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TABLE OF CONTENTS

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Editorial

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Assessment of Ageing-Dependent Effects on Sperm Functions following Semen Cryopreservation

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There has been an increased improvement in the cryopreservation protocol of semen, which contributes to the preservation of genetic resources.[1,2] Cryo-induced damage to spermatozoa is attributed to membrane deterioration caused mainly by the formation of intracellular ice and increased oxidative stress, resulting in a loss of the sperm fertilizing ability.[3] It is noteworthy that ageing-dependent processes in frozen-thawed semen occur in different sperm compartments, and are associated with membrane modifications of specific lipid-protein interactions, activation of an apoptosis-like mechanism and reduced genomic integrity, which consequently compromise sperm cryo-survival.[4,5] Moreover, ageing is a natural process occurring in sperm cells during semen preservation, and there is no single sperm test that can effectively predict the fertilizing capacity of semen following the different reproductive technologies.[6]

Cryo-induced ageing-dependent processes in spermatozoa have marked effects on different sperm attributes, either directly or indirectly, resulting in impaired fertilizing ability of the post-thaw semen.[1] A plethora of sperm markers have provided evidence of the ageing-dependent effects on sperm cryo-survival.[5] Accordingly, molecular markers, used to detect the sperm ageing-dependent processes associated with the freezing-thawing procedure, have made possible a more widespread analysis of different sperm attributes that are considered as a prerequisite for successful fertilization.[6] Cryo-induced sperm ageing processes have been manifested in compromised sperm motility, mitochondrial membrane potential (MMP), plasma membrane integrity (PMI) and acrosome membrane integrity (AMI), which are the key determinants of semen quality.[1] Even though motility characteristics, analyzed by the computer-assisted semen analysis (CASA) system, reflect several essential aspects of the sperm metabolic activity, it is still unclear whether these variables are reliable to predict fertilization.[4] Moreover, the sperm membranes are an integral part of the acrosome reaction and are involved in the fertilization-related events, so deterioration in the structural and functional integrity of spermatozoa is an obvious sign of the ageing processes, as is evident after cryopreservation.[5] Assessment of the cryo-induced sperm ageing processes, using a combination of different fluorescent probes (lipophilic cationic JC-1 in combination with propidium iodide – PI, JC-1/PI; rhodamine 123, R123/PI; MitoTracker Green, MTG; SYBR-14/PI; carboxyfluorescein diacetate, CFDA/PI; Hoechst 33258, H258; fluorescein isothiocyanate (FITC)-labeled peanut agglutinin, FITC-PNA/PI; FITC-labeled *Pisum sativum* agglutinin, FITC-PSA/PI), has provided more detailed information on several sperm attributes that are required to identify individuals with poor and good semen freezability.[1,5] Furthermore, the ageing-associated decrease in post-thaw sperm viability is concomitant with an increase in lipid peroxidation measured by malondialdehyde (MDA) production, capacitation-like destabilization of sperm membranes (antibiotic chlortetracycline, CTC; Merocyanine 540, M540), apoptotic sperm cells (Annexin-V/PI; Yo-Pro-1/PI; caspase activation) or sperm DNA fragmentation (Comet assay, sperm chromatin structure assay, SCSA; terminal deoxynucleotidyl transferase-mediated dUDP nick end labelling assay, TUNEL; sperm chromatin dispersion assay), which significantly compromises the fertilizing ability of frozen-thawed spermatozoa.[4,5] Despite the effectiveness of these sperm assays, the underlying mechanisms involved in the cryo-induced ageing-dependent changes, within different compartments of the spermatozoon, are still poorly understood.

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Sperm attributes react differently to the cryopreservation conditions among individuals.[6] This has contributed to renewed interest in developing new sperm markers that can be used during routine semen analysis to identify individuals differing in semen freezability.[7] It is envisaged that transcriptome studies on RNA-sequence data will identify markers directly associated with the cryo-induced ageing processes in spermatozoa.[8] Analysis of the ageing processes in gene expression profiles of frozen-thawed spermatozoa will provide new opportunities to develop freezability markers for the improvement in the technology of semen cryopreservation. Supported by a project from the National Science Centre, Poland (2015/19/B/NZ9/01333).

REFERENCES

1. Fraser L, Strzeżek J, Kordan W. Post-thaw sperm characteristics following long-term storage of boar semen in liquid nitrogen. *Anim Reprod Sci.* 2014; 147: 119-127. doi: 10.1016/j.anireprosci.2014.04.010

2. Yeste M. State-of-the-art of boar sperm preservation in liquid and frozen state. *Anim Reprod*. 2017; 14(1): 69-81. doi: 10.21451/1984-3143-AR895

3. Fraser L, Strzeżek J, Wasilewska K, Pareek CS. Sperm DNA damage in relation to lipid peroxidation following freezing-thawing of boar semen. *S Afri J Anim Sci*. 2017; 47(2): 213-218. doi: 10.4314/sajas.v47i2.13

4. Fraser L, Zasiadczyk Ł, Pareek CS. Effects of boar variability on comet-detected sperm DNA damage following cryopreservation. *Anim Prod Sci*. 2016. doi: 10.1071/AN16274

5. Hossain MdS, Johannisson A, Wallgren M, Nagy S, Siqueira AP, Rodríguez-Martinez H. Flow cytometry for the assessment of animal sperm integrity and functionality: State of the art. *Asian J Androl*. 2011; 13: 406-419. doi: 10.1038/aja.2011.15

6. Rodríguez-Martínez H. Can we increase the estimate value of semen assessment? *Reprod Domest Anim.* 2006; 41(Suppl 2): 2-10. doi: 10.1111/j.1439-0531.2006.00764.x

7. Fraser L. Sperm transcriptome profiling for assessment of boar semen freezability. *International Journal of Advanced Scientific Research and Management*. 2016; 1(12): 9-12.

8. Fraser L, Brym P, Pareek CS. Isolation of total RNA from fresh and frozen-thawed boar semen and its relevance in transcriptome studies. *S Afri J Anim Sci.* 2017; 47(1): 56-60. doi: 10.4314/sajas.v47i1.9

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Research

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Evaluation of Total Intravenous Anesthesia by Ketamine-Xylazine Constant Rate Infusion in Dogs: A Novel Preliminary Dose Study

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ABSTRACT

Background and Aims: The use of ketamine-xylazine for total intravenous anesthesia (TIVA) by constant rate infusion (CRI) has not been well-documented in literature. The present study aimed to define the optimal dosage of ketamine-xylazine for TIVA in dogs and its effects on physiological body parameters.
Materials and Methods: Twenty mongrel dogs were randomly assigned to four treatment protocols (n=5): A (0.5 mg kg^{-1}, ketamine+ 0.25 mg kg^{-1}, xylazine and 0.6 mg kg^{-1} h^{-1}, ketamine+0.3 mg kg^{-1} h^{-1}, xylazine), B (5 mg kg^{-1}, ketamine+0.5 mg kg^{-1}, xylazine and 10 mg kg^{-1} h^{-1}, ketamine+1 mg kg^{-1} h^{-1}, xylazine), C (2 mg kg^{-1}, ketamine+0.5 mg kg^{-1} xylazine and 5 mg kg^{-1} h^{-1}, ketamine+0.5 mg kg^{-1} h^{-1} xylazine), and D (2 mg kg^{-1} ketamine+1 mg kg^{-1} xylazine and 10 mg kg^{-1} h^{-1}, ketamine+1 mg kg^{-1} h^{-1} xylazine) for induction and maintenance of anesthesia, respectively.
Results: The anesthesia was deep in protocols B, C, and D (pedal reflex of grade=0), but light in the protocol A (pedal reflex of grade=3). Animals in both protocols B and C showed paddling and tonic clonic convulsions. All the protocols recorded a non-significant increase in the body temperature but; a significant decrease in the respiratory rate. There was a non-significant decrease in the heart rate in protocols A and C; while a non-significant increase was recorded in protocols B and D.
Conclusion: The protocol D could be considered as the optimal dosage of ketamine-xylazine for TIVA by CRI in dogs, with minimal effects on vital body parameters, for extended surgical interventions.

KEY WORDS: Ketamine; Xylazine; Constant rate infusion; Dogs.

ABBREVIATIONS: TIVA: Total Intravenous Anesthesia; CRI: Constant Rate Infusion; IV: Intravenous; BW: Body Weight; BT: Body Temperature; HR: Heart Rate; RR: Respiratory Rate; NMDA: N-methyl, D-aspartate.

INTRODUCTION

In the recent years, intravenous anesthetics are increasingly being used as alternatives to inhalation anesthetic agents. Inhalant anesthetics require a costly anesthetic machine, rather than the exposure of the operating-room personnel to pollution.[1-3] The intravenous agents has a lesser chance of causing cardiovascular and respiratory depression than the inhalation anesthetics.

Total intravenous anesthesia (TIVA) is a useful method of anesthesia in the field; as it does not require specialized equipment or an oxygen delivery system.[4] It is used for surgical procedures that involve the upper airway when the placement of an endotracheal tube interferes with surgery as well as for bronchoscopic examination.[5]

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For longer periods of anesthesia, intravenous anesthetics may be given by intermittent injections or by continuous infusion. The intermittent bolus administration can result in high peak plasma levels and an excessive depth of anesthesia, alternating with periods of inadequate anesthesia, while infusion technique provides a continuous steady-state concentration of the drug, producing a more stable plane of anesthesia.[3,6,7]

Surgical intervention requires an ideal anesthetic, with sufficient analgesic and muscle relaxant effects. Such characteristics may be difficult to be present in a sole agent. So, a combination of drugs is used, which is referred to as balanced anesthesia.[8] Use of a combination of anesthetic agents with different mechanisms of action, has the benefit of using lower doses of individual agents that produce sufficient anesthesia, while reducing the possibility of overdosage risks.[9]

Ketamine is an N-methyl, D-aspartate (NMDA) receptor antagonist. It has a cataleptic, analgesic, and anesthetic action, but no hypnotic properties. Its effect on poor muscle relaxation can be improved by co-administration with sedatives such as xylazine. By its stimulatory effect on the sympathetic nervous system, ketamine counteracts the depressant effects of other anesthetic agents during anesthesia. Xylazine is a α_2-adrenoceptor agonist, with analgesic, sedative, and muscle relaxant effects.[3,10]

Several mixtures of different anesthetic regimens were described in literatures for providing TIVA; however, studies that define doses of ketamine-xylazine for TIVA are lacking.

So, the aim of the present study was to define the optimal ketamine-xylazine dosage for TIVA by constant rate infusion (CRI) in dogs, especially emphasizing on its effects on anesthetic and physiological indices.

MATERIALS AND METHODS

Ethics Statement

The present study was approved by The National Ethical Committee of the Faculty of Veterinary Medicine, Assuit University, Assuit, Egypt. The animals were selected in minimum numbers to obtain valid results.[11]

Experimental Animals

The present study was conducted on twenty clinically healthy adult mongrel dogs, of either sex (ten males and ten, non-pregnant, non-lactating bitches). They were 2 to 3 years old and weighed 12 to 14 kg in body weight. The animals were housed in standard individual cages with food and water ad libitum.

Anesthetic Protocols

Many different dosages from the two anesthetic regimens, ketamine-xylazine, were used in induction and maintenance of anesthesia by CRI in dogs, till an optimal dosage was achieved for an effective, deep anesthesia without any complications or side effects. Dogs were randomly assigned into four treatment protocols (A, B, C, and D), of five dogs each (n=5), based on the dosages of ketamine-xylazine mixture, used for induction and maintenance of anesthesia in each protocol. Dogs in each protocol were evaluated individually, each in a separate day (Table 1).

Drugs

Drugs used were ketamine 5% (50 mg/ml) injection (Sigma-tec Pharmaceutical Industries, SAE, Egypt), and xylazine 2% (20 mg/ml) injection (ADWIA Co. SAE, Egypt).

Anesthetic Technique

Food but not water was withheld from dogs for 12 hours (h) before each treatment protocol. Animals were weighed prior to the dosage calculation. The cephalic vein was catheterized by an 18-gauge, IV catheter. Anesthesia was induced by administration of ketamine-xylazine, mixed in the same syringe, at doses in (mg/kg body weight, IV) based on each protocol. The ketamine-xylazine dosages (ml) for CRI were dissolved in normal saline (0.9% NaCl) as a diluent to a maximum volume of 100 ml, administered using a volumetric infusion pump, P-600 (Atom Medical Corp, Japan). CRI commenced immediately after in-

Table 1: The Anesthetic Protocols of Ketamine-Xylazine TIVA in Dogs.

Protocol	No. of Dogs	Drugs	Induction dosage (mg/kg, IV)	Maintenance dosage (mg/kg/hour)
A	5	Ketamine 5%	0.5	0.6
		Xylazine 2%	0.25	0.3
B	5	Ketamine 5%	5	10
		Xylazine 2%	0.5	1
C	5	Ketamine 5%	2	5
		Xylazine 2%	0.5	0.5
D	5	Ketamine 5%	2	10
		Xylazine 2%	1	1

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duction of the anesthesia and was maintained for 60 minutes (min) in all protocols.

Dosage Calculation for CRI

The following calculations were made according to Luisito[12]:

1. Fluid infusion rate (ml kg^{-1} h^{-1})=maximum volume (100 ml)/ body weight (kg)/desired hours of procedure (1 h).

2. Ketamine CRI dose=Infusion rate of ketamine (mg kg^{-1} h^{-1})÷fluid infusion rate (ml kg^{-1} h^{-1})×maximum volume (100 ml).

3. Xylazine CRI dose=Infusion rate of xylazine (mg kg^{-1} h^{-1})÷fluid infusion rate (ml kg^{-1} h^{-1})×maximum volume (100 ml).

Anesthetic Indices

The following parameters were recorded in each case:

- Onset of anesthesia (sec): Time elapsed from the beginning of induction of anesthesia till the disappearance of pedal reflex.

- Recovery time (min): Time elapsed from the disconnection of the infusion set and the ability of the animal to stand.

- Quality of induction and recovery (smooth or there were any adverse reactions).

- Pedal reflex: By clamping the toe web with a hemostat. Recorded every two second from the end of induction till the onset of anesthesia, and subsequently every ten minutes till the disconnection of the infusion set. It was graded in a scale of (0-3), according to Njoku[13] (Table 2).

- Palpebral reflex: Recorded every ten minutes from the end of induction till the disconnection of the infusion set. It was graded in a scale of (1-4), according to Jena et al[14] (Table 3).

- Side effects.

PHYSIOLOGICAL INDICES

The Body temperature (BT, °C), heart rate (HR, beats/min), and respiratory rate (RR, breaths/min) were measured (using a digital thermometer, stethoscope, and visual observation of excursions of the thorax during respiration, respectively). They were measured at 0 min before induction of anesthesia; to ascertain the baseline values and subsequently at 5, 10, 15, 30, 45, and 60 min following the induction of anesthesia.

Statistical analysis

The values are expressed by Mean±SE. The data was analyzed by one way ANOVA using SPSS 16.00 Software (SPSS Inc., Chicago, IL, USA); significance was designated as $p \leq 0.05$. Means were compared by Tukey *post-hoc* test when a significant difference was detected.

RESULTS

Onset of Anesthesia

Protocol D achieved the shortest onset time of anesthesia, followed by protocols B, C, and A, in the given order, (Table 4). The onset of anesthesia was evident by calming the animal in the absence or decrease in pedal and/or palpebral reflexes.

Quality of Anesthesia

The induction of anesthesia was smooth in all animals of the four protocols. The anesthesia was deep in animals of protocols B, C, and D; where their pedal reflex was of grade=0. The anesthesia was light in the protocol A, with pedal reflex of grade=3. The palpebral reflex was sluggish (grade=3) in all animals, ex-

Table 2: The Scale of the Pedal Reflex in Dogs.

Scale	Pedal Reflex
0	No limb withdrawal at the lock of the 3rd ratchet of a hemostat clamped on the toe web
1	Limb withdrawal at the lock of the 3rd ratchet
2	Limb withdrawal at the lock of the 2nd ratchet
3	Limb withdrawal at the lock of the 1st ratchet

Table 3: The Scale of the Palpebral Reflex in Dogs.

Scale	Palpebral Reflex
1	No change in reflex
2	Moderate reflex
3	Sluggish reflex
4	Absence of reflex

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Table 4: The Onset and Recovery Times of Ketamine-Xylazine TIVA in Dogs.

Protocol	A	B	C	D	*p* value
Onset (sec)	113.33±4.41^{x}	74.67±8.37^{xy}	95.00±21.79^{xy}	45.00±8.66^{y}	0.026
Recovery Time (min)	2.67±0.88^{z}	67.33±1.45^{x}	21.33±6.33^{y}	36.00±4.36^{y}	0.000

cept for animals of the protocol A; their palpebral reflex was moderate (grade=2).

The anesthesia was maintained in all animals of the four protocols for 60 mins by CRI of ketamine-xylazine mixture. Dogs in protocol C showed paddling, retching, tonic-clonic convulsions, at irregular intervals while anesthesia was administered by CRI, apart from poor muscle relaxation. One animal in protocol B showed the same behavioural responses, but only once, 5 min post-induction of the anesthesia. Animals of protocol D did not report any such behavioural responses and were characterized by a stable line of anesthesia with a good muscle relaxation. All animals showed minor salivation as well as lacrimation.

Recovery Time

Recovery was smooth in animals of the protocols A, B, and D. Dogs in protocol C showed excitement during recovery recorded as vocalization. Dogs in protocol B had the longest recovery time, followed by those in protocols D, C, and A in the given order, (Table 4).

Effect on Body Temperature

Dogs in the four protocols of anesthesia A, B, C, and D, exhibited a non-significant increase (p=0.940, 0.988, 0.829, 0.229, respectively) in the body temperature, throughout the maintenance period of anesthesia, at different intervals, (Table 5).

Effect on Heart Rate

The dogs in both protocols, A and C had a non-significant decrease (p>0.05) in the HR throughout the maintenance period of anesthesia, except at 15 mins post-induction for dogs in protocol A, where decrease in HR was significant (p≤0.05). On the contrary, there was an increase in HR in the animals of both protocols, B and D, but was non-significant (p=0.851 and 0.685, respectively). HR was regular except for the animals in protocol C; as they had an irregular HR, (Table 6).

Effect on Respiratory Rate

Dogs in the four protocols of anesthesia recorded a significant decrease (p<0.05) in the respiratory rate throughout the maintenance period of anesthesia, at the different intervals. The respiration was regular and deep in all animals, except for those in protocol C, where the respiration was irregular and shallow, (Table 7).

DISCUSSION

The use of ketamine-xylazine for total intravenous anesthesia by constant rate infusion in dogs has not been well-documented

Table 5: Effect of Ketamine-Xylazine TIVA on Body Temperature in Dogs.

Protocol	Time (min)							
	0	5	10	15	30	45	60	*p* value
A	38.93±0.09	39.10±0.15	39.13±0.15	39.20±0.17	39.20±0.23	39.07±0.20	39.03±0.23	**0.940**
B	39.27±0.22	39.37±0.27	39.43±0.23	39.57±0.27	39.53±0.28	39.47±0.32	39.40±0.30	**0.988**
C	38.77±0.20	38.97±0.20	39.03±0.20	39.03±0.20	38.97±0.23	38.83±0.22	38.57±0.43	**0.829**
D	38.67±0.34	39.13±0.09	39.13±0.09	39.17±0.09	39.17±0.09	39.23±0.12	39.23±0.12	**0.229**
***p* value**	**0.336**	**0.541**	**0.455**	**0.314**	**0.396**	**0.312**	**0.280**	

Table 6: Effect of Ketamine-Xylazine TIVA on Heart Rate in Dogs.

Protocol	Time (min)							
	0	5	10	15	30	45	60	*p* value
A	63.00±11.53^{x}	39.00±5.51^{bxy}	40.00±4.00^{xy}	33.00±4.73^{y}	37.33±4.81^{xy}	38.00±3.06^{xy}	38.67±2.40^{xy}	**0.051**
B	73.00±8.54	100.00±0.00^{a}	89.67±14.84	94.00±15.01	93.33±16.67	99.67±17.89	94.00±16.00	**0.851**
C	86.67±20.70	79.67±7.84^{ab}	78.67±9.33	62.00±8.72	53.33±7.06	70.67±14.67	66.67±6.67	**0.486**
D	45.33±1.33	98.67±16.71^{a}	81.33±18.52	80.00±28.00	70.67±23.13	76.00±18.90	73.33±20.28	**0.685**
***P* value**	**0.208**	**0.007**	**0.099**	**0.133**	**0.124**	**0.104**	**0.101**	

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Table 7: Effect of Ketamine-Xylazine TIVA on Respiratory Rate in Dogs.

Protocol	Time (min)							
	0	5	10	15	30	45	60	*p* value
A	57.33±7.42^{x}	19.33±2.40^{ay}	14.33±1.20^{y}	17.00±1.00^{y}	21.33±1.33^{ay}	18.67±1.33^{y}	17.67±0.88^{y}	**0.000**
B	62.00±12.70^{x}	11.67±0.88^{by}	12.33±0.33^{y}	12.67±0.67^{y}	14.00±1.15^{by}	12.00±1.73^{y}	11.33±1.86^{y}	**0.000**
C	61.33±21.95^{x}	10.00±1.00^{by}	15.00±3.21^{y}	12.00±0.00^{y}	16.00±0.00^{aby}	16.67±1.76^{y}	14.33±1.86^{y}	**0.009**
D	41.33±2.67^{x}	13.33±1.33^{aby}	11.33±0.67^{y}	12.33±2.03^{y}	13.33±2.67^{by}	13.33±2.67^{y}	13.00±2.08^{y}	**0.000**
***P* value**	**0.674**	**0.012**	**0.463**	**0.053**	**0.028**	**0.135**	**0.143**	

in literature. This is the first preliminary experimental study defining the optimal dosages of ketamine-xylazine mixture for TIVA in dogs, and its effects on the physiological body parameters (Heart rate, respiratory rate and body temperature). TIVA by ketamine-xylazine CRI can be adjusted according to the suspected time of operations, which makes it more suitable for extended surgical interferences in canine patients.

Ketamine is a dissociative anesthetic with a central sympathomimetic and parasympatholytic effect; thereby counteracting the depressant effects of other agents used during anesthesia. It is a NMDA-receptor-antagonist and has been used as an anesthetic in both, human and veterinary medicine. Ketamine increases muscle tone and induces spontaneous movement and convulsions. Recovery from ketamine anesthesia is often associated with hyperexcitability.[3,15]

Xylazine is an α_2-adrenergic agonist, associated with a rapid onset, good sedation and analgesia as well as a smooth recovery.[16] Its analgesic and sedative effects are due to central nervous system depression and muscle relaxation is due to the central inhibition of intraneural transmission, as a result of the stimulation of alpha adrenoceptors at the spinal cord and brain, thereby inhibiting the release of neurotransmitters, norepinephrine and substance P.[17-19]

Ketamine has a slow onset after an IV administration (1-5 min).[20] Administration of xylazine in a dose of (1 mg/kg) with ketamine (2 mg/kg) in protocol D for the induction of anesthesia, achieved the shortest onset of anesthesia among the four protocols, even with a higher dose of ketamine (5 mg/kg) in protocol B. This explores how effective is the role of xylazine in potentiation of the anesthetic effect of ketamine. Similar findings were reported by Newkirk and Miles.[16]

Animals in protocol A had a light anesthesia. It may be attributed to the administration of sub-anesthetic doses of both, ketamine-xylazine in induction and maintenance of anesthesia, which makes this protocol more suitable for non-painful surgical procedures, such as wound dressing, clinical, or radiological examinations.

Animals in protocols B, C, and D were in deep anesthesia, but not in the same quality. The reduction in xylazine dosage in both protocols, B and C failed to antagonize the recorded side effects as paddling and tonic-clonic convulsions, which could be attributed to ketamine administration.[3]

Usage of ketamine, in high doses (5 mg/kg) in protocol B, prolonged the recovery time in the dogs that received treatment. The recovery in the protocol C was not smooth. It appeared to be associated with the reduction in xylazine doses in both, induction and maintenance, relative to ketamine doses.

From the findings of the present study, the dosage of ketamine-xylazine for induction and maintenance of anesthesia by CRI in protocol D was the optimal dosage that provided a smooth, safe, effective, and deep anesthesia, which could likely be recommended for different surgical interventions in dogs, apart from the advantage of the possibility to maintain anesthesia for longer durations according to the surgical procedures.

Dogs received the ketamine-xylazine mixture which exhibited a non-significant increase in the body temperature. It may be attributed to ketamine; due to the stimulation of sympathetic nervous system, which increases arteriolar peripheral resistance, that is augmented by increased plasma concentrations of norepinephrine, to the extent that it induces vasoconstriction or at least prevents vasodilation from other anesthetics.[21] All α_2-adrenergic agonists cause vasoconstriction.[22] Similarly, hyperthermia was recorded following ketamine-xylazine administration in dogs by Boscan et al.[23] On the other hand, hypothermia was reported in goats following the use of the same combination according to Afshar et al.[24]

It was clear that the ketamine-xylazine anesthetic mixture produced a dose-dependent influence on HR. The stimulatory effect of ketamine on the heart may be masked by the concomitant use of α_2-adrenergic agonists.[25] The reduction in doses of xylazine-induced bradycardia[3,26] in both protocols, B and D, relatively to ketamine, potentiated the cardiovascular stimulatory effect of ketamine,[3,20,27] which was antagonized in both protocols, A and C by increasing the xylazine dosages.

There was a profound reduction in the respiratory rate in all animals of the four protocols. It could have resulted from the synergistic effect of both, ketamine-xylazine as respiratory depressants.[3,26,28] However, with ketamine, the airway is usually

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well maintained as well as it preserves the laryngeal and pharyngeal reflexes. For this reason, ketamine is a very useful anesthetic agent in areas where there is no oxygen or only limited oxygen available. In addition, it is an effective bronchodilator.[20] Also, it has been found that ketamine preserves the respiratory muscle tone.[29]

CONCLUSION

Ketamine-xylazine combination in the doses of (2 mg/kg, ketamine and 1 mg/kg, xylazine, intravenous) for induction, followed by intravenous constant rate infusion (CRI) of (10 mg kg^{-1}. h^{-1} ketamine and 1 mg kg^{-1} h^{-1} xylazine) for maintenance of anesthesia, could be defined for a TIVA, which could be recommended for extended surgical interferences in dogs. These data must be checked under surgical conditions. Further studies are needed for the exploration of its effects on hematological indexes and blood biochemical parameters.

COMPETING INTERESTS

The author declares that he has no competing interests.

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REFERENCES

1. Hasei M, Hirata T, Nishihara H, Tanigami H, Takashina M, Mori T. Occupational exposure of operating room staff to anesthetic gases during inhaled induction-a comparison with intravenous anesthesia induction. *Masui*. 2003; 52(4): 394-398. Web site. http://europepmc.org/abstract/med/12728490. Accessed January 27, 2017.

2. Matthews NS. Inhalant anaesthetics. In: Seymour C, Duke-Novakovski T, eds. *BSAVA Manual of Canine and Feline Anaesthesia and Analgesia*. 2nd ed. Quedgeley, Gloucester, UK: British Small Animal Veterinary Association, Gloucester, 2007: 150-155.

3. Hall LW, Clarke KW, Trim CM. *Veterinary Anaesthesia*. 10th ed. England: Hacourt Publishers Ltd; 2011: 123-125.

4. Bennett RC. Comparison of TIVA and inhalation anesthesia in practice. Proccedings. The British Small Animal Veterinary Congress 2006.

5. Luisito SP. Total IV Anesthesia. 2011b. Web site. https://www.acvs.org/files/proceedings/2011/data/papers/187.pdf. Accessed January 27, 2017.

6. Macintire DK, Tefend M. Constant rate infusions: Practical use. *NAVC Clinician's Brief*. 2004; 4: 25-28.

7. Musk GC, Pang DS, Beths T, Flaherty DA. Target-controlled infusion of propofol in dogs evaluation of four targets for induction of anesthesia. *Vet Rec*. 2005; 157(24): 766-770. doi: 10.1136/vr.157.24.766

8. Thurmon JC, Short CE. History and overview of veterinary anaesthesia. In: Tranquilli WJ, Thurmon JC, Grimm KA, eds. *Lumb & Jones' Veterinary Anaesthesia and Analgesia*. 4th ed. Hoboken, NJ, USA: Blackwell Publishing Ltd; 2007: 3-6.

9. Ukwueze CO, Eze CA, Udegbunam RI. Assessment of common anaesthetic and clinical indices of multimodal therapy of propofol, xylazine and ketamine in total intravenous anaesthesia in West African Dwarf Goat. *J Vet Med*. 2014. doi: 10.1155/2014/962560

10. Lerche P, Nolan AM, Reid J. Comparative study of propofol or propofol and ketamine for the induction of anesthesia in dogs. *Vet Rec*. 2000; 146(20): 571-574.

11. Office of Laboratory Animal Welfare. A: Institutional Animal Care and Use Committee Guidebook. In: Office of Laboratory Animal Welfare, National Institutes of Health, Department of Health and Human Services Bethesda, Maryland, USA; 2002.

12. Luisito SP. Total IV Anesthesia. 2011a. Web site. https://www.acvs.org/files/proceedings/2011/data/papers/157.pdf. Accessed January 27, 2017.

13. Njoku NU. Effects of maintenance of propofol-ketamine anesthesia with repeat bolus and constant rate infusion of propofol on physiological, biochemical and analgesic indices in dogs. *J Adv Vet Anim Res*. 2015; 2(4): 427-434. doi: 10.5455/javar.2015.b114

14. Jena B, Das J, Nath I, et al. Clinical evaluation of total intravenous anaesthesia using xylazine or dexmedetomidine with propofol in surgical management of canine patients. *Vet World*. 2014; 7(9): 671-680. Web site. http://www.veterinaryworld.org/Vol.7/September-2014/9.pdf. Accessed January 27, 2017.

15. Kastner SBR. Intravenous anaesthetics. In: Seymour C, Duke-Novakovski T, eds. *BSAVA Manual of Canine and Feline Anaesthesia and Analgesia*. 2nd ed. Gloucester, England: British Small Animal Veterinary Association; 2007: 133-149.

16. Newkirk HL, Miles DG. Xylazine as sedative-analgesic for dogs and cats. *Mod Vet Pract*. 1974; 55(9): 677-680.

17. Moye RJ, Pailet A, Smith MW. Clinical use of xylazine in dogs and cats. *Vet Med Small Anim Clin*. 1973; 68(3): 236-241.

18. Saha JK, Xia J, Grondin IM, Engle SK, Jakubowski JA. Acute hyperglycemia induced by Ketamine/xylazine anesthesia in rats: Mechanisms and implications for preclinical models. *Exp Biol Med (Maywood)*. 2005; 230(10): 777-784. Web site. http://journals.sagepub.com/doi/abs/10.1177/153537020523001012. Accessed January 27, 2017.

ISSN 2475-1286 http://dx.doi.org/10.17140/VMOJ-2-114

19. Kolahian S. Efficacy of different antiemetics with different mechanisms of action on xylazine induced emesis in cats. *Iranian J Veterinary Surgery*. 2014; 9(1): 9-16. Web site. http://www.ivsajournals.com/article_5789_864.html. Accessed January 27, 2017.

20. Craven R, Alkhafaji R. Ketamine in Anaesthetic Practice. World Anesthesia Tutorial of the Week. 2006. Web site. www.AnesthesiaUK.com/WorldAnesthesia. Accessed January 27, 2017.

21. Zsigmond EK, Kelsch RC, Kothary SP. Rise in plasma free norepinephrine during anesthetic induction with ketamine. Behav Neuropsychiatry. 1974; 6(1-12): 81-84. Web site. http://europepmc.org/abstract/med/4468764. Accessed January 27, 2017.

22. Pypendop BH, Verstegen JP. Haemodynamics effects of medetomidine in the dog: A dose titration study. *Vet Surg*. 1998; 27(6): 612-622. doi: 10.1111/j.1532-950X.1998.tb00539.x

23. Boscan P, Pypendop BH, Solano AM, Ilkiw JE. Cardiovascular and respiratory effects of ketamine infusions in isoflurane-anesthetized dogs before and during noxious stimulation. *Am J Vet Res*. 2005; 66(12): 2122-2129. doi: 10.2460/ajvr.2005.66.2122

24. Afshar FS, Baniadam A, Marashipour SP. Effect of xylazine-ketamine on arterial blood pressure, arterial blood pH, blood gases, rectal temperature rates in goats. *Bull Vet Inst Pulawy*. 2005; 49: 481-484. Web site. http://www.piwet.pulawy.pl/jvetres/images/stories/pdf/20054/20054481484.pdf. Accessed January 27, 2017.

25. Hopster K, Muller C, Hopster-Iversen C, Stahi J, Rohn K, Kastner S. Effects of dexmedetomidine and xylazine on cardiovascular function during total intravenous anaesthesia with midazolam and ketamine and recovery quality and duration in horses. *Vet Anaesth Analg*. 2014; 41(1): 25-35. doi: 10.1111/vaa.12095

26. Yadav GU, Thorat MG, Bedarkar SN. Efficacy of xylazine as a sedative in cattle. *Veterinary World*. 2008; 1(11): 340. Web site. http://veterinaryworld.org/2008/November/Efficacy%20of%20Xylazine%20as%20a%20Sedative%20in%20Cattle.pdf. Accessed January 27, 2017.

27. Haas DA, Harper DG. Ketamine: A review of its pharmacologic properties and use in ambulatory anesthesia. *Anesth Prog*. 1992; 39(3): 61-68. Web site. http://www.pubpdf.com/pub/1308374/Ketamine-a-review-of-its-pharmacologic-properties-and-use-in-ambulatory-anesthesia. Accessed January 27, 2017.

28. White JM, Ryan CF. Pharmacological properties of ketamine. *Drug Alc Review*. 1996; 15(2): 145-155. Web site. http://www.tandfonline.com/doi/abs/10.1080/09595239600185801. Accessed January 27, 2017.

29. Pavlidou K, Savvas I, Moens YPS, Vasilakos D, Raptopoulos D. The effects of four anaesthetic protocols for maintenance of anaesthesia on trans-diaphragmatic pressure in dogs. *PLoS One*. 2008; 8(10): 1-7. doi: 10.1371/journal.pone.0075341

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Retrospective Study

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Epidemiological Study of Rabies from 2001-2016 in Grenada, West Indies

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ABSTRACT

Objectives: To determine the status of rabies infection in Grenada from 2001 to 2016 in animal species with their locations.
Design and Methods: Cases from animal species submitted for rabies diagnosis to the diagnostic laboratory, St. George's University, School of Veterinary Medicine Grenada, West Indies, between March 2001 and July 2016 were analyzed. Over 3,000 cases of animals suspected or tested for rabies were reviewed. Data included the species, sex, age, and location on the island, vaccination status, test date and the laboratory tests performed. The laboratory tests used for the diagnosis included; direct fluorescent antibody test (DFAT), polymerase chain reaction (PCR) and histopathology on the brain tissue.
Results: Out of 173 animals, 64 were positive, 89 were negative, 9 were inconclusive, 3 were non diagnostic and 8 were excluded due to lack of adequate information. Out of 64 rabies positive animals; 28 were canine, 28 were mongoose, 6 were feline, 1 each were ovine and caprine.
Conclusions: Our results showed that mongoose and dogs pose a threat to humans and other species of animals in Grenada. Stray dog control program coupled with vaccination are helping in reduction of rabies in dogs. A program to control rabies in mongoose in Grenada is warranted.

KEY WORDS: Rabies; Animals; Grenada West Indies; 2001 to 2016.

ABBREVIATIONS: DFAT: Direct Fluorescent Antibody Test; PCR: Polymerase Chain Reaction.

INTRODUCTION

Zoonotic diseases, those which are spread from animals to humans, are estimated to comprise 60% of all diseases afflicting the humans today.[1] Rabies is a zoonotic disease affecting public health, causing about tens of thousands of human deaths annually, such that a majority of the cases are reported particularly among children belonging to the rural parts of developing countries.[2] There are multiple viruses from the genus *Lyssavirus* and family *Rhabdoviridae* that are capable of causing rabies. The viruses associated with this disease include rabies virus, Australian bat lyssavirus and European bat lyssavirus. Rabies virus is transmitted through the saliva of an infected animal when it comes in contact with the mucous membrane or penetrates the skin through scratches and bite wounds.[3,4] It has also been documented to spread less commonly through organ transplant infections.[5,6] Incubation time lasts on an average for about five weeks; however, it can range from a few days to several years. Incubation time varies depending on several viral and host factors including the viral load, the path of viral entry and the proximity of the wounds to the brain. Clinical symptoms of the disease may be implicated as hypersensitivity, increased irritability, aggression, aerophobia and hydrophobia, hypersalivation, vocalization, fever, convulsions and paralysis.

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Rabies virus is transmissible to all mammals and members of the order *Carnivora* and *Chiroptera*, therefore considered as the main reservoir hosts. Canines are the predominant reservoir hosts for the rabies virus globally and are responsible for 99% of the human infections.[2,7] The risk for human transmission from dogs is greatest in Africa and Asia, followed by Latin America.[2]

Rabies is endemic to the island of Grenada and had been clinically suspected as far back as 1902. The first laboratory confirmed case of rabies was reported in 1952 in a Bovine species.[8] Small Indian mongoose (*Herpestes auropunctatus)* were introduced in 27 Caribbean countries including Grenada to control rats and snakes in sugarcane plantations, in the late 19th century.[9] Until 1956, rabies was not laboratory confirmed in mongooses in Grenada, till the time that rabies was diagnosed in a mongoose found dead on the road. Several studies examining rabies in Grenada was conducted nearly 40 years ago and identified mongooses, canines, bovines and felines as the predominantly infected species.[10] The current study is a passive re-examination of the occurrences, patterns, and distribution of rabies infections in animals in Grenada over the last 16 years (2001-2016) to identify targets for preventive care.

MATERIALS AND METHODS

Grenada is a tri-island country located between the Atlantic Ocean and the Caribbean Sea, roughly 100 miles north of Venezuela. The island is separated into 6 different parishes, St. Patrick, St. Mark, St. Andrew, St. John, St. George, and St. David. St. George's University is located at the southernmost tip of the island in the second largest parish, St. George. Cases of animal species suspected for rabies were submitted through SGU small animal hospital, private veterinarians, the animal control unit of Ministry of Health, farmers and pet owners to the St. George's University, School of Veterinary Medicine, Pathobiology Department for rabies diagnosis. All cases submitted between March 2001 and July 2016 were archived from the record of the diagnostic laboratory and included in this study. Over 3,000 cases of animals submitted for necropsy were reviewed and data were collected if the animal was suspected or tested positive for rabies. Data included the species, sex, age, location, clinical signs, vaccination status, test date and the laboratory tests performed for each animal.

In diagnostic laboratory of the School of Veterinary medicine, St. George's University following techniques were employed on the brain tissue for rabies diagnosis;

Histopathology of brain tissue: Cross section of the brain stem and cerebellum was fixed in 10% formalin, processed through paraffin embedding and stained by hematoxylin and eosin. Sections were examined under light microscope for the presence of inclusion bodies in the cytoplasm of neurons. Inclusions were observed bright red in bluish background.

Fluorescent antibody test: Impression smears of cut section of brain stem and the cerebellum were made, fixed in cold acetone and stained with a cocktail of three fluorescein-labeled monoclonal antibodies directed against the nucleocapsid (N) protein of rabies virus manufactured by light diagnostics Rabies DFA reagent, Millipore, Livingston, UK. Slides were viewed under the fluorescent microscope (Nikon Eclipse 80 fluorescence. attachment). Rabies positive smears gave apple color fluorescence.

Polymerase chain reaction (PCR): This was followed as described by Zieger et al.[9] Total RNA was extracted from 30-50 mg homogenized brain tissue (brain stem and cerebellum) using Trizol reagent (Invitrogen, Grand Island New York, USA.). The RT-PCR one step iScript was used on 200-600 ng total RNA per 50 microliter reaction mix. The primers used for amplification targeted a 110 bp fragment of the highly conserved region of the nucleoprotein (N) gene. Primers had following sequence: JW12 5'ATGTAACACCYCTACAATG3' and N 165-146 5-'GCAGGGTAYTTRACTCATA-3'. The RT-PCR and amplification reactions were performed in an Eppendorf Master cycle ProS (Eppendorf AG, Hamburg, Germany). PCR products were visualized using and ethidium-bromide stained 3% agarose gel electrophoresis.

RESULTS

Between March 2001 and July 2016, a total of 173 animals, from 8 different species (91 canines, 46 mongooses, 16 felines, 9 bats, 5 caprines, 3 opposums, 2 ovines, and 1 bovines), were tested for rabies at the St. George's University, School of Veterinary Medicine, Grenada. A total of 64 animals were tested positive for rabies virus out of the 173 tested which included canine (28/91 i.e. 30.76%), mongoose (28/46 i.e. 60.86%), feline (6/16 i.e. 37.5%) ovine (1/2 i.e. 50%) and caprine (1/5 i.e. 20%) (Figure 1). No positive cases were reported in the bats, opposums or bovines. Figure 1 depicts the number of animals tested and the number of positive results for each species. Out of the 173 animals tested, 21 were excluded from the study either because of incomplete submission records,[9] inconclusive results,[9] or non diagnostic samples.[3] The sex of the tested and positive animals between males (31/64) and females (25/64) is included in Table 1. Eight cases, five of which were positive, did not have their sex listed. Yearly distribution of rabies cases have been shown in Figure 2. Majority of the positive cases of rabies were observed in adult animals (47/123), with only a few found in juveniles (8/33), although some did not have their ages listed (9/17). All six parishes reported positive cases for rabies; St. Andrew[12], St. George[15], St. John[11], St. David[9], St. Patrick[7], and St. Mark[4] (Figure 3, Table 2). Commonly reported clinical symptoms included a change in behavior, aggression with unprovoked biting, hypersalivation and dysphagia, ataxia and weakness. Additionally, vomiting, pawing at the face and the inability to close the mouth were also reported. Rarely, dyspnea, nystagmus, muscle tremors were also reported. None of the animals affected by rabies were reported as vaccinated, they were either not vaccinated or their vaccination status was unknown. Only two of the negatively reported cases were indicative of up to date vaccinations. Out of 173 cases tested, 107 were performed during the dry season

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Figure 1: Rabies Cases by Species (2001-2016).

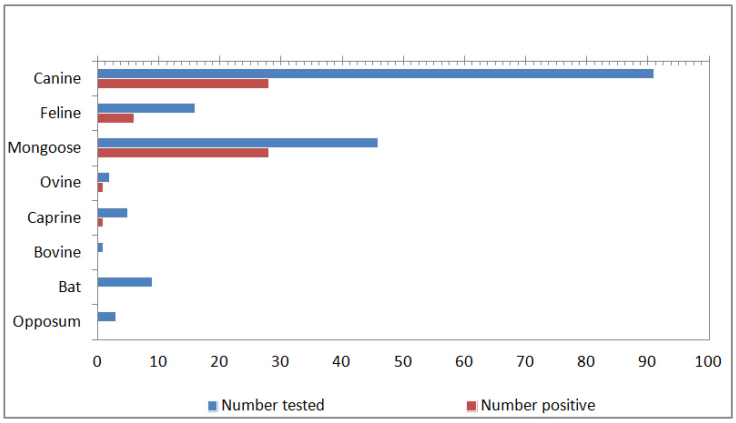

Figure 2: Rabies Cases from March 2001 to July 2016.

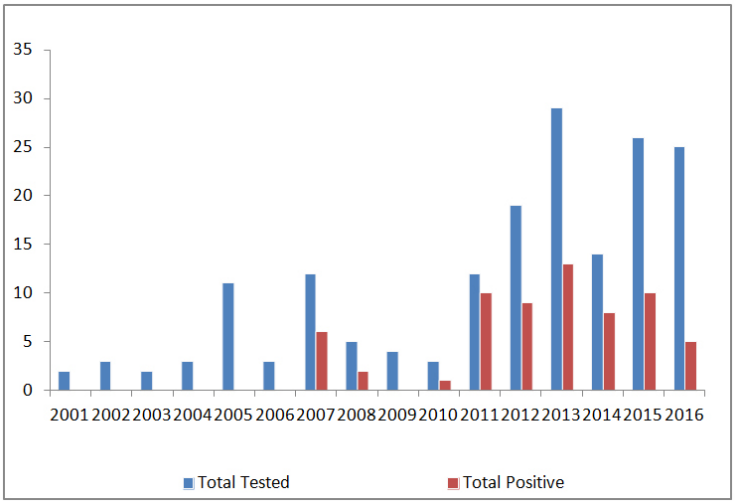

Figure 3: Positive Rabies Cases (2001-2016) in Various Parishes of Grenada.

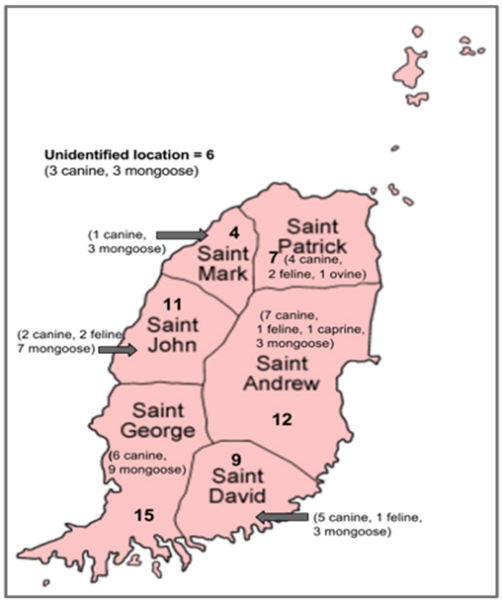

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Table 1: Positive Species Identified by Sex.

Species	Male	Female	Unknown	Total
Canine	21	7	-	28
Feline	1	4	1	6
Mongoose	8	13	7	28
Ovine	1	-	-	1
Caprine	-	1	-	1
Total	31	25	8	64

Table 2: Positive Cases by Species and Location.

Parish	Canine	Feline	Mongoose	Ovine	Caprine	Total
Saint Patrick	4	2	-	1	-	7
Saint Mark	1	-	3	-	-	4
Saint Andrew	7	1	3	-	1	12
Saint John	2	2	7	-	-	11
Saint George	6		9	-	-	15
Saint David	5	1	3	-	-	9
Unidentified location	3	-	3	-	-	6
Total	28	6	28	1	1	64

Table 3: Test Methods Used to Identify rabies Positive Cases (64 Total).

Species	PCR	Histopathology	DFAT+PCR	DFAT+Histopathology	DFAT+PCR+Histopathology
Canine	-	6	11	1	10
Feline	-	-	4	-	2
Mongoose	1	1	23	1	2
Ovine	-	-	1	-	-
Caprine	-	-	-	-	1
Total	1	7	39	2	15

(December-June) and 66 were performed during the rainy season (July-November). Out of 64 cases that tested positive for rabies, 37 occurred in the dry season (58%) and 27 occurred in the rainy season (42%). Three tests were used to identify animals as positive or negative for rabies infection: Direct fluorescent antibody testing (DFAT), polymerase chain reaction (PCR), and histopathology. A breakdown of the tests used to identify positive cases from each species is shown in Table 3. A combination of DFAT and PCR were the most commonly used method, thereby becoming an almost exclusive technique of diagnosis 2013 onwards. Nine samples examined by histopathology alone were considered as inconclusive based on the clinical implications of meningoencephalitis without a confirmation of the presence of Negri bodies. The collected data showed a positive trend for a number of animals tested as well as the number of animals testing positive over the 16 year period (Figure 2). The greatest number of animals both tested for and positive for rabies was in 2013.

DISCUSSION

Previously published reports on clinical surveys of rabies in Grenada from 1952 show that among the domestic species, bovine and canines were primarily affected,[11] however it is believed that reported cases for canines in Grenada have been declining in terms of diagnosis with the institution of vaccination clinics,[10,12] The previous survey reports indicated annual variation in the number of rabies positive cases in various species of animals.[11] No explanation for this variation is found in the reports. In the current survey (2001-2016), up to 2006, no rabies positive animals were observed. However, a large number of animals were diagnosed positive for rabies during 2011 to 2016. This increase in the number of identified positive cases could be attributed to the application of reliable diagnostic procedures in the laboratory to test for rabies, established since 2011 at the St. George's University. Before 2010, this diagnosis was based primarily on the histopathology of the brain tissue.

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In an extensive epidemiological study of rabies in mongoose, conducted in Grenada during the 1960's and 1970's, over half of the human population was exposed to rabies as a result of contact with a mongoose.[10] Data on human exposure and administration of post exposure prophylaxis could not be acquired for the current study. However, in this study, 16 reports (8 canines, 5 felines, 3 mongooses) indicated animals testing positive for rabies to be attacking and biting humans. Since the mongoose is a wildlife species, they are less likely than domestic species and family pets, to be caught and brought in for testing. Although less likely to bite when rabid, ruminants can still transmit the virus to humans through direct contact with their saliva.[5] The lower number of reported cases in this study involving ruminants makes the interpretation of previous data difficult.

Six species of bats including *Artibeas jamaicensis* have been tested for rabies in Grenada during 1968-1977; one bat (in *Artibeas jamaicensis)* was found positive by FAT and mouse inoculation test. Rabies neutralizing antibodies were detected in all six species tested with 40.55% in *A. jmaicensis*.[13] However, since there are no reports of vampire bats in Grenada, the role of other species of bats in Grenada is not known in rabies transmission to humans.[8,13] Only 7 bats were reported in this study, all of which were negative for rabies virus as identified with DFAT and confirmed with PCR. One case of rabies was reported in a *Didelphis opossum* in 1969.[14] Three opossums, another wildlife species in Grenada, were also included in this study and were diagnosed negative by DFAT and confirmed with PCR. More studies need to be conducted with larger sample sizes before bats and opossums can be ruled out as a reservoir or host species for rabies in Grenada.

Although, there is a slight discrepancy in positive cases reported between the dry (58%) and wet (42%) seasons, the significance of this discrepancy needs to be determined. An increase in canine rabies has been observed in North and South America during the spring months in coordination with the mating season and the close interaction between dogs.[15] Grenada's climate is tropical with minimal fluctuations in temperature and decrease in rainfall seen during the months of December to June. It is believed that the lack of temperature variability leads to the disruption of typical breeding seasons in canines in Grenada. However, the hypothesis needs to be confirmed.

The laboratory methods used for the diagnosis of positive rabies case are presented in Table 3. The results indicate that use of DFAT and PCR were most sensitive. The use of DFAT and PCR on all rabies suspected cases is recommended for the rabies diagnosis.

The majorities of dogs on the island are free-roaming or are kept tied outside. In the present survey, a higher incidence of rabies in male dogs was recorded. Further study is warranted to identify the cause of higher incidence of rabies in male dogs. Free-roaming dogs or dogs kept outside are more likely to come in contact with humans. These dogs are also more likely to come in contact with wildlife species, in particular mongoose, which act as host reservoirs for the disease. Contrary to dogs, in this study, more female mongoose was tested positive for rabies. Further study on the behavior of mongoose may explain the higher incidence of female positivity of rabies in this species.

Stray dog control programs, in coordination with vaccination programs, have been helping to decrease canine rabies reservoirs in many Latin American countries since the 1980's.[16,17] Current programs of rabies control in Grenada include veterinary and government facilitated vaccination clinics for animals, education of individuals and children, pre-exposure vaccination of the risk individuals, and post-exposure prophylaxis for humans. However, a regular surveillance system to report the suspected cases of rabies in human and animals is essential for the prevention and control of this fatal disease.

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COMPETING INTEREST

The authors declare that there is no competing interest.

REFERENCES

1. Eloit M. Message from the World Organization for Animal Health. *Weekly Epidemiological Record.* 2016. Web site. http://apps.who.int/iris/bitstream/10665/254403/1/WER9113_177-178.pdf. Accessed March 20, 2017.

2. World Health Organization (WHO) Media center (2016) Rabies fact sheet. Web site. http://www.who.int/mediacentre/factsheets/fs099/en/. Accessed July 19, 2016.

3. Australian Government: Department of Health. Rabies virus and other Lyssavirys (including Australian bat Lyssavirus) exposures and infections. CDNA national guidelines for public health units. Updated 04 Jul 2014. Web site. http://www.health.gov.au/internet/main/publishing.nsf/content/cdna-song-abvl-rabies.htm. Accessed July 19, 2016.

4. Susan S, Ahmad F, Pooneh R. Naser E, Nader H, Peyvand B. Six fatal cases of classical rabies without biting incidents, Iran 1990-2010. *J Clin Virol.* 2012; 54(20): 251-254.

5. Susan F, Shaeila Z, Erika J. US acquired human rabies with symptoms onset and diagnosis. 2012. Web site. https://www.cdc.gov/mmwr/preview/mmwrhtml/mm6139a1.htm. July 19, 2016.

6. Bronnert J, Wilde H, Tepsumethanon V, Lumlertdacha B, Hemachudha T. Organ transplantations and rabies transmis-

ISSN 2475-1286 http://dx.doi.org/10.17140/VMOJ-2-115

sion. *J Travel Med*. 2007; 14: 177-180. doi: 10.1111/j.1708-8305.2006.00095.x

7. Macpherson CNL, Meslin, FX, Wandeler AI. *Dogs, Zoonoses, and Public Health.* Boston, MA, USA: CABI; 2013.

8. Everard COR, James AC, DaBreo S. Ten years of rabies surveillance in Grenada, 1968-1977. *Bull Pan Am Health Organ.* 1979; 13(4): 342-353.

9. Zieger U, Marston DA, Sharma R, et al. The phylogeography of rabies in Grenada, West Indies, and implications for control. *PLoS Negl Trop Dis.* 2014; 8(10): 1-10. doi: 10.1371/journal.pntd.0003251

10. Everard COR., Baer G, James A. Epidemiology of mongoose rabies in Grenada. *J Wildl. Dis*. 1994; 10: 190-196.

11. Thomas D, Delgado A, Lefrancois T, Shaw J, Louison B. Exploring factors related to dog owners' willingness to vaccinate their pets against rabies in Grenada. 2012. Web site. http://www-old.caribvet.net/en/vep-project/epidemiology-survey/exploring-factors-related-to-dog-owners%E2%80%99-willingness-to-vaccinate-th. Accessed March 20, 2017.

12. Thomas D, Delgado A, Louison B, Lefrancois T, Shaw J. Examining dog owners beliefs regarding rabies vaccination during government funded vaccine clinics in Grenada to improve vaccine coverage rates. *Pre Vet Med.* 2012; 110(3-4): 563-569. doi: 10.1016/j.prevetmed.2013.02.009

13. Price JL, Everard COR. Rabies virus and antibody in bats in Grenada and Trinidad. *J Wildl Dis.* 1977: 13: 131-134. doi: 10.7589/0090-3558-13.2.131

14. Everard COR, Murray D, Gilber PK. Rabies in Grenada. *Trans R Soc Trop Med Hyg.* 1972; 66(6): 878-888. doi: 10.1016/0035-9203(72) 90123-x

15. Malaga H, Lopez NE, Gambirazio C. Canine rabies seasonality. *Int J Epidemiol.* 1979; 8(3): 243-245. doi: 10.1093/ije/8.3.243

16. Vigilato MAN, Clavijo A, Knobl T, et al. Progress towards eliminating canine rabies: Policies and perspectives from Latin America and the Caribbean. *Philos Trans R Soc Lond B Biol Sci*. 2013; 368(1623): 20120143. doi: 10.1098/rstb.2012.0143

17. Vigilato MAN, Cosivi O, Knöbl T, Clavijo A, Silva HMT. Rabies Update for Latin America and the Caribbean [letter]. *Emerg Infect Dis* [Internet]. 2013; 19(4): 678-679. doi: 10.3201/eid1904.121482

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Case Report

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Human Chorionic Gonadotrophin Treatment for Sexual Immaturity in a Male Ibex (*Capra Pyrenaica*)

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ABSTRACT

Gonadotrophin deficiency, a condition usually present from birth, can cause delayed puberty in humans and domestic animals, but this has not been confirmed in wild mountain ruminants. During the rutting season (December), signs of sexual immaturity were detected in a 2.5 year-old male Iberian ibex (*Capra pyrenaica*) maintained in captivity. Cryptorchidism of the right testicle was diagnosed, along with complete adherence of the urethral process to the prepuce. The animal was treated with five injections of 500 UI hCG, administered intramuscularly over four weeks (two injections four days apart in the first week, and then one injection per week). Upon completion of the treatment, the right testicle completed its descent into the scrotum, and the urethral process detached from the preputial mucous membrane. A semen sample was collected which revealed oligoasthenospermia, confirming hCG to be at least partially effective in the stimulation of spermatogenesis in ibexes with gonadotrophin deficiency.

KEY WORDS: Wild goat; Ibex; Testes; hCG; Puberty

ABBREVIATIONS: hCG: Human Chorionic Gonadotrophin; RIA: Radioimmunoassay; TUMASG: Transrectal Ultrasonic-Guided Massage of the Accessory Sex Glands.

INTRODUCTION

In wild ruminants, sexual maturation concludes in puberty. The end of the prepubertal period marks the beginning of the reproductive capacity. Puberty, which only occurs in animals showing sufficient body development, coincides with the species' period of mating activity; at this time, photoperiod-induced morphological changes occur in the reproductive tract, spermatogenesis is initiated, and sexual behaviour is first shown. In the Iberian ibex; however, social interactions appear to play an important role in the establishment of puberty, sometimes delaying reproductive behaviour until the age of 2-3years.[1] In humans and domestic animals, puberty can be delayed due to gonadotrophin deficiency, a condition usually present from birth, but this has not been confirmed in wild mountain ruminants. The aim of this case report was to evaluate the efficacy of human chorionic gonadotropin treatment (hCG) to increase the plasma testosterone concentrations in a male Iberian ibex with sexual immaturity.

CASE HISTORY

Iberian ibex sperm samples are routinely collected and cryopreserved at the Animal Reproduction Department of the INIA (Madrid, Spain); the donor animals are kept in captivity as a part of the conservation program. Sperm are collected after anesthetization with 50 µg/kg intravenous detomidine (Domosedan; Pfizer Inc., Amboise Cedex, France), 0.5 mg/kg ketamine hydrochloride (Imalgene 1000; Rhône Mérieux, Lyon, France), and 0.5 mg/kg tiletamine-zolazepam (Zoletil 100; Virbac España S.A., Barcelona, Spain), maintained with 1.5% isoflurane (Isobavet; Intervet/Schering Plough Animal Health, Madrid, Spain) in oxygen (flow rate 2.5

VETERINARY MEDICINE
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L/min) administered *via* an endotracheal tube (all animals are placed in the lateral recumbent position). A pulse oximeter is used to monitor the condition of animals throughout. After clipping the hair around the penis and cleaning the surrounding area, the latter organ is manually made to protrude, and maintained protruded with the help of gauze placed caudal to the glans. Semen is obtained by transrectal ultrasound-guided massage of the accessory sex glands (TUMASG) combined with electroejaculation.[2] Anaesthesia is then reversed *via* the administration of 0.7 mg/kg yohimbine hydrochloride (Sigma, St. Louis, MO, USA) (half of the dose injected intravenously, the other half intramuscularly). The animals recover fully within 16 min.

During the rutting season (December), signs of sexual immaturity were detected in a 2.5 year-old-male Iberian ibex, from which sperm could not be collected by the normal procedure. A lack of inguinoscrotal migration of the right testicle was detected by palpation of the testes, and right cryptorchidism was diagnosed. In addition, the urethral process completely adhered to the preputial mucous membrane (Figures 1A and 2A). To complete the examination, the testicular diameter and the echotexture and size of the accessory sex glands were determined by transscrotal and transrectal ultrasonography respectively, employing a 7.5 MHz linear array probe (Prosound 2, Aloka CO., LTD, Tokyo, Japan). The non-descended testicle was smaller than the left testicle (diameter 1.49 cm compared to 1.86 cm), and hypoechogenic. The accessory sex glands were small[3] but normal in appearance (bulbourethral gland area 0.25 cm^2, mean seminal vesicle area 0.21 cm^2).

The ibex was treated with five injections of 500 UI hCG (VeterinCorion®, DivasaFarmavic S.A, Barcelona, Spain) administered intramuscularly for the first 4 weeks (2 injections 4 days apart in the first week, and then one injection weekly). Blood samples from the jugular vein were collected in lithium heparin collection tubes (BD Vacutainer®, Becton Dickinson Co., Plymouth, UK) once per week to assess the plasma testosterone concentrations by radioimmunoassay (RIA); single assay; sensitivity 0.06 ng/ml; intra-essay CV 11.5%. Blood samples were centrifuged at 1900 g for 15 min and the plasma was immediately separated and stored at -20 °C until determination of the testosterone concentration by RIA.

Upon completion of the treatment, the animal was anesthetized once again, the above-mentioned examinations repeated, and a new attempt to collect the semen was made. The right testicle was found to have completed its descent into the scrotum and the urethral process had detached from the preputial mucous membrane (Figures 1B and 2B). The right testicle now had a normoechogenic echotexture, and had reached a diameter of 1.58 cm (the diameter of the left testicle was 1.97 cm) (Figure 3). The bulbourethral glands remained the same size, but the mean area of the seminal vesicles had increased to 0.26 cm^2. The collected semen sample showed a very low sperm concentra-

Figure 1: A) Before hCG Treatment the Urethral Process was Completely Adhered to the Preputial Mucous Membrane. B) After Treatment the Urethral Process had Detached from the Preputial Mucous Membrane.

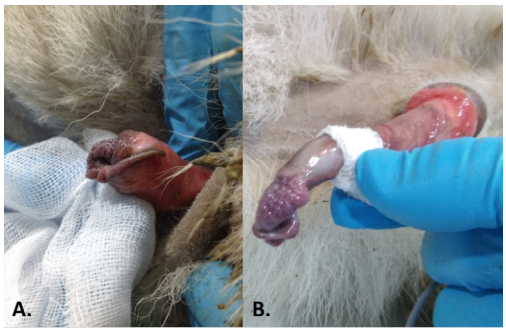

Figure 2: A) Cryptorchidism of the Right Testicle before Treatment with hCG. B) The Right Testicle Descended after Treatment with hCG.

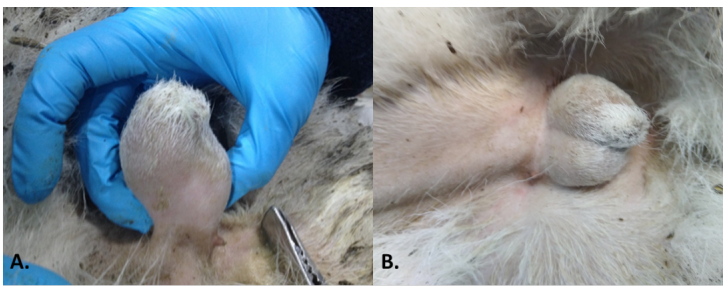

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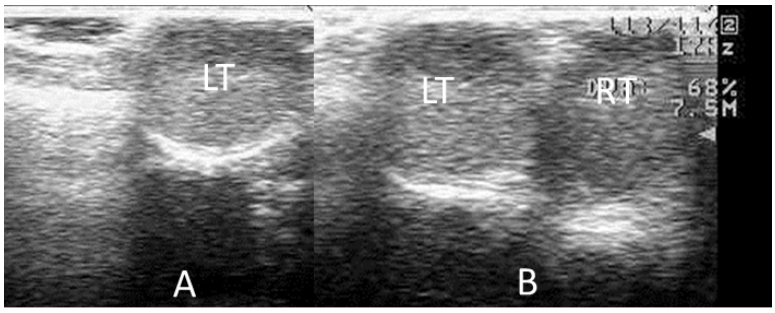
Figure 3: A) Left Testicle (LT) before Treatment with hCG. B) Left (LT) and Descended Right Testicle (RT) after Treatment with hCG.



tion, and most sperm were non-motile (oligoasthenospermia). Motility was assessed using a phase contrast microscope (Nikon Eclipse model 50i; negative contrast). Before treatment, plasma testosterone concentration was maintained on basal values, but reached a value of 8.0 ng/mL at 24 h after each hCG administration, falling again to basal values before the next measurement.

This study was conducted according to procedures approved by the INIA Ethics Committee (reference number CEEA 2014/027), and appropriate guidelines were followed in accordance with the Spanish Policy for Animal Protection (RD53/2013), which conforms to European Union Directive 86/609 regarding the protection of animals used in scientific experiments. The INIA Ethics Committee approved the entire study.

DISCUSSION

Natural adhesions of the urethral process and glans penis to the prepuce are common in prepubertal lambs and goats.[4] However, in a 2-year-old Iberian ibex male, it is a sign of sexual immaturity. In bovids, the urethral process becomes free under the influence of testosterone, and the penis separates from the preputial mucosa. This separation never occurs in early-castrated lambs[4]: the fusion between the urethral process and the preputial mucous membrane persists when lambs are castrated during the prepubertal period.[5] This suggests that the rise in plasma testosterone in animals approaching puberty is involved in the aforementioned separation.

Plasma testosterone concentrations vary with season. In normal, mature ibexes, the testosterone concentration remains at baseline during winter and spring, but begins to rise in September, reaching a maximum (8 ng/mL) in October and November, before falling again to basal levels in January.[3] Testosterone stimulates the growth of the vaginal process, the secretion of calcitonin gene-related peptide by the genitofemoral nerve (thus providing directional guidance to the gubernaculum), and then the regression of the gubernaculum, and the constriction of the inguinal canal.[6] Males that produce insufficient testosterone suffer from reduced fertility, but in some mammalian species, this can be improved with hormone therapy.[7,8] For instance, hCG can be used to increase follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels, which then stimulates the Leydig cells to secrete testosterone.[8] After injecting hCG into bulls, plasma LH has been reported to increase after a few minutes, stimulating testosterone production.[9] The present results suggests that in ibexes, the administration of hCG can induce a temporary increase in plasma testosterone, which can help the testes relocate to the scrotum.

Although, the post-treatment sperm concentration of the present ibex was low (oligospermia), and the sperm cells were mostly immotile, their simple appearance shows that hCG treatment stimulated spermatogenesis. The fact that treatment with hCG allowed the undescended testis to reach the scrotum, together with the increase in the size of both testes and the seminal vesicles, suggests that the sexual immaturity of this ibex was related to a gonadotrophin deficiency.

CONCLUSION

This is the first report of a sexual immaturity process in an ibex species with adhesions of the urethral process to the prepuce and cryptorchidism. hCG administration was effective to treat these alterations. More frequent treatment with hCG should be tested to determine whether full sperm functions can be recovered.

ACKNOWLEDGEMENTS

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CONFLICTS OF INTEREST

The authors declare they have no conflicts of interest.

REFERENCES

1. Santiago-Moreno J, Gómez-Brunet A, Toledano-Díaz A, Pulido-Pastor A, López-Sebastián A. Social dominance and breeding activity in Spanish ibex (*Capra pyrenaica*) maintained in captivity. *Reprod Fertil Dev*. 2007; 19(3): 436-442. doi: 10.1071/RD06122

ISSN 2475-1286 http://dx.doi.org/10.17140/VMOJ-2-116

2. Santiago-Moreno J, Castaño C, Toledano-Díaz A, Esteso MC, López-Sebastián A. Cryopreservation of aoudad (*Ammotragus lervia sahariensis*) sperm obtained by transrectal ultrasound-guided massage of the accessory sex glands and electroejaculation. *Theriogenology*. 2013; 79(2): 383-391. doi: 10.1016/j.theriogenology.2012.10.011

3. Coloma MA, Toledano-Díaz A, Castaño C, et al. Seasonal variation in reproductive physiological status in the Iberian ibex (*Capra pyrenaica*) and its relationship with sperm freezability. *Theriogenology*. 2011; 76: 1695-1705. doi: 10.1016/j.theriogenology.2011.07.001

4. Smith MC, Sherman DM. *Goat Medicine*. 2nd ed. Hoboken, NJ, USA: Wiley-Blackwell; 2009.

5. Belonje PC. Observations on the post-natal development of the penis in Merino ram lambs and wethers: The possible relationship to the passage of urinary calculi. *J S Afr Vet Assoc*. 1965; 36 (3): 381-383. Web site: http://hdl.handle.net/10520/AJA00382809_3438. Accessed April 9, 2017.

6. Amann RP, Veeramachaneni DNR.Cryptorchidism in common eutherian mammals. *Reproduction*. 2007; 133(3): 541-561. doi: 10.1530/REP-06-0272

7. Karaman IM, Kaya C, Ozturk M, Pirincci N, Yimazgumrukcu G, Tuken M. The effects of human chorionic gonadotrophin on normal testicular tissue of rats: Dose-dependence and reversibility. *BJU Int*. 2006; 97: 1116-1118. doi: 10.1111/j.1464-410X.2006.06139.x

8. Oliveira LR, Homma TK, Woloszynek RR, Brito VN, Longui CA. Gonadal response after a single-dose stimulation test with recombinant human chorionic gonadotropin (rhCG) in patients with isolated prepubertal cryptorchidism. *Basic Clin Androl*. 2016; 26: 13. doi: 10.1186/s12610-016-0039-2

9. Katongole CB, Naftolin F, Short RV. Relationship between blood levels of luteinizing hormone and testosterone in bulls, and the effects of sexual stimulation. *J Endocrin*ol. 1971; 50: 457-466. doi: 10.1677/joe.0.0500457

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Research

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Endoscopic Evidences of Upper Respiratory Tract Disorders in Horses and Donkeys

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ABSTRACT

Background: Upper respiratory tract (URT) obstructive disorders are a common problem among horses and are often associated with poor performance and respiratory noise during exercise.
Aim: The study is proposed to visualize the upper respiratory tract of horses and donkeys by endoscopic examination, aiming to highlight and clarify the disease conditions that causing respiratory distress and subsequenly affect negatively on the work and sports ability of these animals.
Materials and Methods: The present study employed the endoscopic examinations on the upper respiratory tract of 45 stallions, 30 mares, 10 foals and 38 donkeys, suffered from upper respiratory tract distresses. These animals were of various ages and weights.
Results: Investigation of these animals proved that the upper respiratory tract disorders were numerous in horses and prevailing in donkeys. The pharyngeal region was more affected than the other parts of the upper respiratory tract. In addition, squamous cell carcinoma, cyst and granuloma in the guttural pouches have been taken care of, regardless of its rare occurrence. The ethmoidal glandular cyst acini, pharyngeal mycosis, and pharyngeal tonsillitis were considered critical evidences for the study.
Conclusion: Endoscopy of the upper respiratory tract of horses and donkeys should be a standard diagnostic technique for all upper respiratory disorders in equine.

KEY WORDS: Horses; Donkeys; Guttural pouches; Upper respiratory disorders; Equine endoscopy.

ABBREVIATIONS: URT: Upper Respiratory Tract; DDSP: Dorsal Displacement of Soft Palate; PLH: Pharyngeal Lymphoid Hyperplasia; EE: Epiglottic Entrapment.

INTRODUCTION

The equine respiratory tract is a highly specialized system facilitating the movement of large volumes of air in and out of the lungs each minute. Any structural or functional disorder associated with the lungs can lead to exercise intolerance and poor performance.[1]

Respiratory disease was identified in 40% to 42% of racehorses presented for poor performance evaluation. Of the horses identified with respiratory dysfunction, dorsal displacement of the soft palate was the most commonly identified respiratory disorder, affecting 29% to 35% of racehorses. Laryngeal hemiplegia was identified in 17% to 24% of the racehorses. Pharyngeal collapse was identified in 27% in one study and in only 1.5% in another study. Retrospective analysis of sport horses presented for poor performance also identified respiratory disease disorders in 54 of 77 (70%) sport horses. Laryngeal hemiplegia was identified in 40% of these horses. Dorsal displacement was identified in only 11%, and pharyngeal collapse was identified in 30%. Results of the retrospective analysis of sport horses suggest that respiratory compromise may be over-represented in this population. Additionally, laryngeal hemiparesis

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and pharyngeal collapse represent most of the respiratory problems in sport horses.[2]

Respiratory diseases are the result of exposure to dust and other airborne irritants. Besides; bacteria, mold and ammonia levels. Excess nasal discharge, coughing, sneezing and mucus secretion are all signs that a horse may be suffering from respiratory problems.[3]

Endoscopy is become routine over the last decades because it an excellent tool for diagnosis of the respiratory disorders. It facilitates visualization of inaccessible areas of the pharynx, larynx, guttural pouches and trachea.[3-5]

Also, the upper respiratory endoscopy is used for diagnosing many of the respiratory disorders namely dorsal displacement of soft palate (DDSP), epiglottic entrapment, roaring, infections and taking samples for bacterial cultures and histopathological examinations. Biopsy samples are assumed for diagnosing many types of tumors, inflammatory processes, as well as removal of foreign objects that have been inhaled into the respiratory system.[6]

The aim of the current study is directed toward the endoscopic examination of upper respiratory tract throughout highlighting and clarification of the disease conditions that are met with the respiratory distressed horses and donkeys, as these diseases or abnormalities negatively affect work and sports ability. Consequently, the study will assist to pave away for the hopeful and future treatment of such disease evidences.

MATERIAL AND METHODS

The present work employed the endoscopic examination of the upper respiratory tract of 45 stallions, 30 mares, 10 foals and 38 donkeys suffering from upper respiratory tract distress. These animals belonged to various age groups and weights (Table 1). The study was conducted following the receipt of the approval from the Animal Ethics Committee of Faculty of Veterinary Medicine, Cairo University, Giza, Giza Governorate, Egypt.

Prior to the endoscopic examination using equine flexible video-endoscope (Eickemyer 3000 Vmec, Germany), each animal was subjected to fasting for 4-6 hours and withheld from water consumption for 4 hours. Each animal twitched and stood in the stanchion. Vicious animals were injected with Xylazine HCl (Xyla-Ject 2%®, Adwia Co. SAE- Egypt; 1.1 mg/kg. b. wt) used as a sedative to mediate their long-term examination.

The nasal passage, pharynx, larynx and trachea up to the carina were examined to record any structural and functional abnormalities concerning the guttural pouch entry and drainage. To perform the endoscopic visualization of the guttural pouch through the working channel, biopsy forceps were used as a guide to pass through the cartilaginous flap. The endoscope was then pushed forward and rotated to roll the fibrocartilage axially, which enabled the endoscope to enter smoothly. As the endoscope passed through the opening, the salpingopharyngeal fold was clearly recognized in Figure 1a, 1b, 1c and 1d.

The endoscope brush was inserted into the orifice of Foley catheter until the tip of the catheter. The stinted Foley catheter was introduced into the nasopharynx through the nasal cavity contralateral to the side of the distended guttural pouch as demonstrated in Figure 2a. The endoscope was introduced through the ipsilateral nasal cavity until the pharyngeal ostium to visualize the affected guttural pouch. Using the endoscopic biopsy forceps, the working channel was taken out and the tip of the Foley catheter was passed through the pharyngeal ostium of the guttural pouch such that the balloon on the Foley catheter was distended with water or saline solution as in Figure 2b. The catheter was left *in situ* in a donkey and in a foal as demonstrated in Figure 2c and 2d.

The degree of pharyngeal lymphoid hyperplasia (PLH) was graded as: Grade 0 - a few small white follicles over the dorsal pharyngeal wall; Grade 1 - mainly small white follicles with occasional larger pink follicles over the dorsal pharyngeal wall and extending laterally to the level of the guttural pouch ostia; Grade 2 - overing pink and white follicles covering the entire dorsal and lateral pharyngeal walls and often also involving the dorsal surface of the soft palate; and Grade 3 - large pink,edematous follicles covering all visible mucosa of the pharynx and sometimes including polyps according to the criteria described by Raker & Boles.[7]

The degree of laryngeal hemiplasia (LH) was graded as Grade 0 - synchronous full abduction and adduction of the left

Table 1: Demonstrating the Number, Age and Body Weight of Animals of Study.

Animal	Number		Age/year	Body weight/kg
	Normal	Diseased		
Foals	2	8	6 months-3 years	100 - 250
Stallions	20	25	11-17	450 - 600
Mares	21	9		
Donkeys	24	14	3-6	150 - 250
Total	67	56		

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Figure 1: Showing the Technique Applied for Guttural Pouch Entry.
a. A Biopsy Forceps was Approached to the Cartilaginous Flap
b. The Biopsy Forceps was Inserted Underneath the Cartilaginous Flap
c. Opening of the Cartilaginous Flap to Enter the Guttural Pouch.
d. The Biopsy Forceps Introduced into the Guttural Pouch.

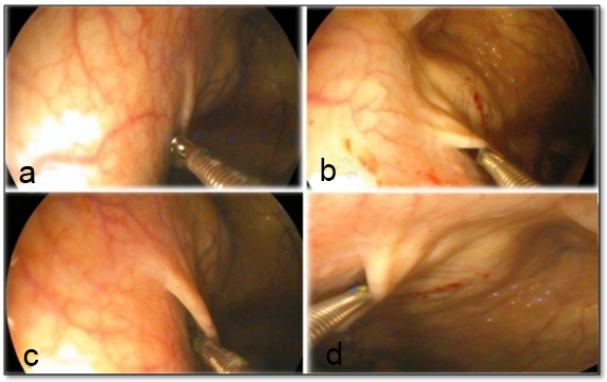
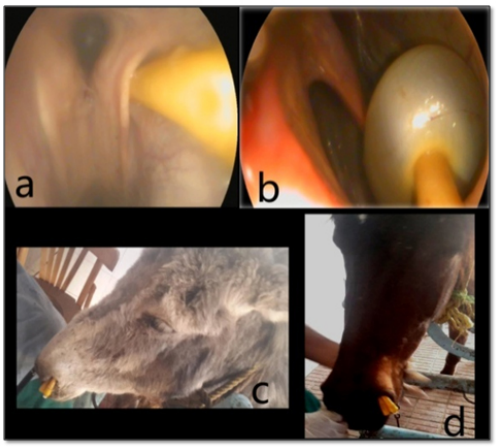


Figure 2: Illustrating the Foley Catheter Placement for Drainage of Guttural Pouch.
a. The Foley Catheter was Introduced into Cartilaginous Flap.
b. The Foley Catheter was Inflated
c. The Foley Catheter was Exteriorized from the Nostrils of a donkey.
d. The Foley Catheter was Placed Out of a horse Nostril.



and right arytenoid cartilages; Grade 1 indicates asynchronous movement (hesitation, flutter, adductor weakness) of the left arytenoid cartilage during any phase of respiration. Full abduction of the left arytenoid cartilage is (as compared to the right) inducible by nasal occlusion or swallowing. Grade 2 indicates asynchronous movement (hesitation, flutter, adductor weakness) of the left arytenoid cartilage during any phase of respiration. Full abduction of the left arytenoid cartilage cannot be induced and maintained by nasal occlusion or swallowing. Grade 3 is marked by asymmetry of the larynx at rest and no substantial movement of the left arytenoid cartilage during any phase of respiration; and Grade 4 is characterized by laryngeal asynchrony or asymmetry not included in any one of the previous grades.[8]

The degree of dorsal displacement of soft palate (DDSP) was graded according to the following criteria: Grade 0 - displacement is not elicited by swallowing; Grade 1 - displacement is elicited by swallowing, but is restored to the normal position after one subsequent swallowing movement; Grade 2 - displacement is elicited by swallowing, but is restored to the normal position after two or more subsequent swallowing movements; Grade 3 - displacement occurs easily in no association with swallowing. The degree of epiglottic entrapment (EE) was graded according to the following criteria: Grade 0 - not found; Grade 1 - the lateral part of the epiglottis alone is covered with the arytenoepiglottic fold; Grade 2 - the tip of the epiglottis alone is covered with the arytenoepiglottic fold; Grade 3 - almost the entire epiglottis is covered with the arytenoepiglottic fold, the assessment of appearance of disorders other than upper respiratory tract disorders was depended upon the description of.[9]

The endoscopic biopsy samples and the specimens of humane euthanized animals were fixed in formol saline 10%, washed, dehydrated, cleared, embedded in paraffin wax sectioned and stained with hematoxylin and eosin (H&E) following,[10] then examined microscopically.

Sterile swab was taken from the growth in the palatopharyngeal fossa of a stallion and sent to the laboratory. Slide culture technique was used to detect fungal morphology after growth in Sabouraud dextrose agar plate.[11]

All data were collected and evaluated statistically by using an Microsoft Office Excel package 2007.[12]

RESULTS

Clinical Appraisal

The endoscopic examination of the 123 animals reported were categorized into 56 diseased animals constituting a percentage of (45.5%), encompassing 25 stallions (44.6%), 9 mares (16%), 8 foals (14.4%) and 14 donkeys (25%). The remaining 67 animals constituting a percentage of (54.5%) were found to be endoscopically normal; including 20 stallions (29.9%), 21 mares (31.3%), 2 foals (3%), and 24 donkeys (35.8%) as shown in Graph 1. The number, age and body weight of the animals in the study were recorded in Table 1. The lesions detected during endoscopic examination were shown in Table 2. In addition, the lesions encountered in donkeys were demonstrated in Table 3.

Disorders of Ethmoid Bone

Ethmoidal fibromatous swelling: The 2 foals in the diseased conditions constitute (14.4%) of the affected foals and 1 stallion represents (44.6%) of the total number of diseased stallions. Endoscopically, the ethmoidal fibromatous swellings were marked as lesions originating from the ethmoidal labyrinth at the caudal

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Graph 1: Showing the Percentage of the Diseased and the Normal Animals.

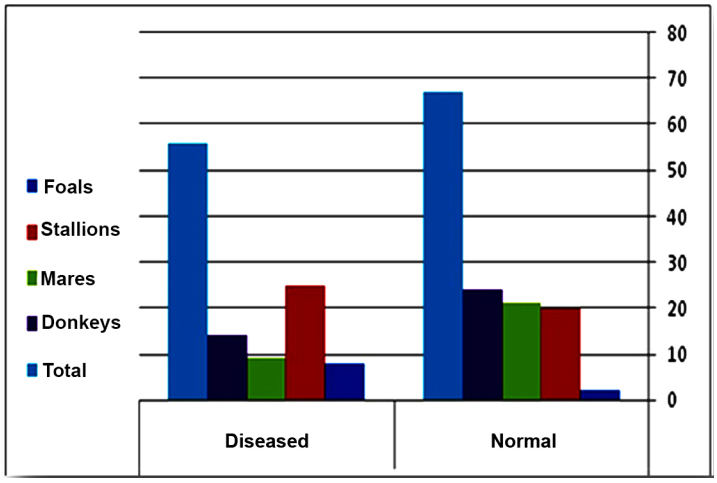

Table 2: Showing the Lesions Encountered in Animals of Study.

No. of Stallions	No. of Mares	No. of Foals	Lesions
3	-	-	Epiglotic entrapment
1	-	-	Dorsal displacement of soft palate
6	2	-	Pharyngitis
1	-	-	Fungal growth in the palatopharyngeal recess
1	-	-	Squamous cell carcinoma in guttural pouch
-	1	-	Cyst formation in guttural pouch
1	-	-	Fibrocartilagenous mass in the trachea
1	1	-	Enlarged tracheal spike
1	1	1	Lymphoid hyperplasia
9	3	-	Recurrent laryngeal neuropathy
-	1	2	Pharyngitis and tracheitis
1	-	2	Ethmoidal Fibromatous swelling
-	-	1	Guttural pouch tympanitis
-	-	1	Rostral displacement pharyngeal arch
-	-	1	Ethmoidal glandular cystic acini

Table 3: Demonstrating the Lesions Encountered in Donkeys.

No. of donkeys	Lesions
1	Tracheal collapse
13	Pharyngitis and tracheitis

aspect of the middle meatus. The lesion grew rostrally into the nasopharynx. The histopathological picture of these swellings showed normal histological structure of the cartilage (a), bone (b), and the fibromatous growth (c) represented by the swelling as displayed in Figure 3.

Ethmoid glandular cyst acini: In Figure (4), the right nasal passage mucosal lining was found to be swollen in the region of the middle meatus anterior to the turbinate and this swelling prevented viewing of the ethmoid turbinate *via* the middle meatus as a large mass protruded below the base of the middle nasal ethmoid conchae (a). Slight trauma to this structure by the end of the endoscope induced further hemorrhage. The lateral radiographic projection, revealed presence of ovoid-shaped densities super-imposed to the ethmoid region that confirmed the location and extent of this mass (b). On performing post-mortem exami-

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Figure 3: Showing the Endoscopic View of the Cystic Dilatation in the Ethamoid Bone (a), the Lateral Radiographic Picture (b) (White Arrows), the PM Depiction (Red Arrow) (c) and the Cystic Dilatation (cs) of the Glandular Acini (d).

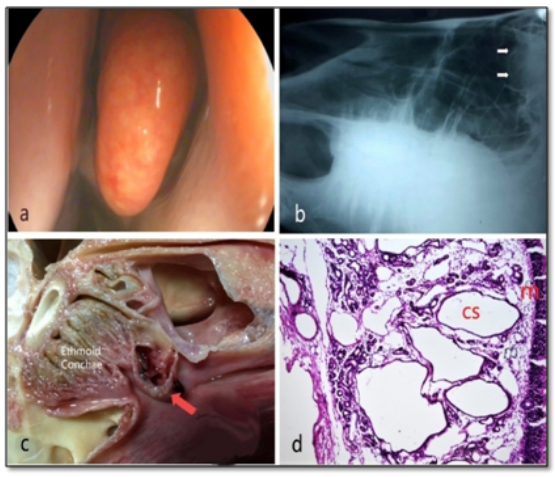
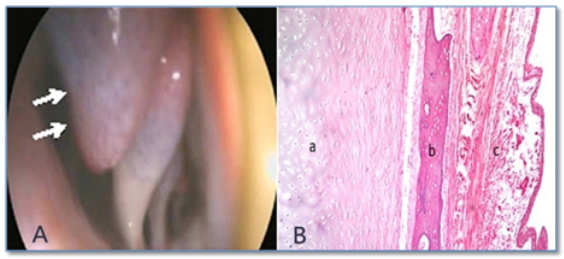


Figure 4: Illustrating Ethmoidal Fibromatous Swelling in a Yearling foal (A) and the Histopathological Section (B) Showing the Normal Cartilage (a), Normal Bone (b), and the Fibromatous Growth (c).



nation, the ethmoid indicated the presence of the remnants of clotted blood and a thin lining membrane (c). On the histopathological section (d) the ethmoid cystic dilatation presented in the glandular acini (cs) with inflammatory cells infiltration (m) at the lamina propria of mucosa H&E×16.

Pharyngeal Disorders

Pharyngeal mycosis (Asperiglosis): During pharyngeoscopy of a senile stallion (Figure 5), a whitish growth was observed in the palatopharyngeal recess (a). Microbiological examination of the collected samples revealed the presence of *Aspergillus* sp. Hyphae (b). During the PM examination, this was considered as a fistula between the guttural pouches and the dorsal pharyngeal recess as a sequel to the guttural pouch mycosis.

Lymphoid hyperplasia: In a horse and a foal, grade 1 lymphoid hyperplasia was characterized by the presence of small white follicles in multiple areas over the dorsal pharyngeal wall as demonstrated in Figure 6a. In a horse, grade 2 lymphoid hyperplasia was characterized by many small white lymphoid follicles located over the dorsal and lateral walls of the pharynx and extended to the level of the guttural pouch opening. They were marked by pink and glistening follicles as displayed in Figure 6b. In a horse, grade (3) lymphoid hyperplasia presented as many large coalescing follicles covering the lateral and dorsal pharyngeal wall in some of the soft palate as shown in Figure 6c. In another horse, grade (4) lymphoid hyperplasia was marked by pink, glistening follicles covering the pharynx and the dorsal surface of the nasopharynx such that these follicles coalesced into larger follicles as shown in Figure 6d.The histopathological examination of these follicles revealed abundant amount of lymphocytic cell aggregations as displayed in Figure 6e.

Pharyngitis: Seven horses, 14 donkeys and 1 foal suffered from pharyngitis and tracheitis indicating some mucopurulent discharge with and without hemorrhage in the nasal cavity, scat-

Figure 5: Showing the Endoscopic View of Fungal Growth (a) and *Aspergillus* sp. Hyphae in Culture (b).

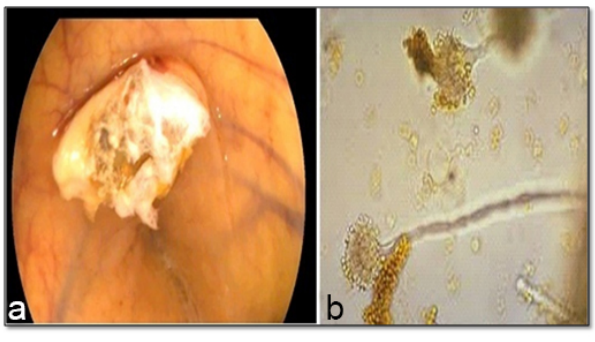


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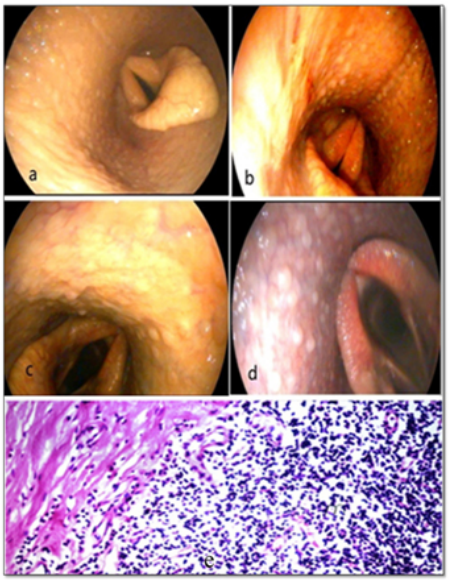
Figure 6: Demonstrating Grades of Lymphoid Follicles; Grade-1 (a), Grade-2 (b), Grade-3 (c), Grade-4 (d) and the Histopathological Picture of Lymphoid Hyperplasia (e)



tered at the conchae and or the nasal septum as illustrated in Figure 7a, 7b and 7c. The histopathological examination revealed abundant amount of inflammatory cell aggregations as shown in Figure 7d.

Corniculitis: Inflammation of the corniculate processes of the larynx (Figure 8a and 8b) was observed in a donkey suffering from continuous coughing. Histopathological examination reports indicated hypertrophy in the mucosal epithelium with inflammatory cells located under the lamina propria (m) as shown in Figure 8c.

Epiglotitis: In a donkey, inflammation of the epiglottis was observed during examinationas is evident in Figure 9a. The mucosal layer showed normal histological structure while,indicating the presence of focal circumscribed round granuloma - like formation of lymphoid cells as displayed in Figure 9b.

Pharyngeal tonsillitis: The inflammation of the lymphoid follicles was located dorsolaterally to the pharynx, between the pharyngeal openings of the auditory tubes. It appeared as a large swelling, protruded into the pharyngeal cavity as shown in figure 10a and 10b. The histopathology sections showed lymphoid hyperplasia as shown in Figure 10c.

Recurrent Laryngeal neuropathy (RLN): Following endoscopic examination, the grading system of arytenoid motility determines the incidence of clinical RLN. Grade 1 as in Figure 11 is viewed in 3 stallions and 1 mare where the adductory and

Figure 7: Showing the Mucopurulent Exudates in the Nasal Cavity of a Horse (a) a Donkey (b) and a Foal (c) and the Inflammatory Cells Infiltration in Mucosa (d) H&Ex40, Resulted from Equine Influenza.

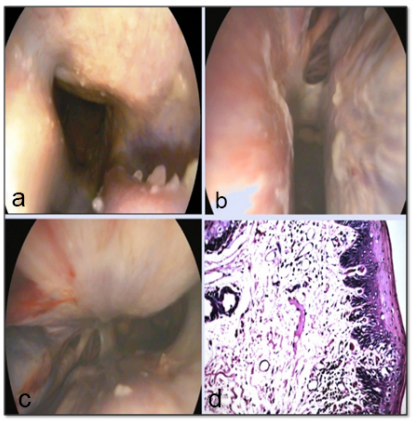


Figure 8: Showing Corniculitis (a) with Hypertrophy in Mucosal Epithelium (b) with Inflammatory Cells in Underlying Lamina Propria (mu) H&Ex40.

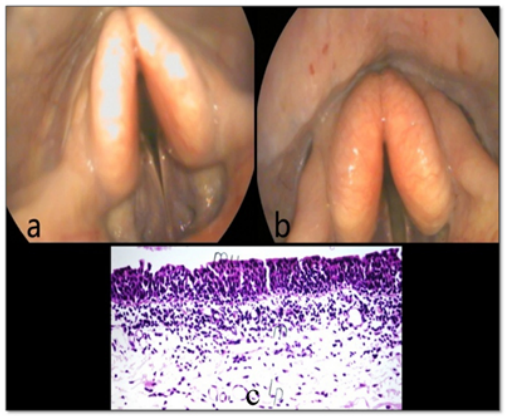


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Figure 9: Showing the Endoscopic Picture of Epiglottitis (a), and the Focal Lymphoid Cells and Granuloma Formation (b) in the Lamina Propria of Mucosal Layer (m).

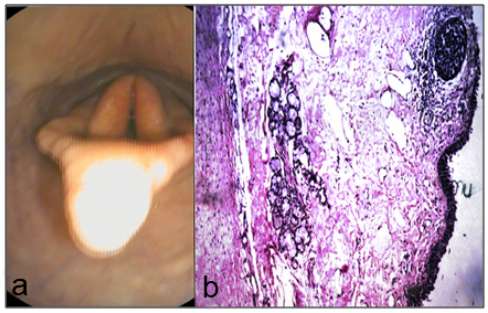
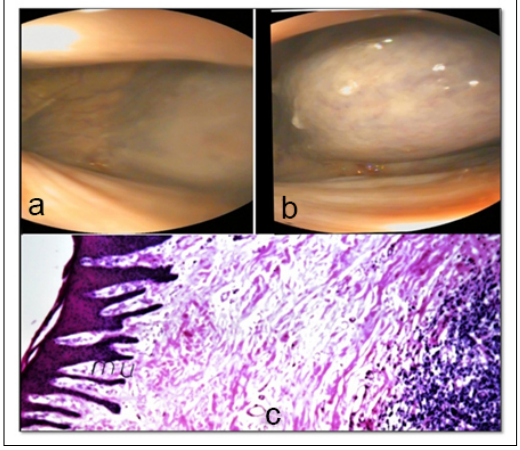


Figure 10: Demonstrating the Various Endoscopic Views of Pharyngeal Tonsillitis (a & b) and Histopathological Section, the Tonsil showing Lymphoid Hyperplasia (c).



abductory movement is asymmetric. As the endoscope is introduced through the right nasal passage, the right arytenoid may appear less abducted and *vice versa*. Grade 2 is endoscopically recognized in 2 stallions and 1 mare; transient flutter or delayed abduction is seen in the left arytenoid. Grade 3 is endoscopically recorded in 1 stallion and 1 mare, the left arytenoid abduction activity is reduced when compared with the right, with periods of prolonged asymmetry, particularly during quiet movements. Grade 4 is endoscopically determined in 3 stallions. The left arytenoids are not full abducted and during adduction, the right arytenoid crossing the midline with some residual movement is observed.The histopathological examination demonstrated in Figure 12 revealed normal histopathological structure of mucosa (mu) and a glandular structure (g) in grade 1 observed in the 3 stallions and a mare. The arytenoid showing focal inflammatory cells aggregation in lamina propria of mucosa in grade 2 was observed in 2 stallions and a mare (Figure 13).

Epiglottic entrapment: In a senile horse, epiglottic entrapment was recognized during the advent of the flexible endoscope, whereas the glosso-epiglottic and aryepiglottic folds are unidentified as illustrated in Figure 14.

Figure 11: Illustrating the Degrees of Recurrent Laryngeal Hemiplasia; First Degree (a), Second Degree (b), Third Degree (c), and Forth Degree (d).

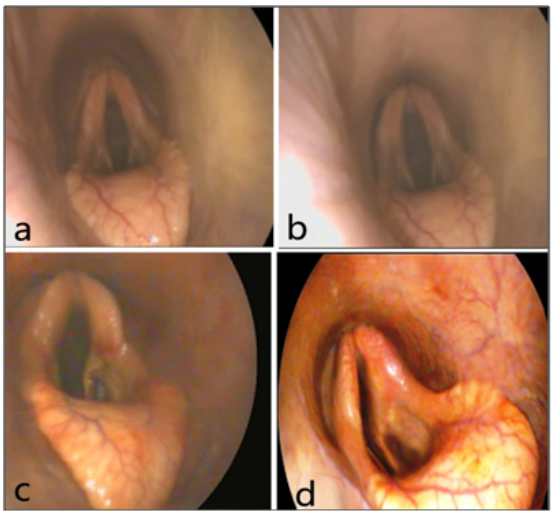


Figure 12: The Histopathological Changes of Arytenoids in Grade 1 Recurrent Laryngeal Neuropathy.

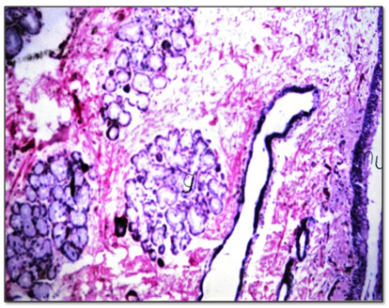
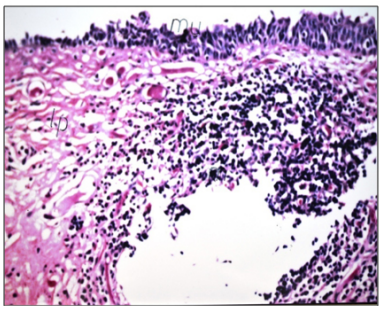


Figure 13: The Histopathological Changes of Arytenoids in Grade 2 Recurrent Laryngeal Neuropathy.



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Rostral Displacement of the Palatopharyngeal Arch (RDPA): A 3-year-old foal exhibited coughing during dancing as observed by endoscopic visualization of the respiratory tract. Pharyngitis and dorsal displacement of the palatopharyngeal arch were obvious observations as shown in Figure 15.

Guttural pouch squamous cell carcinoma: An old horse exhibited the clinical signs including the swelling in the parotid region, dyspnea, dysphagia, and pneumonia. The endoscopic examination of their guttural pouches revealed that one horse suffered from squamous cell carcinoma accompanied with metastasis to the retropharyngeal lymph nodes which resulted inthe enlargement and collapse of the dorsal roof of the nasopharynx and in turn led to dyspnea and dysphagia.

In Figure 16, the squamous cell carcinoma was located in between the stylohyoid bone and internal carotid artery (a). The histopathological changes included the visualization of multiple clusters of neoplastic epithelial cells with eosinophilic cytoplasm and prominent nucleoli together with abundant interstitial infiltration of lymphocytes and mono-nuclear cells (b).

Guttural pouch cyst: Another observation showed cyst formation in the guttural pouch in Figure 17. Endoscopically, the cyst was located in the lateral compartment of the guttural pouch. It was an ovoid shaped structure that contained clear fluid inside, similar to the polyp-like mass which measured 6 cm in length and 4 cm in width and its wall was traversed by vasculatures as shown in (a). Histopathologically, the cyst was a benign growth lined with stratified squamous epithelium between 2 to 8 cells thick. Parallel to the epithelium was a dense layer of fibrous connective tissue. The fibrous connective tissue also formed loose whorls around the blood vessels and was more pronounced in the deeper tissue layer as demonstrated in (b).

Guttural pouch granuloma: As demonstrated in Figure 18, the guttural pouch of a donkey showed a focal circumscribed round mass (a). The histopathology of the trans-endoscopic biopsy samples (b) revealed the formation of granuloma with a necrosed center (m) in the mucosal layer (mu).

Figure 14: Demonstrating the Epiglottic Entrapment in a Horse.

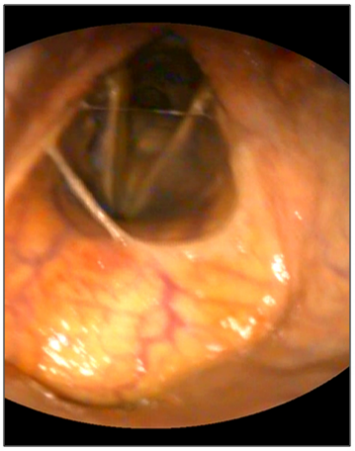

Figure 15: Showing Rostral Displacement of the Palatopharyngeal Arch (Black Arrow) and Adducted Position of the Right Arytenoid Cartilage (White Arrow).

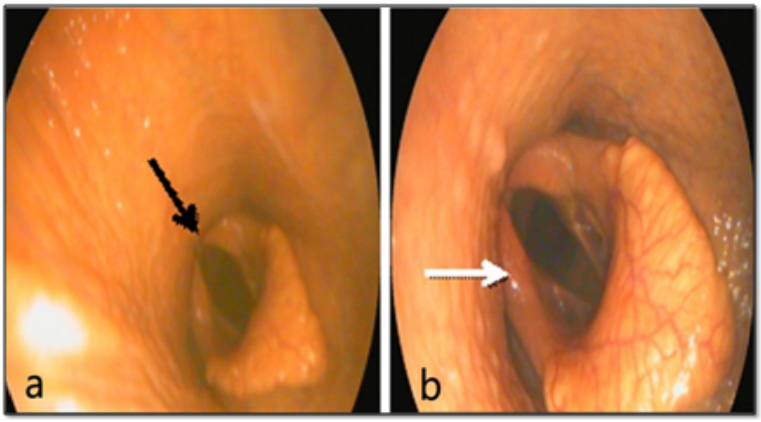

Figure 16: Showing the Location of The Squamous Cell Carcinoma (a) and the Histopathological changes (b) which Revealed Multiple Cluster of Neoplastic Epithelial Cells with Eosinophillic Cytoplasm and Prominent Nucleoli together with Abundant Interstitial Infiltration of Lymphocytes and Mononuclear Cells.

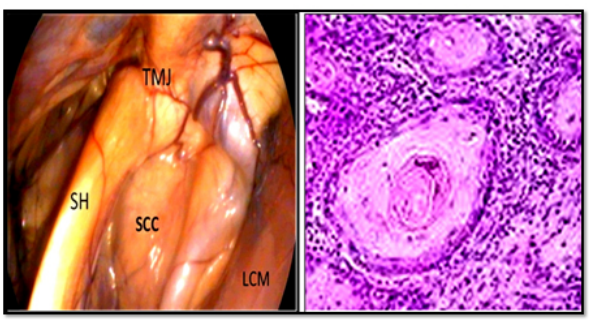

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Figure 17: Showing the Cystic Structure in the Lateral Compartment of the Guttural Pouch of a Stallion (a) and the Cyst was Histopathologically lined by a Layer of Pseudostratified Epithelium (b).

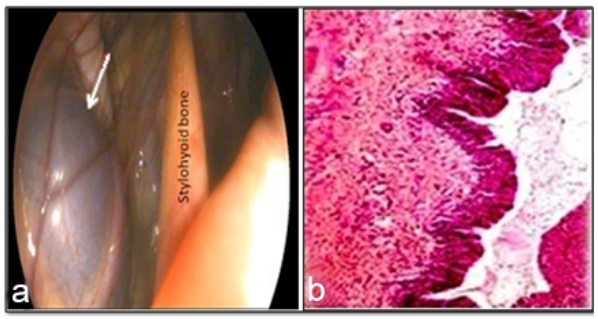
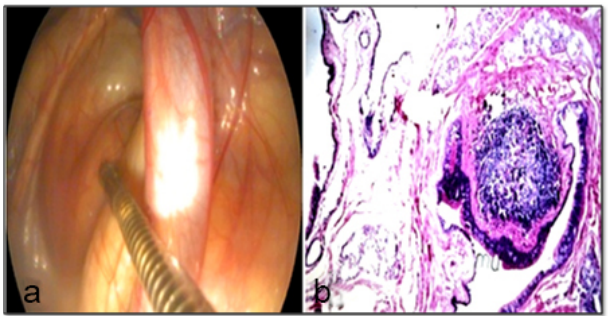


Figure 18: Illustrating theGranulomatous Mass (a) and its Formation in the Histopathological Section (b) Stained with H&Ex16.



Guttural pouch tympanitis: In Figure 19, the clinical picture of a foal suffering from bilateral tympanitis was described in (a), collapse of the dorsal pharyngeal wall and compression of the nasopharynx as the lateral walls moved medially by the distention of the pouches during endoscopic examination was displayed in (b), the infected material within the left guttural pouch was illustrated in (c), and the indwelled and inflated Foley catheter was shown in (d) introduced within the pouch to facilitate irrigation and drainage *via* percutaneous gutturocentesis (e).

The tympany resolves immediately after the Foley catheter enters the guttural pouch, provided that only one guttural pouch is affected. If tympany of the contralateral guttural pouch becomes apparent after the guttural pouch was decompressed, a second catheter needed to be inserted through the pharyngeal ostium of that guttural pouch.

Disorders of Trachea

Pharyngitis and tracheitis: 1 mare, 3 foals and 10 donkeys were diagnosed with pharyngitis and tracheitis using the endoscopic examination. The affected animals showed some mucopurulent discharge with and without hemorrhage in the nasal cavity, scattered at the conchae and or the nasal septum as illustrated in Figure 20a, 20b and 20c.

Tracheal collapse: During the endoscopic examination of a donkey; the trachea appeared to be misshaped dorsoventrally and flaccid resulting in a collapse at the distal cervical part of the trachea. The circumferential or lateral luminal narrowed with stenosis as shown in Figure 21a and 21b.

Tracheal fibroma: As shown in Figure 22, an ovoid mass (22a) was viewed by performing tracheoscopy of a stallion, as an elevated swelling in the midcervical portion of the trachea (22b),of the dimensions 1.5 cm×2 cm (width×length). The histopathological sections of the biopsy samples (22c and 22d) revealed an excessive formation of fibrocytes in the dermal layer and accumulation of inflammatory cells in the lamina propria with the intact mucosal surface (22e).

Tracheal spike: As demonstrated in Figure 23, spike-shaped

Figure 19: Showing Clinical Picture of Guttural Tympanitis (a), Endoscopy of Swelled Pouch in the Pharyngeal Cavity (b), Presence of Pusy Material Inside the Medial Compartment (c), Applied and Inflated Foley Catheter within the Pouch (d), and the 3-year Foal after Irrigation of the Pouch *via* the Indwelled Foley Catheter (e).

Figure 20: Showing Various Forms of Tracheitis in a Mare (a), in a Foal (b), and in a Donkey (c).

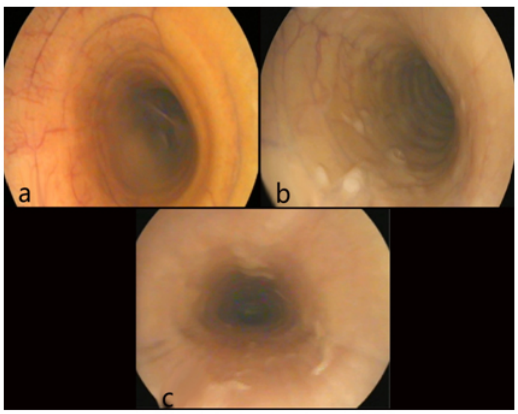


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Figure 21: Showing Tracheal Colapse in a Donkey (a) , and the Dorso-ventral (Blue Arrows) Lumen Reduction at the Distal Cervical Part (b).

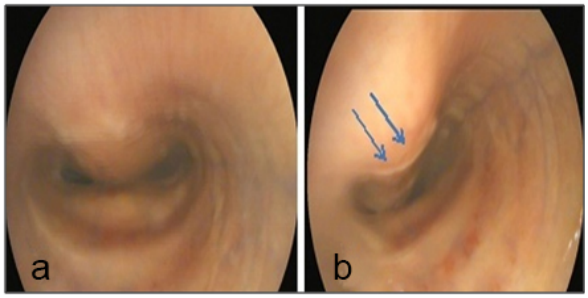


Figure 22: Showing the Ovoid Mass (a), Located at Midcervical Portion of the Trachea (b), Biopsy Sampling (c), the Sampled Site (d), and the Fibrous Connective Tissue Formation in the Underlying Dermis, and the Inflammatory Cells Infiltration in the Lamina Propria (e).

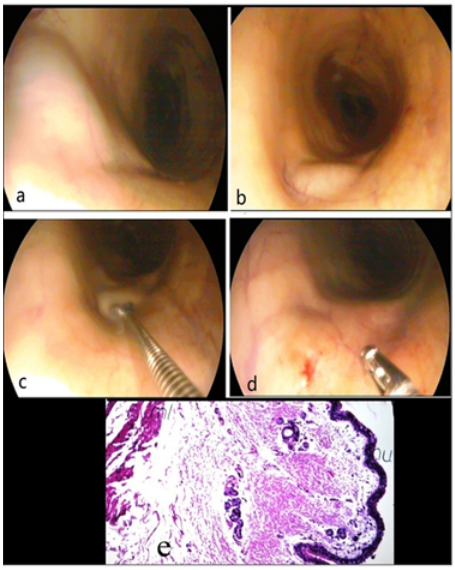
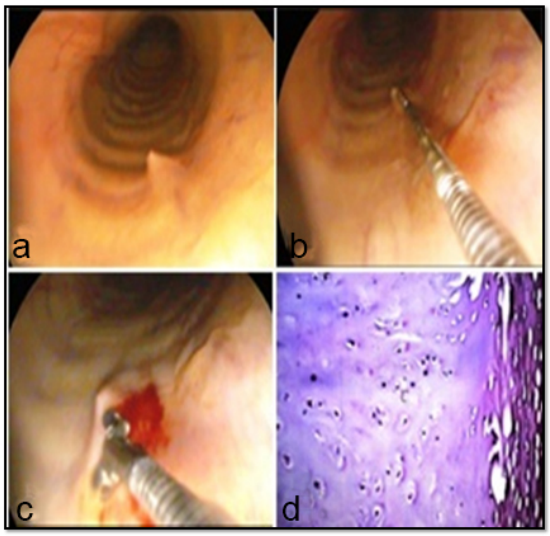


Figure 23: Demonstrating the Tracheal Spike. Normal Endoscopic Appearance (a), Location of the Spike (b), Biopsy Sampling (c), and the Normal Cartilaginous Structure (d).



granulomatous lesions appeared as sporadic nodules (23a) and were observed in the trachea of a stallion and a mare at the proximal tracheal lumen nears the larynx (23b). The histopathological examination of the collected endoscopic samples (23c) showed normal cartilaginous structure and mucous surface (23d).

DISCUSSION

The present study provided evidences of some disorders or disease conditions in 25 stallions (44.6%), 9 mares (16 %), 8 foals (14.4%), and 14 donkeys (25%) exhibited remarkable distress in the upper respiratory tract (URT). Diagnosis was based on case history, clinical appraisal, and physical examination, besides endoscopic, microbiologic and histopathologic investigations.

Donkeys are still used as work animals and play an important role in the economy of the developing countries, but surprisingly little information is available relating to the structure and functional anatomy of the upper airways of this species.[13] Conversely, enormous information have been published for horses in this respect.[14] Therefore the present study included both horses and donkeys to settle down the endoscopic and anatomic depictions variation.

The three cases of ethmoidal fibromatous swellings were considered an incidental visualization throughout the endoscopic examination of the upper respiratory tract in one stallion (represents 44.6% of the total diseased stallions) and two foals (constitute 14.4% of the affected foals). Endoscopically, the lesions grow rostrally into the nasopharynx, and accompanied clinically with respiratory distress and cough. However, another three cases of ethmoid swelling were diagnosed osteoma and an osseous fibroma by Cilliers et al.[15]

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The ethmoid glandular cyst acini in a senile stallion was detected during endoscopic examination, that appeared as large mass protruded below the base of the middle nasal ethmoid conchae. Radiologically and histopathologically revealed ethmoid cystic dilatations with glandular acini with inflammatory cells infiltration in between the lamina propria of mucosa. Similar findings have been reported in a senile stallion by endoscopic visualization and radiographic examination.[16,17] Furthermore, this lesion is not likely a neoplastic process as it does not demonstrate uncontrolled, progressive cell multiplication, which is a hallmark of neoplasia as mentioned by Bell et al.[18]

Pharyngeal asperiglosis in the palatopharyngeal recess has been observed during pharyngeoscopy in a stallion based on the microbiological examination. Similar lesion was located more anteriorly and resulted in the development of a fistula between the right and left guttural pouches and the dorsal pharyngeal recess as reported by Jacobs and Fretz.[19]

Pharyngeal lymphoid hyperplasia (PLH) is a condition occurs in horses involving proliferation of one of the equine tonsillar structures. In the present work, it has been diagnosed in a stallion, two foals, and a mare during endoscopic examination.[20] It was obvious that the pharyngeal tonsil was first effects when upper respiratory infections occurred. This might be due to its common access to pathogens either through inhalation *via* the nasopharynx or ingested through the oropharynx. During the acute phase, the follicles were edematous and hyperemic, with slight ulceration on the most prominent portion. The accompanying nasal discharge is initially serous, then converted into mucopurulent. Concurrently, pharyngeal follicular hyperplasia involves the proliferation primarily of one of the five tonsillar structures in the equine. Its anatomical placement and diffuse nature predisposes the pharyngeal tonsil to inflammation. This inflammation begins acutely due to viral agents and progresses to a chronic nature by the addition of air turbulence and mechanical irritation, secondary bacterial invaders, and caustic agents such as smoke inhalation. Clinically, the most common signs are inspiratory dyspnea and exercise intolerance. Endoscopic exam reveals varying grades of hyperplasia, from a few scattered, white follicles to hyperemic, edematous follicles and polyps involving the entire visible pharyngeal mucosa.[21]

Pharyngitis has been recognized in five senile stallions and two old mares. Although it is very common in young horses, where its prevalence decreases as horses become old and develop immunity.[22] Pharyngitis is an airway inflammation. It is more common in horses and caused by infection that originated due to bacteria and viruses, as well as environmental agents such as endotoxin, ammonia, respirable dust, and metal.[2] It is important to evaluate the extent of pharyngeal inflammation, which classified into four grades based on the degree of severity.[23,24] Endoscopic examination of diseases of the upper airway associated with poor racing performance.[25]

The pharyngeal tonsils are lymphoid-filled follicles on the dorsum of the pharynx are most often seen in young horses, their number and size decreasing at 2 to 3 years of age, and only a few being present at 4 or 5 years.[26]

The etiology of epiglottitis is unknown. But possible predisposing factors include pharyngeal inflammation, intermittent dorsal displacement of the soft palate, epiglottic entrapment, and trauma from ingestion of foreign bodies.[27]

Endoscopically, epiglottitis results in edema, reddening, and thickening of the epiglottis and aryepiglottic fold. Extensive swelling and marked discoloration of the mucosal tissue that attaches loosely to the lingual surface of the epiglottis is common, and swelling of the lingual surface may cause mild to marked dorsal elevation of the epiglottic axis. Cartilage at the tip of the epiglottis may be exposed, resulting in granulation tissue formation. Chondritis of the epiglottic cartilage can develop and may result in epiglottic deformity during healing.[28]

Tympany is usually unilateral, but bilateral cases have been reported, diagnosis is based on clinical examination, endoscopy, with or without radiography, or percutaneous gutturocentesis. The latter technique should be performed with great care, as there is risk of damage to nervous and vascular tissues.[29]

Recurrent laryngeal neuropathy (RLN) is considered one of the upper airway obstructive diseases. The knowledge of its etiology, pathogenesis, methods of assessment and the critical evaluation of treatment.[30] True hemiplegia, where one-half of the larynx, almost invariably the left side, shows no active abductory or adductory motility, can be determined easily at rest. However, controversy still exists regarding the clinical significance of asymmetric or asynchronous movements of the arytenoid cartilages, it is more common in large breed-horses and has been associated with the height of horses.[31] However, the disease is considered to affect predominantly large young male animals.[32] A horse with small epiglottis can perform well and a horse with a very robust large looking epiglottis can still displace its palate.[33]

The principle means of differentiating entrapment of the epiglottis by a redundant arytenoepiglottic fold from elongation of the soft palate is through endoscopic examination of the larynx and pharynx. When entrapment of the epiglottis occurs the arytenoepiglottic folds become redundant and curl over the epiglottis obscuring only the anterior border of the epiglottis from the view of the observer. However, when elongation of the soft palate occurs the epiglottis is lying ventral to the soft palate and it is therefore completely lost from the observer's view.[34]

Rostral displacement of the palatopharyngeal arch (RDPA), over one or both of the corniculate processes of the arytenoids that appears malformed, does not abduct well, or both. Abnormal arytenoid movement in most cases might be a result of abnormal anatomy of laryngeal cartilages and muscles, not restriction by the palatopharyngeal arch. The long-term prognosis for horses with this problem is poor, these coincide with.[35]

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Horses with DDSP described as making a characteristic gurgling sound during expiration.[1] Recently, it has suggested that up to 30% of horses with DDSP make no audible abnormal respiratory sounds at exercise.[36]

The tracheal spikes have recognized by endoscopic viewing and proved normal by the histopathological examination. Moreover, the etiology of these spikes is unknown and not associated with clinical signs.[37]

The endoscopic findings showed tracheal collapse in a donkey as dorso-ventral flattening of tracheal ring in the thoracic part of the trachea and this result is agreed with findings of Ohnesorge et al.[38] Also, it was found in thorax of ponies and donkeys that resulted from dorsal ligament flaccidity.[39] It occurred after traumatic damage to cartilage rings or the intrathoracic portion of the trachea showed transient collapse in horses affected with severe pulmonary disease as result of raised intrathoracic pressures.[40] However, chronic airway disease, malformation of the hyaline cartilage rings, or trauma resulted in abnormal collapse of the tracheal lumen.[41] Tracheal collapse in older donkeys because of age related degeneration in tracheal ring/cartilage or secondary to other respiratory diseases that caused an increase in respiratory effort; typically collapse occurred in animals with chronic recurrent airway obstruction or pulmonary fibrosis.[42]

CONCLUSION

The present investigation recommends the use of video-endoscopy for regular examination, assessment and tracing the health for appropriate diagnosis and management of upper respiratory system in horses and donkeys. The horse's upper respiratory disorders are numerous and more popular than donkey's disorders. Moreover, the present study has proved the occurrence and the superiority of pharyngeal disorders in horses than donkeys. Endoscopic examination of guttural pouches needs the use of biopsy instrument as a guide to allow advance of the endoscope through the auditory tube and into guttural pouch. The presence of squamous cell carcinoma and cyst formation in the guttural pouches of horses and granuloma in the guttural pouch of a donkey has claimed to be foremost records.

Future studies on endoscopic evaluation of respiratory system and application of accessories are hoping to ameliorate the surgical interferences of such upper respiratory tract disorders in horses and donkeys.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interests and no financial interests related to the substance of this manuscript.

REFERENCES

1. Beard W. Upper respiratory causes of exercise intolerance. *Vet Clin North Am Equine Pract.* 1996; 12(3): 435-455. doi: 10.1016/S0749-0739(17)30266-3

2. Elizabeth JD, Benson BM. Diagnosis of upper respiratory tract diseases in the performance horse. *Vet Clin Equine*. 2003;19: 51-62. doi: 10.1016/S0749-0739(02)00066-4

3. Gerard PM, Wikins AP. Respiratory tract chapter 3. In: Southwood LL, Wilkins PA, eds. *Equine Emergency and Critical Care Medicine.* Boca Raton, Florida, United States: CRC Press; Abingdon, UK: Taylor and Franics Group; 2015: 253-303.

4. Barakzai S. Endoscopy of upper respiratory In: *Handbook Equine Respiratory Endoscopy*. 3rd ed. Missouri, USA: Elsevier Saunders; 2007: 20-30.

5. Ducharme NG. Pharynx. In: Auer JA, Stick JA, eds. *Equine Surgery*. 4th ed. Missouri, USA: Elsevier Saunders. 2012: 569-591.

6. Fulton IC, Anderson BH, Stick JA, Robertson JT. Larynx. In: Auer JA, Stick JA, eds. *Equine Surgery*. 4th ed. Missouri, USA: Elsevier Saunders; 2012: 592-623.

7. Raker CW, Boles CL. Pharyngeal lymphoid hyperplasia in the horse. *J Equine Med Surg*. 1978; 2: 202-207.

8. Hackett RP, Ducharme NG, Fubini SL. The reliability of endoscopic examination in assessment of arytenoid cartilage movement in horses Part 1: Subjective and objective laryngeal evaluation. *Vet Surg*. 1991, 20: 174-179. doi: 10.1111/j.1532-950X.1991.tb00331.x

9. Hobo S, Matsuda Y, Yoshida K. Prevalence of upper respiratory tract disorders detected with a flexible video-endoscope in thoroughbred racehorses. *J Vet Med Sci*. 1995; 57: 409-413. doi: 10.1292/jvms.57.409

10. Bancroft DJ, Cook CH, Stirling RW, Turner DR. *Manual of Histological Techniques and their Diagnostic Application*. Edinburgh, Scotland: Churchil Livingston; 1996.

11. Barakzia S. Trachea and bronchia In: *Handbook of Equine Respiratory Endoscopy*. Missouri, USA: Elsevier Saunders 2007: 97-89.

12. McCullough BD. Assessing the reliability of statistical software: Part II. *The American Statistician*. 1999; 53(2): 149-159.

13. Flora EF, Lindsay D, Hilary MC. An anatomical and endoscopic study of the nasopharynx and larynx of the donkey (*Equus asinus*). *J Anat*. 1986; 144: 123-132.

14. Budras KD, Sack WO, Rock S, Horowitz A, Berg R. Schlütersche verlags gesellschaft hannover [In German]. In: Stubbs G, eds. *Anatomy of the Horse*. 6th ed. Germany: Courier Corporation; 2012.

15. Cilliers I, Williams J, Carstens A, Duncan NM. Three cases of osteoma and an osseous fibroma of the paranasal sinuses of

ISSN 2475-1286 http://dx.doi.org/10.17140/VMOJ-2-117

horses in South Africa. *J S Afr Vet Assoc*. 2008; 79(4): 185-193.

16. Etherington WG, Vasey JR, Horney FD. Ethmoid hematoma of the equine. *Can Vet J*. 1982; 23: 231-233.

17. Laing JA, Hutchins DR. Progression ethmoidal hematoma in horses. *Aust Vet J*. 1992; 69: (3): 57-58.

18. Bell BTL, Baker GJ, Foreman JH. A Progressive ethmoid hematoma: Background, clinical signs, and diagnosis. In. *Compendium on Continuing Education for Practicing Veterinarian*. 1993; 15: 1101-1110.

19. Jacobs KA, Fretz PB. Fistula between the guttural pouches and the dorsal pharyngeal recess as a sequela to guttural pouch mycosis in the horse. 1982; *Can Vet J*. 23: 117-118.

20. Raker CW, Boles CL. Pharyngeal lymphoid hyperplasia in the horse. *J Equine Med Surg*. 1978; 2: 202-207.

21. Auer DE, Wilson RG, Groenendyk S. Pharyngeal lymphoid hyperplasia in Thoroughbred racehorses in training. *Aust Vet J*. 1985; 62: 124-126. doi: 10.1111/j.1751-0813.1985.tb07259.x

22. Auer JA, Stick JA. *Equine Surgery* 3rd ed. St. Louis, Missouri, USA: Saunders Elsevier; 2012: 544-550.

23. Holcombe SJ, Jackson C, Gerber V, et al. Stabling is associated with airway inflammation in young Arabian horses. *Equine Vet J*. 2001; 33(3): 244-249. doi: 10.2746/042516401776249606

24. Robinson NE. Inflammatory airway disease: Defining the syn- drome: Conclusions of the Havenmeyer workshop. *Equine Vet Edu*. 2003; 15: 61-63. doi: 10.1111/j.2042-3292.2003.tb00216.x

25. Holcombe SJ. Tracheal mucus is associated with poor racing performance in Thoroughbred horses, in Proceedings. *Am Assoc Equine Pract*. 2004; 50: 172-173.

26. Raker CW. Diseases of the guttural pouch. *Mod Vet Pract*. 1976; 549-552. Web site. http://repository.upenn.edu/cgi/viewcontent.cgi?article=1067&context=vet_papers. Accessed May 2, 2017.

27. Hawkins JF. Tulleners EP. Epiglottitis in horses: 20 cases. *J Am Vet Med Assoc*. 1994; 205: 1577-1580.

28. Davenport-Goodall CLM, Parente EJ. Disorders of the Larynx. *Vet Clin North Am Equine Pract*. 2003; 19: 169-187. doi: 10.1016/S0749-0739(02)00072-X

29. Berbish EA, Senna NA. Guttural pouch empyema and tympany: Diagnosis and management. *Vet Med J Giza*. 1999; 47(4): 561-574.

30. Hahn CN. Horner's syndrome in horses. Equine Veterinary Education. 2003; 15: 86-90. doi: 10.1111/j.2042-3292.2003.tb00222.x

31. Lane JG, Bladon B, Little DRM, Naylor JRJ, Franklin SH. Dynamic obstructions of the equine upper respiratory tract. Part 1: Observations during high speed treadmill endoscopy of 600 Thoroughbred racehorses. *Equine Vet J*. 2006a; 38: 393-399. doi: 10.2746/04251640677840058

32. Hillidge CJ. Interrpretation of laryngeal function tests in the horse. *Vet Rec*. 1986; (19)118: 535-536. doi: 10.1136/vr.118.19.535

33. Cook WR. Procedure and technique for endoscopy of the equine respiratory tract and eustachian tube diverticulum. *Equine Vet J*. 1970; 2: 137-152. doi: 10.1111/j.2042-3306.1970.tb04177.x

34. Aitken MR, Parente EJ. Epiglottic abnormalities in mature non race horses 2011; 23 cases (1990-2009). *J Am Vet Med Assoc*. 2011; 238(12): 1634-1638. doi: 10.2460/javma.238.12.1634

35. Dixon PM. A review of the role of the epiglottis in equine upper airway obstruction. *Equine Vet Educ*. 1995; 7(3): 131-139. doi: 10.1111/j.2042-3292.1995.tb01210.x

36. Embertson RM. Evaluation of the Young Horse Upper Airway. Reprinted in the IVIS website with the permission of the AAEP 1998; 44(34-38).

37. Martin BB, Reef JR, Parente VB, Sage AD. Causes of poor performance of horses during training, racing, or showing: 348 cases (1992-1996). *J Am vet med Ass*. 2000; 216: 554-558. doi: 10.2460/javma.2000.216.554

38. Ohnesorge B, Gehlen H, Deegen, E. Disorders of the trachea in horses. *International Veterinary Information Service*. 2002; (3): 315-402.

39. Rush B, Mair T. Noncontagious diseases of the upper respiratory In: *Equine Respiratory Disease*. 1st ed. Hoboken, NJ, USA: Blackwell Science; 2004: 150

40. Tate LP. Chapter 40 surgery of the trachea. In: Hawkins JF, ed. *Advances in Equine Upper Respiratory Surgery Hawkins*. 1st ed. Hoboken, NJ, USA: John Wiley & Sons; 2015:261-269.

41. Barnett TP, Hawkes CS, Dixon PM. Tracheal Resection and Anastomosis after Traumatic Tracheal Stenosis in a Horse. A merican College of Veterinary Surgeons. *Vet J*. 2014; 49(1): 108-115.

42. Thiemann AK. Respiratory disease in the donkey. *Equine Vet Educ*. 2011; 10: 2042-3292. doi: 10.1111/j.2042-3292.2011.00292.x