09%) in age group I cattle may be due to the fact that most calves are kept indoors hence have low chance to cercariae exposure.

The higher prevalence of schistosomosis in this study in female cattle than male cattle is not in line with the previous study [15] in Bahir Dar .The reason for the higher prevalence in female than that of male in cattle is that cows were in stress of lactation and pregnancy even though the two sexes are equally exposed to the disease because they graze at the same time in the same place [23].

In the postmortem examination of slaughtered animals to determine the adult Schistosome worms from portal veins were examined and incised. 12.5% (n= 10) were found to be positive but only 6.25% (n= 5) of the 80 cattle were positive in coproscopic examination. The reason for this difference in prevalence of schistosomosis between abattoir survey and coproscopical examination may be due the fact that trematodes are intermittent egg layers which are dependent on the age of animal [3].

5. Conclusion and recommendations

Bovine Schistosomosis is one of the endemic disease condition in the study area that deserve serious attention in the future even though there has been little recognition of its veterinary significance, cattle Schistosomosis does cause significant loss through out the world. This is due to the nature of the disease, which usually occurs at sub clinical level with long term effect on animal growth and productivity and increase susceptibility to other parasitic or bacterial diseases. It is, therefore, important to obtain more information on natural schistosomes' infection in cattle in general, and on the evaluation of the host–parasite relationship

under condition of challenge in particular. Despite the fact that there was no significant difference between male and female cattle, it should be allowed to graze the cattle at the same time and the same place to avoid transmission in between because females give a high economic value as compared to males.

Based on this study, the following recommendations are forwarded.

- ✓ Schistosomosis should be taken in to consideration as a one of the major limiting factor to livestock productivity in Fogera District; hence any Endeavour towards animal disease control strategy must include it in the priority list.
- ✓ Further detailed studies are needed to gather a rich database both on the parasite and its vector, which will be useful to envisage a cost effective and sound Schistosomosis control measure in the area.
- ✓ Farmers should get educated about the transmission of the disease at least to tell them not to let their cattle freely in swampy area and supply dry feeds sometimes.
- ✓ Cross breeds should be kept indoors and supplying of clean water should be performed to prevent infection as they are highly sensitive to the disease. And also different ages and breed groups should not graze together.
- ✓ Available means in snail control and disease monitoring could be implemented as a short term activity. Indigenous knowledge deserves investigation in this regard. The native Ethiopian plant phytoplancca dodecandora, locally known as Endod which is considered as potent molluscicide for the control of human Schistosomosis, could also be effectively used against intermediate host of *S. bovis.*

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CVJ CLUJ VETERINARY JOURNAL

Article

Analysis of reproductive hormones and morphometric attributes in Thamankaduwa White male cattle

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Abstract: Despite a few attempts in exploring genetic variability, management systems, and morphometric descriptions, Thamankaduwa White cattle in Sri Lanka have not been subjected to any evaluation of their endocrinological distinctiveness, especially related to reproduction. The main aims of the present study were to: (1) investigate the dynamics of circulating insulin-like peptide 3 (INSL3) and testosterone in Thamankaduwa bulls during development, and (2) assess the association among INSL3, testosterone and selected morphometric parameters, namely, body weight, height at withers, body length, chest girth of Thamankaduwa White cattle in Sri Lanka. The blood samples were collected from male animals (n = 41) under three age categories; 3-6 months (Group I; n = 12), 6-12 months (Group II; n = 14) and > 12 months (Group III; n = 15), along with their morphometric measurements. Serum INSL3 and extracted testosterone concentrations were measured by using a competitive ELISA. The detection ranges of INSL3 and testosterone assay were 0.078-80 ng/mL and 0.04-40 ng/mL, respectively. Intra- and inter-assay coefficient of variations of INSL3 and testosterone assays were 6.9% (n = 6) and 16.4% (n = 6), and 12.5% (n = 3) and 11.9% (n = 4), respectively. Serum INSL3 and testosterone concentrations ranged between 1.44 - 19.85 ng/mL, and 0.003 ng/mL - 2.81 ng/mL, respectively. The mean serum INSL3 concentrations did not differ between Gr. I and II ($p > 0.05$) but were significantly high ($p < 0.05$) in Gr. III. There was a significant association ($R^2 = 0.65$; $p < 0.05$) between serum INSL3 and testosterone concentrations in Thmankaduwa White males. No strong associations were observed among hormones and the morphometric parameters tested. In conclusion, the dynamics of INSL3 and testosterone concentrations were compatible and correlated with each other in Thamankaduwa White male cattle.

Keywords: ELISA, INSL3, morphometric attributes, native white cattle, testosterone

1. Introduction

Sri Lankan native white cattle is mostly described as one of the local zebu cattle types which originated from a cross between local Sri Lankan cattle with imported Indian cattle [1]. This is also known as the Thamankaduwa White cattle which is specifically characterized by its white coat, black color tail switch, and hooves. This cattle type is predominately available in the Eastern, South Eastern, and North Central regions of Sri Lanka [2,3]. Even though the genetic resources, management practices, morphometric analysis and milk attributes of Thamankaduwa White were little addressed [3- 7], the reproduction physiology of these animals has not been investigated to sufficient depth yet.

Insulin-like peptide 3 (INSL3), along with testosterone, is a predominate secretory product of Leydig cells of mature testes as well as in fetuses of all mammals [8]. INSL3 concentrations have been discovered in many mammals including humans [9-12], beef cattle [13-15], Norwegian Red bulls [16], dogs [17], goats [18-21], and sheep [22]. Furthermore, [23] reported that the set of body and reproductive tract morphometry was beneficial in assessing the growth of young bulls.

Lack of investigations on endocrinological changes in Thamankaduwa White cattle left with no comparison of the reproductive efficiency of this native cattle type with other cattle breeds. Thus, the existing background creates a research gap in identification of

Received: 24.11.2023
Accepted: 23.12.2023
Published: 30.12.2023

DOI: 10.52331/cvj.v28i3.59

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endocrine changes and assess the associated morphometric changes to support the breeding programs involved in this important native cattle type in Sri Lanka. The present study aimed to: (1) To measure the circulating INSL3 and testosterone concentrations during development in Thamankaduwa White male cattle (2) To examine the relationship between each hormone concentration and morphometric parameters in Thamankaduwa White male cattle in Sri Lanka.

2. Materials and Methods

2.1. Animals, blood sampling and body measurements

Thamankaduwa White male animals (n = 41) raised in six smallholder semi-intensively managed farms in Chenkalady veterinary range (Latitude: 7° 46' 59.99" N and Longitude: 81° 35' 59.99" E), Batticaloa District, Eastern Province, Sri Lanka were used to this study. Blood samples were drawn from healthy animals of three age groups (Group I: 3–6 months, n = 12; Group II: 6-12 months, n = 14; Group III: more than 12 months, n = 15), and all were apparently normal. Samples were collected from jugular vein puncture in to plane vacutainer tubes and were centrifuged at 2000 × g for 20 minutes just after brought them into the laboratory. The Serum was separated and stored in microcentrifuge tubes at -20 °C until the hormone assays. Morphometric parameters were simultaneously measured during the collection of blood samples. The chest girth (CG), height at withers (HW), and body length (BL) were measured by using a flexible measuring tape, while the body weight (BW) was calculated using the weigh band (Dalton Supplies Ltd.) according to the obtained chest girth. The Ethical clearance for the study was obtained from the Research Ethics Committee, Faculty of Agriculture, University of Peradeniya, Sri Lanka (ECC/2023/R/062).

2.2. Hormone assays

2.2.1. INSL3 assay

Serum INSL3 was measured using a homologous bovine competitive enzyme immunoassay (EIA) as described by [13] for cattle and [21] for goats, with modifications. Eight-well strips (SPL Life Sciences, South Korea) were coated with 100 µL/well by using anti-mouse IgG (5 µg/mL in 0.05 M sodium bicarbonate; pH 9.7; KPL Lab Inc.) and kept for 2 hours at room temperature. The wells were then drained and washed three times by using 200 µL/well of washing saline (Sodium Chloride). Non-specific binding sites were blocked by using assay buffer 200 µL/well (AB I; 0.01 M Phosphate buffer which contained 0.15 M sodium chloride, 0.25 % skim milk and ProClin 950 (Sigma-Aldrich); pH 7.4) and kept overnight at 4 °C for blocking the wells which were drained immediately before the assay. Then, 50 µL of each standard or serum sample and 50 µL of anti-bovine INSL3 (1: 4000; a generous gift from Prof. E. E. Bullesbach, Medical University of South Carolina, USA) were dispensed into each well and kept two hours for incubation at room temperature followed by 50 µL of biotinylated human INSL3 peptide (1 ng/mL in AB I, 1: 5000; a generous gift from Prof. N. Kawate, Osaka Metropolitan University, Japan) dispensing and allowing again one hour for incubation. Subsequently, the wells were drained and washed 3 times with 200 µL/well of saline (0.15 M sodium chloride containing 0.05 % Tween 20). Then, 100 µL of HRP-labeled streptavidin (100 ng/mL in AB I, 1: 5000; KPL Lab Inc.) was added to each well and kept for 30 min at room temperature for incubation. After 30 min the wells were drained and washed three times using saline and kept another 30 min with 100 µL of substrate solution containing the 3,3′,5,5′-Tetramethylbenzidine (TMB; Sigma-Aldrich). Finally, the reaction was stopped by adding 50 µL of 2 M sulfuric acid, and the optical density (OD) was measured at 450 nm with a 630 nm reference using a microplate reader (UT-2100C, MRC, Israel).

The minimum detection limit of the assay was 0.078 ng/mL and the sensitivity range was 0.078 - 80 ng/mL. Intra and Inter-assay coefficient of variations were 6.9 % (n = 6), and 16.4 % (n = 6), respectively.

2.2.2. Testosterone extraction

Testosterone extraction prior to the testosterone assay was performed according to the previously described protocol by [17], with modifications. Briefly, various concentrations of testosterone standards (0.01-

40 ng/mL) were diluted with the assay buffer (AB II; 0.01 M Phosphate buffer containing 0.15 M sodium chloride, 0.1 % BSA and 0.02 % Proclin 950; pH 7.4). Native white cattle serum samples (250 µL/ sample) were dispensed into glass tubes and mixed with 2.5 mL of diethyl ether by vortexing for 5 min followed by centrifugation at 3500 rpm for 5 min to separate the upper ether phase from the lower water phase and kept at -18 °C allowing the lower water phase to freeze. Then, the upper phase was separated into another glass tube, and allowed to evaporate using a heat block at 40 °C. After that, the dried extracts were dissolved in 250 µL of AB II by vigorous vortexing. Simultaneously, the standards were also extracted.

2.2.3. Testosterone assay

Testosterone concentrations were measured in the same set of samples using the method described by [13, 17] previously. In brief, previously coated wells with 100 µL/well of anti-rabbit IgG polyclonal antibody (2µg/mL in 0.05 M Sodium bicarbonate; pH 9.7; KPL Lab Inc.) and blocked by AB II, were drained just before the assay, and 50 µL of extracted testosterone standards or extracted samples, 50 µL of HRP-labeled testosterone (1: 1600; Cosmo Bio Co., Ltd., Japan) and 50 µL of anti-testosterone rabbit polyclonal antibody (1: 1500; Cosmo Bio Co., Ltd., Japan) were added and kept two hours for incubation. Then, the wells were drained and washed three times by using washing saline 200 µL/well following the step of adding 100 µL/well substrate containing TMB and kept for another 30 min. The reaction was stopped by adding 50 µL of 2 M sulfuric per each well and the optical density was measured as similarly mentioned in the INSL3 assay.

The minimum detection of the assay was 0.04 ng/mL and the detection was reliable between 0.04 - 40 ng/mL. Intra and Inter-assay coefficient of variation and percentage recovery were 12.5 % (n = 3), 11.9 % (n = 4), and 91.8 % (n = 2), respectively.

2.3. Statistical Analysis

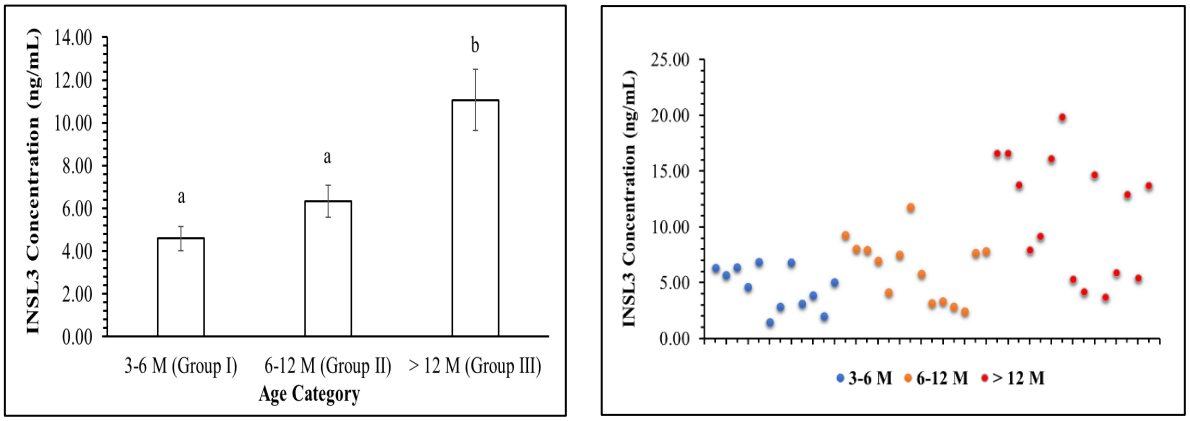
The changes in individual and mean INSL3 and testosterone hormone concentrations with age were assessed. Differences in mean INSL3 and testosterone concentrations among age groups (age in months) were compared using pairwise comparisons of the generalized linear models (GZLM; SPSS version 25.0, IBM Corporation, Somers, NY, USA) procedure by the least significant difference (LSD) post hoc test. Best regression curves among hormone concentrations (INSL3 and testosterone), and morphometric parameters were estimated by using the curve estimation procedure (SPSS version 25.0, IBM Corporation, Somers, NY, USA).

3. Results

3.1. Serum concentrations of INSL3 and testosterone in Thamankaduwa White male cattle

The values obtained for intra- and inter-assay CV and percentage recovery were within the acceptable range for both EIAs used during the present study. INSL3 standards had uniform inhibition at the same concentrations in the sensitivity range and the B/B0 values for serially diluted samples were parallel to the standards [13].

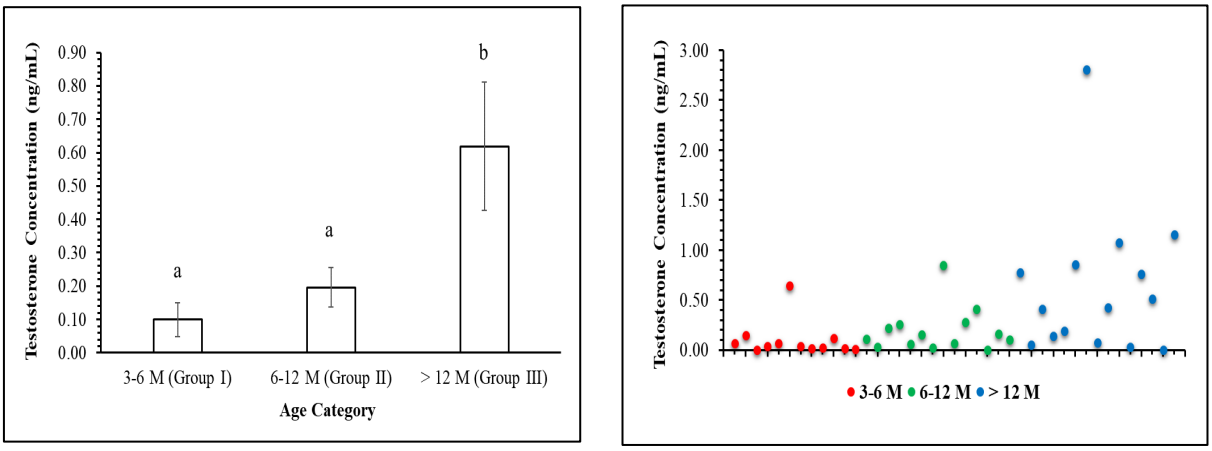
The serum INSL3 concentrations during the development of Thamankaduwa White cattle ranged between 1.44 to 19.85 ng/mL. The mean INSL3 concentration increased from the age group I (4.58 ± 0.55 ng/mL; n = 12) to age group II (6.34 ± 0.75 ng/mL; n = 14) ($p > 0.05$) with an increment factor of 1.4, and from age group II to age group III (11.06 ± 1.43 ng/mL; n = 15) ($p < 0.05$) with an increment factor of 1.7 (Figure 1a). The highest individual concentration of INSL3 (19.85 ng/mL) was observed in group III whereas the lowest (1.44 ng/mL) was observed in group I (Figure 1b).



(**a**) (**b**)

Figure 1. (a) Mean serum INSL3 concentration (mean ± SEM); (b) Individual INSL3 dynamics among age group I (3-6 M; n = 12), II (6-12 M; n = 14) and III (> 12M; n = 15) of native white cattle. [a-b] Mean with different superscripts significant at $p < 0.05$

Serum testosterone concentrations also followed the same pattern of dynamics of INSL3 concentrations (Figure 2A). The mean testosterone concentration was increased from age group I (0.10 ± 0.05 ng/mL) to age group II (0.20 ± 0.06 ng/mL) but it was not statistically significant. However, it was increased ($p < 0.05$) from age group II to III (0.62 ± 0.19 ng/mL). The highest individual concentration of testosterone (2.81 ng/mL) was observed in group III whereas the lowest (0.04 ng/mL) was observed in group I (Figure 2B).



(**a**) (**b**)

Figure 2. (a) Mean serum testosterone concentration (mean ± SEM); (b) Individual testosterone dynamics among age group I (3-6 M; n = 12), II (6-12 M; n = 14) and III (> 12M; n = 15) of native white cattle. [a-b] Mean with different superscripts significant at $p < 0.05$

3.2. Regression analyses among INSL3, testosterone and morphometric measurements

In the Thanakaduwa White male animals, the R^2 value of the of the best regression curve was 0.65 (n = 41, $p < 0.05$; Figure 3).

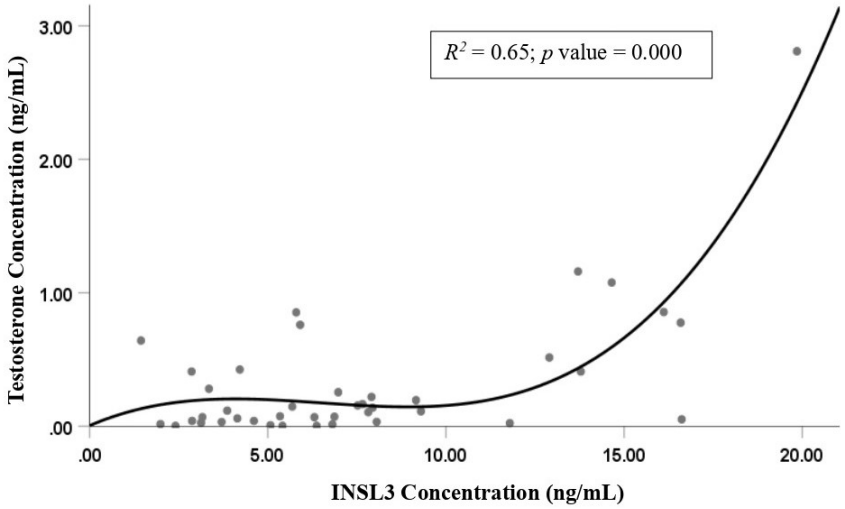


Figure 3. Best regression curves between serum concentrations of insulin-like peptide 3 (INSL3) and testosterone in native white cattle bulls during development (0 to > 12 months of age; n = 41)

Despite the weak associations, significant relationships were observed between the serum INSL3 concentration and BW, CG, and BL ($p < 0.05$) except with HW ($p > 0.05$) (Table 1). Similarly, there were weak associations noticed between the serum testosterone level and BW, CG, HW, and BL ($p < 0.05$; Table 2). However, the body length exhibited the highest significant association with INSL3 among all the tested characteristics, while the chest girth showed the highest significant association with testosterone.

Table 1. Estimated R^2 values and p values of best regression curves between serum INSL3 concentrations and morphometric characteristics of native white cattle bulls in Sri Lanka (*$p < 0.05$; n = 41)

	Body weight	Height at withers	Chest girth	Body length
R^2 value	0.193*	0.183	0.213*	0.321*
p value	0.045	0.055	0.030	0.002

Table 2. Estimated R^2 values and p values of best regression curves between serum testosterone concentrations and morphometric characteristics of native white cattle bulls in Sri Lanka (*$p < 0.05$; n = 41)

	Body weight	Height at withers	Chest girth	Body length
R^2 value	0.250*	0.245*	0.259*	0.236*
p value	0.013	0.015	0.011	0.018

4. Discussion

In the present study, we report to the best of our knowledge, the first measurement of circulating INSL3 and testosterone in Thamankaduwa White cattle and the association between those hormones and morphometric measurements. [21] suggested that the measurement of both INSL3 and testosterone in the same animal may provide an added benefit in assessing Leydig cell function due to its differential patterns of regulations. [13] found that there was an effect of age on INSL3 concentrations in prepubertal Japanese black beef bulls. Those findings further revealed that the plasma INSL3 concentration did not differ significantly from prepubertal (3 to 6 months) to early pubertal age (6 to 12 months) followed by a significant elevation from early to late (12 to 18 months) and late to post-pubertal age (18 to 22 months). Additionally, [16] reported that, even though the mean serum INSL3 concentrations didn't significantly differ over age

categories 2-3, 4-5, 6-7, 8-10, and 11-13 months, respectively, there was an increasing trend over time where the highest concentration was observed at 8-10 months age category in Norwegian Red bulls. Hence, the present findings of INSL3 concentrations of Thamankaduwa White cattle type in Sri Lanka were closely aligned with the previous findings of Japanese black beef bulls whereas it was more or less similar to the findings of Norwegian Red bulls. Furthermore, the INSL3 dynamics followed similar trends as found in the present study in male sheep [22], male Saanen goats [24], Jamnapari X Local crossbred goats [21], and Kottukachchiya crossbred goats [25] during development. However, the serum INSL3 concentrations were very high just after the birth of humans and sharply declined within a few months thereafter and one year of age [26], and continued until 10 years followed by a sharp increment during puberty [9,12]. Even in rats, the serum INSL3 concentrations were in minor quantities during 2 days before and after birth which continued until 10 days after birth followed an increment until puberty [27,28]. However, the decrement trends of serum INSL3 concentrations between infancy to puberty seemed to be absent in Thamankaduwa White cattle males. This is in strong agreement with the previous findings of Japanese black beef bulls as well [13].

Serum testosterone concentrations also followed the same pattern of dynamics as INSL3 concentrations during the present study. There was a marked increment of testosterone concentration of Japanese black beef bulls from prepubertal (3-6 months) to early pubertal age (6-12 months) though it wasn't observed significant increment from early to late phase (12-18 months) [13]. Even though there was no evidence on the age at puberty of Thamankaduwa White bulls, [29,30] reported that the average age at puberty of Bos indicus beef bulls ranged from 16-18 months. In Brahman bulls, the serum testosterone level increment was observed between 12-14 months [23]. The increment of serum concentrations of both INSL3 and testosterone during puberty was suggested due to the HPG axis triggering during this particular age in mammals [8,12]. Hence, the present marked increment of INSL3 and testosterone concentrations from age group II to III in Thamankaduwa White cattle could be suggested due to puberty during this age. Furthermore, the testosterone of Saanen bucks did not differ in all those three age groups as considered in the present study, nevertheless, a significant increment was observed in Kottukachchiya crossbred bucks from below 06 months age category to 6-12 months age category [24,25]. [21] observed a significant drop in serum testosterone concentration on the 23rd week after birth and a three-fold increment on the 28th week after the birth of Jamnapari X Local crossbred goats. There was no difference from the 3-6 months phase to the 6-12 months phase. However, a remarkable elevation from the 6-12 months phase to the 12-24 months phase in male sheep [22]. Thus, the present findings for serum testosterone dynamics in Thamankaduwa White cattle in Sri Lanka were not in agreement with those findings recorded for Japanese black beef bulls and more or less similar to the findings of Saanen and Kottukachchiya crossbred buck. Nevertheless, the findings were comparable with those observed in male sheep during development. However, the minor concentrations of testosterone detected, compared to the serum INSL3 concentrations in Thamankaduwa White cattle were comparable to previous studies reported for the livestock species during development [18,22,24,25], except Japanese black beef bulls [13].

The R^2 value of the association between INSL3 and testosterone hormone concentrations in Thamankaduwa White cattle bulls in the present study was higher ($p < 0.05$) than that of the Japanese black beef during development. [13] concluded that the high R^2 value inferred a similarity in releasing patterns of those two hormones both of which are produced by Leydig cells. It further observed a higher R^2 value for the association between these two hormones during birth to 3 months of age than that of the period around pubertal age (3 to 22 months).

The morphometric measurements of Withers height and chest girth of Thamankaduwa White cattle during the present study were comparable with the previous findings [3,31]. All the farms visited to collect the samples are being managed under a semi-intensive system. The cattle are allowed to graze in nearby lands during the morning as a herd just after the milking and confined to a paddock during the night time. Since the morphometric measurements were taken without proper restraining facilities at the field conditions, the weak associations between the tested reproductive hormones and body measurements could be attributed to the precision of the measurement taken. Therefore, the weigh band was used to take the body weight in all groups even though it could be effectively used for mature animals. However, [32] found that the prediction of live weight using the heart girth measurements is acceptable from the prepubertal to postpubertal ages of cattle. [23], reported that there were positive correlations between testosterone level and girth ($r = 0.38$; $P < 0.01$), body weight ($r = 0.38$; $P < 0.01$), right testicle ($r = 0.23$; $P < 0.05$), left testicle ($r = 0.21$; $P < 0.01$) and testicular volume ($r = 0.22$; $P < 0.008$) in Brahman male cattle.

Owing to the unexpected practical difficulties, the association between scrotal circumference (SC) with serum INSL3 and testosterone levels in Thamankaduwa White cattle males could not be assessed in the present study. Therefore, further studies are recommended to assess the association among INSL3, testosterone and SC, because it has been already proven that there is a significant association between INSL3 and SC in several mammals including humans, Norwegian Red bulls, and several breeds of goats [12,16,21, 24,25].

5. Conclusions

Serum INSL3 and testosterone concentration dynamics were highly compatible and strongly correlated with each other in Thamankaduwa White male cattle. Future studies could aim at comparing reproductive hormones and reproduction-related attributes such as scrotal circumference and sperm quality parameters which would be beneficial to understand the Leydig cell functionality during the sexual development of Thamankaduwa White male cattle.

Author Contributions: Conceptualization, S.T.D. De Silva, M. Pagthinathan, G.L.L.P. Silva and I.N. Pathirana; methodology, S.T.D. De Silva, M. Pagthinathan, G.L.L.P. Silva and I.N. Pathirana; formal analysis, S.T.D. De Silva, I.N. Pathirana; investigation, S.T.D. De Silva and O.B. Gunawardena; writing—original draft preparation, S.T.D. De Silva; writing—review and editing, M. Pagthinathan, G.L.L.P. Silva and I.N. Pathirana; supervision, M. Pagthinathan, G.L.L.P. Silva and I.N. Pathirana.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of the Faculty of Agriculture, University of Peradeniya, Sri Lanka and approved by the Ethics Committee of the Faculty of Agriculture, University of Peradeniya, Sri Lanka (ECC/2023/R/062).

Data Availability Statement: All the relevant data is available in the manuscript.

Acknowledgments: The authors are grateful to Prof. N. Kawate from Osaka Metropolitan University, Japan for his generous support in providing critical reagents used in enzyme immunoassays. The authors thank Dr. (Mrs.) K.A. Viveka, Mr. N. Manoharan and V. Sukumar from the Department of Animal Production and Health, Chenkalady, Sri Lanka and Mr. P. Pragashan from Department of Animal Science, Faculty of Agriculture, Eastern University, Sri Lanka for their utmost support during the sampling.

Conflicts of Interest: The authors declare no conflict of interest.

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CVJ CLUJ VETERINARY JOURNAL

Case Report

Complete small colon ablation and fixation of the mesocolon to the internal anal sphincter due to prolapse in a young draft horse

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Abstract: A two-year-old draft stallion was referred for evaluation of a type IV prolapse. A thorough physical examination followed by blood tests was performed to assess the situation. Following the examination, it was concluded that the protruded small colon was devitalised, measured approximately one and a half meters and mesenteric and vascular injury were present. A standing surgery approach was chosen for the present case, in which the affected tissue was excised and the remaining mesentery was ligated to the internal anal sphincter to decrease pressure during straining in the physiological act of defecation. Six months later, after an uneventful recovery, the stallion was in good health and performing its reproductive duties. To our knowledge, this is the first report of a mesenteric fixation to the internal anal sphincter in a horse. The study confirms that this technique is a feasible method that can be used in the complete ablation of the small colon in prolapses.

Keywords: small colon, prolapse, mesocolon, colonic fixation, ablation

Received: 15.11.2023
Accepted: 25.12.2023
Published: 30.12.2023

DOI: 10.52331/cvj.v28i3.58

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1. Introduction

In horses, rectal prolapse frequently develops because of conditions that also cause prolonged and intense straining, such conditions usually have their origin in the gastrointestinal sphere some examples are intestinal parasitism, diarrhea, colitis, and proctitis. Other times, prolapse can develop secondary to conditions that increase abdominal pressure, such as dystocia, constipation, colic, urinary tract obstruction, retained fetal membranes, foreign bodies, or obstructive rectal tumors. [1-8] There are factors that can predispose to rectal prolapse and they are usually related to the lack of good function in the rectum and the anal sphincter, such as loss of tone in the anal sphincter, loose attachments of the mucous membrane to the muscular coat of the rectum, or loose attachments of the rectum to perirectal tissues. [4] There are four different kinds of rectum and small colon prolapse in horses. Only the mucosa and submucosa prolapse through the anus in type I, but all layers of the rectal ampulla do so in type II. A variable portion of the small colon prolapses through the anus in type III, and the peritoneal rectum as well as a variable portion of the small colon prolapse through the anus in type IV. Most

dystocia mares have this latter kind. [1-6] Due to mesenteric and vascular damage, the prognosis is guarded to poor for types III and IV but good for types I and II. As soon as the prolapse is longer than 30 cm, a descending mesocolon rupture should be suspected. Additionally, the anal sphincter itself may mechanically compress, reducing venous return. [3],[4],[6] To avoid postponing the choice to intervene surgically, prompt detection of a ruptured mesocolon is essential. The likelihood of a favorable outcome can be decreased if the necrosis of the intestine is allowed to progress to the point of peritonitis. The absence of a viable distal small colon that can be accessible and connected to the proximal portion of the small colon via anastomosis is another potential issue with this condition. [6]

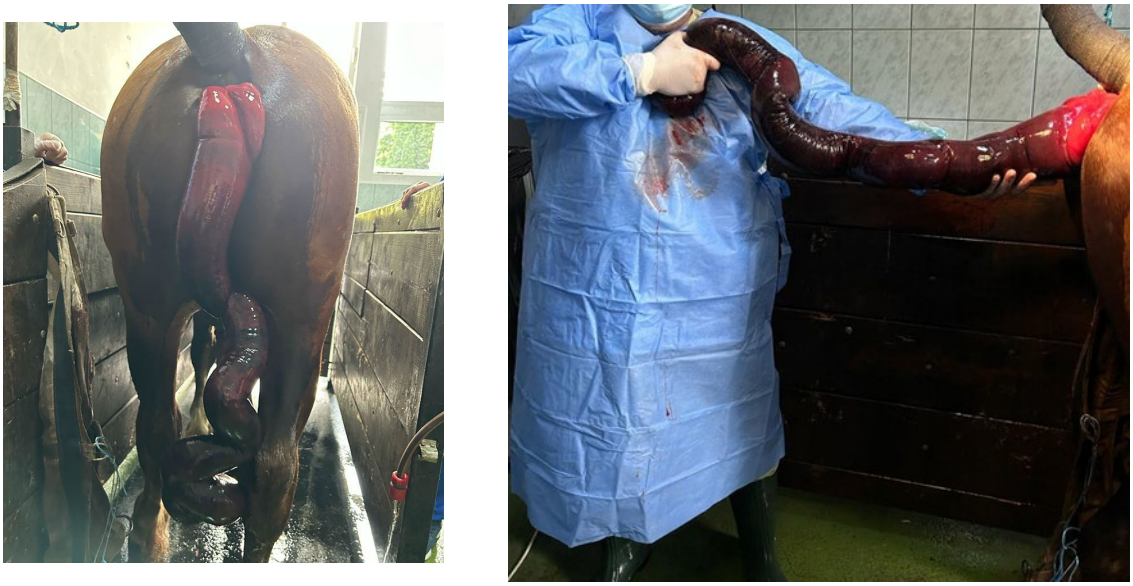
2. Case presentation

2.1. History

A two-year-old stallion was referred to the veterinary center at Cluj-Napoca Faculty of Veterinary Medicine Equine Clinic for assessment following a rectum and small colon prolapse. According to reports, the prolapsed intestine extended approximately 1.5 meters. The owner reported that the stallion entangled itself with the rope that it was tied with, fell, and struggled unsuccessfully to get back up until the morning when it was found.

2.2. Clinical findings

Upon arrival, the stallion was in moderate pain, depressed but stable. Rectal temperature could not be taken. Mucosal membranes were pink, heart rate was 50 bpm, respiratory rate was 18 rpm, capillary refill time of 3 seconds, and the extremities had a normal temperature without perceptable pulse on the digital arteries. A type IV rectal prolapse (Figure 1.a.) measuring approximately 1.5 meters in length and with an edematous, red-purple color was observed extending to a point below the hock (Figure 1.b.). A complete blood count, chemistry profile, and electrolyte panel were performed. The tests revealed mild metabolic acidosis, slight ionic calcium and sodium imbalance, and blood glucose elevation. The creatine kinase levels were elevated up to 6 times over the superior limit.



(**a**) (**b**)

Figure 1. Preoperative assesment (**a**) Type IV rectal prolapse with an edematous, red-purple color extending to a point below the hock; (**b**) measuring approximately 1.5 meters

2.3. Preoperative management

A catheter was aseptically inserted in the jugular vein and flunixin meglumine (1.1 mg/kg, Niglumin®) was administered. Cefquinome (1 mg/kg, Cobactan) was administered intramuscularly. Afterward, the stallion was guided into a padded induction stall in preparation for the standing surgery to prevent accidents in case it became necessary to undergo general anesthesia due to pain and inability to remain in a standing position. There, fluid therapy was instituted with isotonic saline (NaCl 0.9%) and preparations for the surgery were started. The tail was braided and a tail bandage was applied to keep tail hairs out of the surgical field and to minimize contamination. The caudal gluteal region and the area at the base of the tail were clipped and aseptically prepared. An epidural anesthesia was performed using 8 ml of mepivacaine HCl (Mecain® 10 mg/ml) in the sacrococcygeal space using an 21 gauge hypodermic needle. The anal sphincter was additionally infiltrated with 40 ml of lidocaine HCl (Lidobel®) for further analgesic effect. The prolapse was resolved using standing resection and anastomosis of the rectal prolapse as described in the literature [1][5] with the addition of fixating the mesocolon to the internal anal sphincter.

2.4. Treatment

To sustain the prolapse during dissection, two catheter stylets were inserted perpendicularly in the anal sphincter and healthy mucosa in order to insert stay sutures. Full circumferential incisions were made in the intussusceptions's exterior and inner walls, with a No. 24 scalpel blade. (Figure 2.) Along the way, bleeding mesenteric arteries were ligated with AssuCryl® PGA No. 2. At this point, the remaining mesentery was ligated to the internal anal sphincter using a simple interrupted suture pattern with AssuCryl® PGA No. 2. (Figure 3.) Afterward, a whole thickness simple interrupted pattern was used to appoint the proximal and distal ends. (Figure 4.)

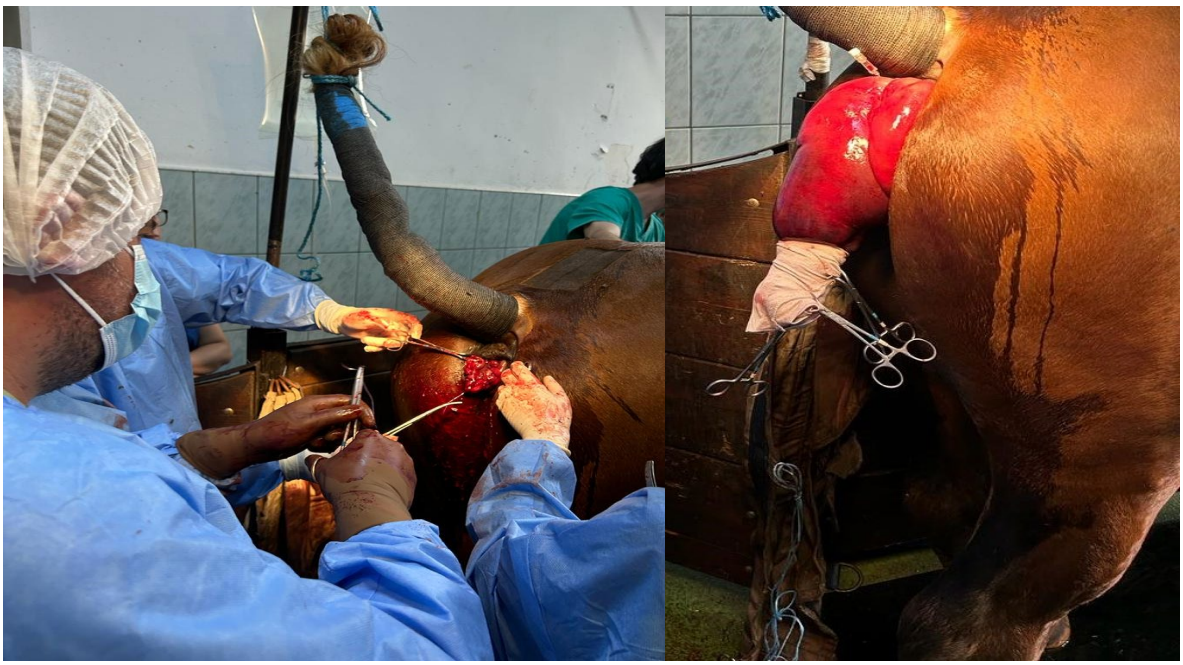


Figure 2. Full circumferential incisions in the exterior and inner walls

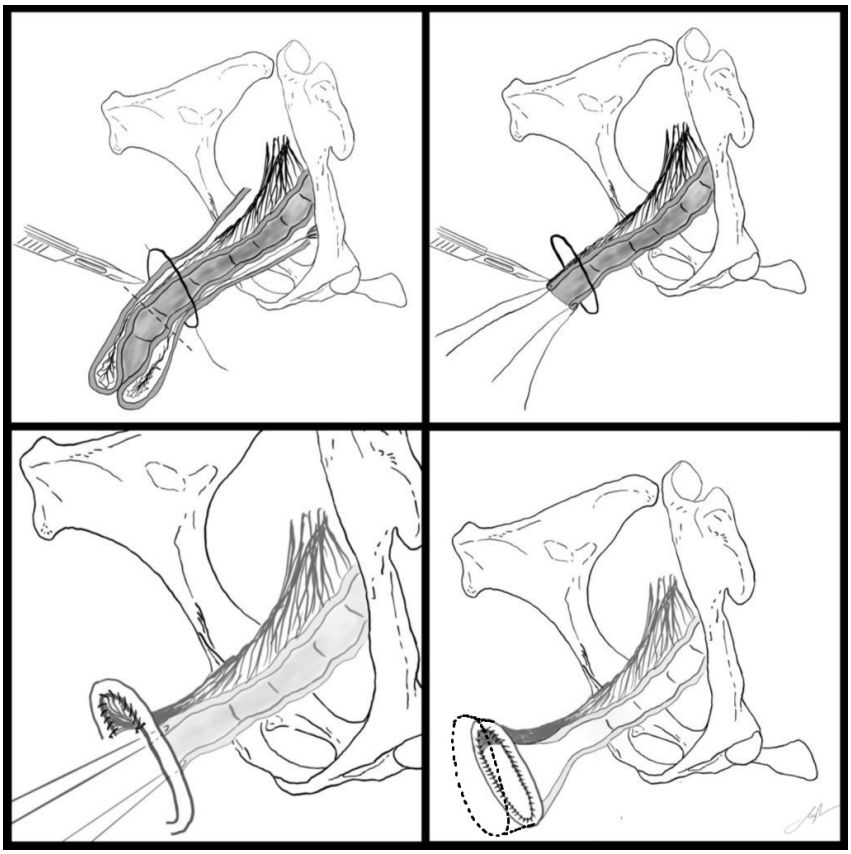


Figure 3. Schematic representation of the surgery (a) Small colon prolapse; (b) Full circumferential incision and stay sutures; (c) the mesentery ligated to the internal anal sphincter using a simple interrupted suture pattern; (d) end result, red arrow: internal anal sphincter; blue arrow: external anal sphincter

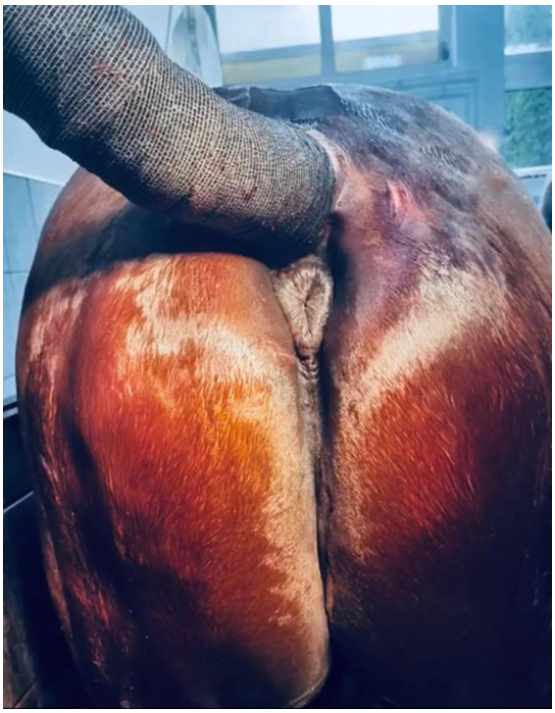


Figure 4. Postoperative result

2.5. Postoperative Care

After the surgery was completed, the stallion was admitted into the clinic for postoperative care for six days. immediately post-surgery, the stallion was administered 20 ml of tetanus antiserum, and a nasogastric tube was placed. Via the nasogastric tube, the horse was administered daily 3 liters of mineral oil for five days. Flunixin meglumine (1.1 mg/kg, Niglumin®) and cefquinome (1 mg/kg, Cobactan) were administered intravenously respectively intramuscularly daily for the entire duration of the stay in the clinic.

2.6. Postoperative Results

After a week spent in the clinic, the stallion was discharged and transported back to its owner's stable. The owner agreed to regular telephonic questionnaires about the horse's evolution. The recuperation was reported to be uneventful up to the 6 months postoperatively mark and normal reproductive duties were resumed.

4. Discussion

Ischaemic damage of the small colon can occur secondary to a rectal prolapse,[6] in the case we presented, the mesentery was stretched and presented multiple tears thus disrupting the vascular supply to the terminal small colon. The interruption of the blood supply to the section of the small colon that has prolapsed must be considered while treating intussusception and small colon prolapse. [9] The patient must be closely watched for indications of peritonitis if intussusception of the small colon is suspected, and additional testing by a midline exploratory laparotomy may be necessary.[9] Jacobs KA et al suggested that if the small colon loses its blood supply, a colostomy should be considered because it is not surgically accessible enough to remove the small colon and connect it to the rectum.[9] The surgeon did not opt for a colostomy because we were able to connect the remaining intestine with the rectum and secure it with the remaining mesentery to the internal anal sphincter. It is the author's belief that securing the mesentery offered better stabilisation and decreased pressure during straining in the physiological act of defecation. Similar approaches are used in humans with rectal prolapses, the differences consisting in the type of material used to achieve a better fixation. The rectum is fixated via a prosthetic rectopexy based on the notion that rectopexy via adhesion and fibrosis is viable, and that mesh fixation would be more successful than a simple suture.[10] Materials including fascia lata, nylon, polypropylene, marlex, polyvinyl alcohol, and polytape are utilized in the development of meshes and other prostheses to facilitate fixation.[10] It was proven that using these methods improves fecal incontinence in most cases, however, opinions on the effects regarding constipation are divided.[10] In the case we are presenting, the owner did not report any episodes of constipation, normal bowel movement, and defecation being restored. The study confirms that this technique is a feasible method that can be used in the complete ablation of the small colon in prolapses.

Author Contributions: Cristian Mihăiță Crecan, Valeria Ciulu-Angelescu and Cosmin Petru Pește an had equal contribution. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding

Institutional Review Board Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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