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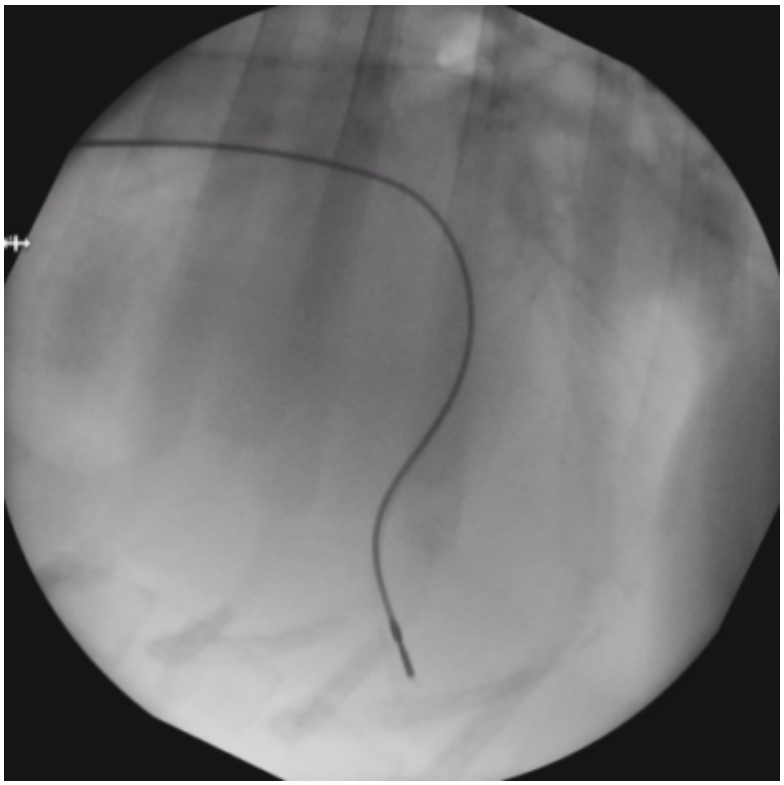

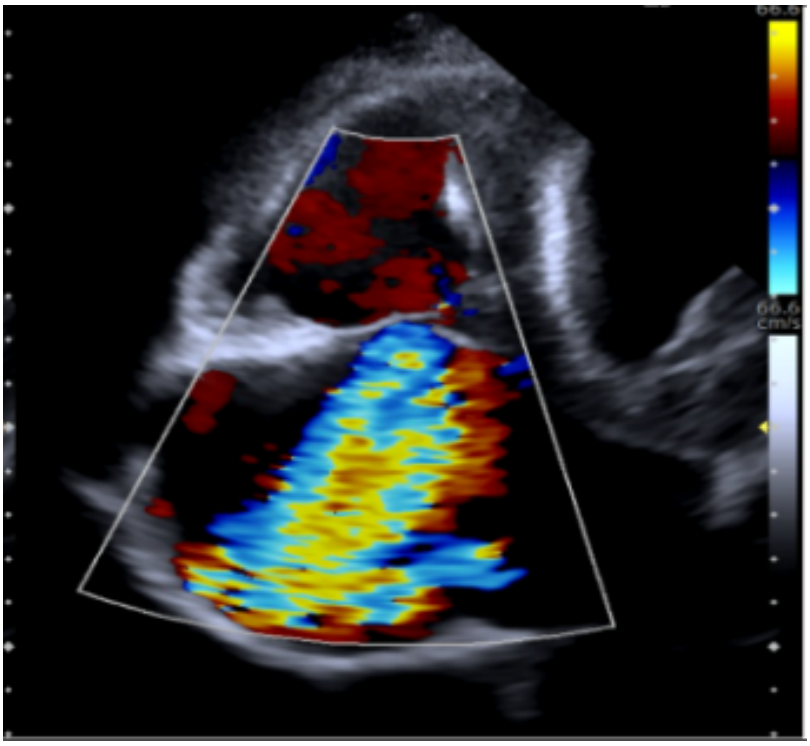

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Article

Horse Personality Simplified: A Scientific Approach to Equine Temperament

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Abstract: The article titled "Horse Personality Simplified: A Scientific Approach to Equine Temperament" discusses the complexity of horse personality and its significance in training, welfare, and human-horse interactions. The study aims to propose a simplified classification of horse personality into four types: energetic/reliable, energetic/unreliable, passive/reliable, and passive/unreliable. Using a systematic literature review, the authors identified 24 key behavioral traits commonly used to describe horse temperament. A questionnaire was administered to horse handlers and veterinarians to evaluate the correlation between these traits and the proposed personality types.The results suggest that horse personality can be effectively categorized using these four types, based on behavioral traits such as energy level and reliability in response to humans. This model offers a practical framework for improving human-horse relationships, facilitating safer and more efficient handling, and optimizing training and care strategies. The study also emphasizes the potential for future research to explore physiological correlations with these personality types, such as heart rate variability and hormonal changes, to further understand horse temperament. The authors conclude that this simplified approach provides a useful tool for veterinarians, equestrian professionals, and researchers, offering a straightforward method for assessing horse behavior and temperament in various contexts.

Keywords: equine, behavior, personality, temperament.

1. Introduction

Horse personality is a complex and multifaceted subject that has garnered increasing attention in the fields of ethology, veterinary science, and equine psychology. Just as in humans, personality in horses refers to the consistent patterns of behavior, emotion, and interaction that characterize individual animals. Understanding these personality traits is crucial for improving training methods, enhancing animal welfare, and fostering effective human-horse relationships [1].Horses, as social animals, exhibit a wide range of personality traits, influenced by genetic, environmental, and social factors [2].

Research indicates that horse personality can manifest in various dimensions, such as sociability, competitiveness, and emotional reactivity. These traits not only affect a horse's behavior in social settings and during training but also have implications for their health and well-being. For example, horses with more docile personalities may respond better to conventional training techniques, while those with higher reactivity may require more specialized approaches [3]. Studies suggest that genetic factors play a significant role in determining personality traits in horses. Selective breeding for specific traits has influenced temperament, with certain breeds exhibiting more docile or more spirited behaviors [1].

Early experiences, such as handling and socialization during the critical developmental stages, are vital in shaping a horse's personality. Studies have shown that positive interactions with humans and other equines can enhance confidence and reduce anxiety,

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leading to healthier behavioral profiles [4]. Conversely, negative experiences can lead to fearful or aggressive tendencies, which can complicate training and care [2]. Horses are herd animals, and their personality can be affected by their social environment. The establishment of hierarchies within a group can influence individual behavior [5]. It's essential to note that a horse's physical health can also impact its personality. Pain, discomfort, or illness can lead to behavioral changes, such as aggression or withdrawal. Thus, a comprehensive understanding of horse personality must take into account their physical health and emotional well-being [6]. Moreover, understanding horse personality has practical applications beyond training; it can inform breeding decisions, herd management practices, and therapeutic approaches in equine-assisted activities [2]. As equestrian sports and therapy programs grow in popularity, recognizing the individuality of horses becomes essential to optimize their performance and ensure their well-being [1]. Several methods have been developed to assess horse personality scientifically. One widely-used approach is the Horse Personality Questionnaire (HPQ), which evaluates horses based on five personality traits: Agreeableness, Neuroticism, Extroversion, Gregariousness towards People, and Gregariousness towards Horse [3]. Observational studies in natural and controlled settings also contribute to understanding how horses respond to different stimuli and social situations [6].

Innovative research has employed tools such as behavioral tests, where horses are exposed to novel objects or scenarios to evaluate their reactions. These observations can help categorize personalities into similar profiles, facilitating more effective management and training practices aligned with each horse's unique characteristics [5]. Understanding horse personality has profound implications for training and behavior management. Trainers who recognize and respect a horse's personality traits can tailor their training methods to align with the horse's natural tendencies, thereby improving learning outcomes and enhancing the horse's well-being [4].

For example, a horse that is naturally curious may thrive in a training regimen that involves exploration and problem-solving, while a more anxious horse might benefit from a gradual desensitization approach [2]. Moreover, acknowledging personality can help foster better relationships between horses and their handlers. Horses that are understood through the lens of their individual personalities are more likely to develop trust and rapport with humans, leading to safer and more enjoyable interactions, whether in recreational riding, competitive sports, or therapeutic settings [6].

However, the multitude of behavioral traits used to describe horse personality make it difficult to categorize them as clearly as human personality types, such as choleric, sanguine, melancholic, and phlegmatic. The aim of this study was to propose a standardized nomenclature of horse personality types by merging all characteristics found throughout literature into only four types: energetic/reliable, energetic/unreliable, passive/reliable, and passive/unreliable.

2. Materials and Methods

Study design

The study was designed in two stages. In the first stage we performed a thorough research of the literature in order to determine the most common behavioral traits attributed to horses by other scientists in their studies. Secondly, we created a questionnaire comprising the most common behavioral traits identified and asked respondents to assign behavioral traits with four personality types. Lastly, we analyzed data to detect whether there is consensus among the responders and if horse personality could be easily described by only four personality types.

Literature research

We performed a systematic search of the literature using the key words horse, personality, traits, temperament and we were able to summarize the behavioral traits used by other researchers to define horse personality.

Questionnaire

Questionnaires were administered online via a secure survey platform (Typeform) to facilitate ease of access and data management. Data were collected electronically and checked for completeness and consistency before analysis. All data were anonymized and securely stored in accordance with ethical research guidelines and data protection regulations.

The participants were asked to associate (match/assign) as many behavioral traits they considered (table 1) with the proposed temperament types 1. energetic&reliable 2. energetic&unreliable 3. passive&reliable 4. passive&unreliable.

The questionnaire encompasses two types of questions: one is related to the level of energy (Energetic & Passive) and the other to the reactivity to humans (Reliable & Unreliable).

The questionnaire was sent to 1300 subjects covering the areas of interest in first time human-horse encounter (FTHHE) : (1) students from the Faculty of Veterinary Medicine and Faculty of Animal Sciences from USAMV Cluj-Napoca, Romania (2) veterinarians, members of Romanian Equine Veterinarian Association and (3) people involved in horse handling, riding, training, and breeding from 78 equestrian facilities in Romania. The questionnaire is represented in Table 1.

Table 1. The questionnaire distributed to respondents listed each of the 24 behavioral traits used to describe horse behavior in the literature, with each trait displayed in a row on the left. Participants were asked to assign each behavioral trait to one of the personality types listed in the first row using the online platform, TypeForm.

		Energetic / reliable	**Energetic / unreliable**	**Passive / reliable**	**Pasive / unreliable**
1	Intelligent				
2	Calm				
3	Energetic				
4	Slow				
5	Skittish				
6	Dominant				
7	Anxious				
8	Attentive				
9	Friendly				
10	Reactive				
11	Solitary				
12	Tensed				

13	Suspicious
14	Patient
15	Obedient
16	Stubborn
17	Cautious
18	Reliable
19	Aggressive
20	Fearfull
21	Nervous
22	Social
23	Cooperative
24	Intelligent

3. Results

3.1. Literature research

We conducted a comprehensive review of 80 papers, identifying 24 commonly cited behavioral traits used by the authors. The selection of these traits was based on their frequency of occurrence across the literature. Each of the identified traits appeared more than 10 times in relation to horse temperament, indicating their relevance and significance in the field.

3.2. Questionnaire

Out of the 1,300 questionnaires distributed, 1,260 were validated as complete and correctly filled. The results are presented in Figure 1 as the total number of respondents per option, and in Figure 2 as the percentage of respondents per option.

Figure 1. Number of responders that associated one behavioral trait with one of the personality types. A&C – active and confident; A&U – active and unconfident; P&C – passive and confident; P&U – passive and unconfident.

nr. total	INTELLIGENT	CALM	ENERGETIC	SLOW	SKITTISH	DOMINANT	ANXIOUS	PLAYFUL	ATTENTIVE	FRIENDLY	REACTIVE	SOLITARY	TENSED	SUSPICIOUS	PATIENT	OBEDIENT	STUBBORN	CAUTIOUS	RELIABLE	AGGRESSIVE	FEARFULL	NERVOUS	SOCIAL	COOPERATIVE
A & C	1242	239	1247	13	101	1123	60	1184	1156	1189	342	9	127	18	356	667	61	11	1144	189	391	75	1202	743
A & U	1008	10	958	63	1021	302	1135	441	164	14	1098	504	1210	67	41	29	428	1165	554	1123	1184	545	454	145
P & C	54	1222	25	1235	25	983	17	365	50	1252	27	454	22	668	1235	1221	513	62	844	22	34	6	956	946
P &U	76	315	88	1121	1096	63	1089	1201	76	21	1159	1134	712	1096	71	126	1109	1084	36	983	1005	1199	123	53

Figure 2. Percentage of responders that associated one behavioral trait with each personality type proposed. A&C – active and confident; A&U – active and unconfident; P&C – passive and confident; P&U – passive and unconfident.

%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
A & C	99	19	99	1	8	89	5	94	92	94	27	1	10	1	28	53	5	1	91	15	31	6	95	59
A & U	80	1	76	5	81	24	5	35	92	1	27	40	10	5	28	2	5	92	91	89	31	43	95	12
P & C	4	97	2	98	2	78	1	29	4	99	2	36	2	53	98	97	41	5	67	2	3	0	76	75
P &U	6	25	7	89	87	5	86	95	6	2	92	90	57	87	6	10	88	86	3	78	80	95	10	4

4. Discussion

From the outset, we made it clear that this proposal is not intended to resolve the ongoing debate over horses' temperamental and personality traits, nor to add further confusion to a field where much remains unclear. The discussion around defining temperament and personality requires robust scientific approaches, not only for equids but for many other species as well.

Our aim is to introduce simple and effective markers for regular human-horse interactions, prioritizing the safety and welfare of both parties. As the results indicate, the proposed typologies are straightforward and easily understood, suggesting both simplicity and effectiveness. The consistent alignment of subjects with the behavioral characteristics of the proposed types demonstrates their coherence across diverse and large groups. Additionally, the use of terms such as "energetic," "confident," "passive," and "non-confident" resonates with people interacting with horses, as it aligns with their expectations and is easy to grasp.

Another potential application of this model lies in studies related to horse behavior, especially where grouping horses with different temperamental or personality types presents a complex challenge. Measuring emotional states—such as reactions to humans or the environment, anxiety, fear, arousal, etc.—across a wide variety of breeds and individuals with varying levels of reactivity, energy, and habituation to humans requires a method for sorting horses into consistent groups.

Of course, there will always be some degree of subjectivity in classifying horses, and there are numerous traits that could be used to group them. Several studies on equine emotional and behavioral characteristics have proposed different temperamental styles, yet there remains significant inconsistency in the variables used. Establishing a common language is a critical first step toward creating a foundation for future research. Using these temperament types to explore correlations between physiological states—such as heart rate variability (HRV) or hormonal changes—and behavioral patterns within specific temperamental groups could provide valuable insights.

As with research into human temperament and personality, defining behavioral traits such as novelty seeking, harm avoidance, reward dependence, neuroticism, agreeableness, extraversion, openness, and conscientiousness often involves a margin of subjectivity [7,8].A key issue in the debate surrounding general factor models of temperament is whether these factors represent substantive phenomena or are merely methodological artifacts or statistical byproducts [9].

Until further progress is made in clarifying the nature of temperament, we wish to underscore the simplicity and efficiency of this model, which can yield positive outcomes in first-time horse-human encounters. Beyond the scientific foundation, the ability to communicate critical information quickly between horse handlers can significantly enhance both human safety and equine welfare. Condensing important behavioral traits into just two descriptive words is essential for the FTHHE. In veterinary schools, equestrian centers, and for certain horse handlers, approaching an unfamiliar animal is a common occurrence. Basic information about a horse's natural reactivity and energy level, delivered promptly, can make the difference between a positive or negative interaction.

Future Directions refer to implementing the proposed typology in veterinary schools and equestrian centers as part of the routine vocabulary used in horse-human interactions. Conducting further behavioral studies using these temperament categories to assess whether statistical data reveal strong correlations between these traits and physiological responses.

5. Conclusions

This study introduces a simplified approach to categorizing horse temperament and personality traits, focusing on ease of understanding and practical application in human-horse interactions. By reviewing existing literature and identifying commonly cited behavioral traits, we developed a set of temperamental types—energetic, confident, passive, and non-confident—designed to offer a straightforward framework for assessing horses in various contexts.

The results demonstrate that these proposed typologies are coherent, easy to understand, and applicable across diverse groups. The consistency with which participants aligned behavioral traits with the proposed types underscores their potential as effective markers for horse temperament. Furthermore, this model facilitates quick and efficient communication between horse handlers, which can significantly improve both human safety and horse welfare, particularly in situations where immediate assessment of a horse's reactivity and energy level is crucial.

While the field of equine temperament research remains complex, and subjective factors will always play a role in classification, this proposal offers a practical tool for use in equestrian centers, veterinary schools, and other settings. Future research should focus on validating these categories through physiological data, exploring correlations between temperament types and measurable biological markers such as heart rate variability and hormonal changes.

In summary, the simplicity and practicality of this model offer valuable insights into horse-human interactions and present a foundation for further study into the behavioral and physiological aspects of equine temperament.

Author Contributions: Conceptualization D.M.; methodology, D.M.; software, M.T.; validation, I.P..; formal analysis, S.D; investigation, C.C; data curation, D.M.; writing—original draft preparation, D.M.; writing—review and editing, M.T; visualization, C.C.; supervision, I.P.; project administration, D.M.;. All authors have read and agreed to the published version of the manuscript".

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

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Article

Nutritional Value, Microbiological Safety, and Mycotoxin Risk of Black Soldier Fly Larvae: Implications for Dog Nutrition

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Abstract: This study comprehensively evaluates the crude chemical composition, microbiological dynamics, and mycotoxin contamination in *Hermetia illucens* larvae (Black Soldier Fly Larvae, BSFL), aiming to assess their suitability as a sustainable protein source. Proximate analysis revealed a high protein content (43.22%), along with significant fat levels (19.99%) and moderate fiber content (12.05%), predominantly chitin. Mycotoxin analysis indicated safe levels of aflatoxin B1 (1.29 µg/kg) and deoxynivalenol (6.0 µg/kg), with undetectable levels of ochratoxin A, ensuring compliance with feed safety standards. Microbiological assessments across developmental stages identified a progressive increase in microbial load, in adults. The predominant microbial species included *Enterococcus* spp., *Klebsiella aerogenes*, *and Escherichia coli*. Thermal treatment via microwave drying significantly reduced microbial contamination, although *Enterococcus* spp. remained detectable post-treatment. These findings highlight BSFL's potential as a nutritionally valuable ingredient in animal feed, particularly due to their high protein and fat content. However, further refinement of microbial decontamination strategies is necessary to enhance safety, ensuring their optimal use in food and feed applications.

Keywords: insect, dog, nutrition, analysis

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1. Introduction

The global demand for sustainable and high-quality protein sources is increasing, driven by population growth, environmental concerns, and the need to reduce reliance on conventional livestock production. Insects, particularly Black Soldier Fly Larvae (BSFL), have emerged as a promising alternative protein source for both animal feed and human consumption due to their high nutritional value and low environmental footprint [1,2]. BSFL are rich in protein, essential fatty acids, and micronutrients, making them an attractive candidate for various applications [3], including pet food [4]. In particular, insect-based ingredients are gaining attention in the pet food industry, where dog nutrition is being re-examined through the lens of sustainability and alternative protein sources [5]. The Black Soldier Fly (*Hermetia illucens)* is a non-pest species of *Diptera* native to tropical and temperate regions worldwide [6]. It has gained significant attention for its unique ability to convert organic waste into valuable biomass, reducing environmental waste while producing nutrient-dense larvae [7]. The life cycle of *H. illucens* is divided into several key stages: egg, larva, pupa, and adult, with each stage playing a vital role in its biological and ecological success [8].

The use of Black Soldier Fly Larvae in dog nutrition is increasingly being explored due to the high protein content and essential fatty acids comparable to traditional protein

sources like chicken or fish. BSFL offers additional benefits, including being rich in medium-chain fatty acids, such as lauric acid, which can promote immune function and improve skin and coat health in dogs [9]. As dogs require high-quality protein for muscle maintenance and overall health, BSFL represents a nutritionally balanced and environmentally sustainable ingredient that could reduce the ecological footprint of pet food production. Moreover, BSFLs are hypoallergenic, making them a potential solution for dogs with food sensitivities to common proteins like beef or poultry [10]. However, for BSFL to be integrated into dog food, microbiological safety is a critical concern. Insects, like BSFL, naturally harbor microbes, which can include both beneficial bacteria and harmful pathogens such as *Salmonella* spp. [11] and *Escherichia coli* spp. [12]. Ensuring that BSFL-derived products are microbiologically safe is essential for preventing health risks in pets [13]. Various rearing conditions and substrates, like wheat bran, can influence microbial loads [14]. By evaluating microbial populations at different developmental stages, from larvae to adults, and using thermal treatments to reduce microbial contamination in BSFL powder, this study aims to ensure that BSFL meets the stringent safety standards required for dog food ingredients. Additionally, the presence of mycotoxins, which can pose a risk to pet health, is assessed to ensure compliance with pet food safety guidelines.

This study focuses on BSFL reared on wheat bran, exploring their chemical composition, microbial dynamics, and mycotoxin contamination to assess their suitability as a safe and nutritious ingredient for dog food. By integrating the nutritional benefits and addressing safety concerns, this research supports the potential of BSFL as a sustainable, eco-friendly solution for the pet food industry.

2. Materials and Methods

Black Soldier Fly Larvae (BSFL), adults, pupae and eggs were purchased from an industrial facility located in Brasov County, Romania. The larvae were delivered dried and were subsequently ground to a fine powder in preparation for further analyses. Chemical Composition Analysis: The powder was analyzed for crude protein, fat, moisture, fiber, NFE (nitrogen-free extract) and ash content, following standard procedures. Mycotoxin Testing: The powder was also assessed for the presence of mycotoxins, including aflatoxins and ochratoxins, to ensure compliance with safety standards for pet food ingredients. Nutritional Value: samples were homogenized before analysis to ensure consistency. The gross chemical composition, including moisture, crude protein, crude fiber, crude fat, NFE (nitrogen-free extract) and ash content, was determined following the procedures outlined in the AOAC official methods. To analyze the chemical composition of Black Soldier Fly larvae, specific devices and methods are employed. Dry matter content is determined using a drying oven, such as the Memmert Universal Oven UF. Crude protein is measured via the Kjeldahl method, utilizing a semi-automatic device like the Kjeltec 8400 Analyzer Unit. The ether extract is obtained through the Soxhlet method, using a Soxhlet apparatus. Crude ash was quantified using a muffle furnace in accordance with standard incineration methods. The Nitrogen-Free Extract (NFE) was calculated by subtracting the sum of the crude protein, ash, ether extract, and crude fiber from 100% of the dry matter, as per standard proximate analysis methods. This calculation provides an estimate of the carbohydrate content.

Microbiological Analysis: To evaluate the microbiological safety and monitor microbial dynamics throughout the developmental stages, a total of 8 samples were examined. One sample was taken from each key life stage of the larvae, including adults, live eggs, first and second-instar larvae, third and fourth-instar larvae, pupae, and microwave-dried larvae. Each sample was analyzed for microbial load and species composition to determine the progression of microbial communities during larval development and post-processing treatment.: *Eggs: Purchased immediately after oviposition. *First Instar: Purchased 3 days post-hatching. *Second Instar: Purchased 8 days post-hatching. *Third Instar: Purchased 12 days post-hatching. *Fourth Instar: Purchased 18 days post-hatching. *Fifth Instar: Purchased on the day of slaughtering (22 days post-hatching). *Pupae: Purchased at 24 days post-hatching. *Adults: Purchased immediately upon emergence. The samples were analyzed using quantitative and qualitative methods. Quantitative analysis: The serial dilution method was employed to estimate the number of microorganisms. Initially, 0.5 g of the sample was diluted in 4.5 ml of sterile saline to obtain a 1:10 dilution. Serial dilutions (up to 10^{-5}) were prepared similarly. From each dilution, 0.5 ml were plated on nutrient agar (Merck, Darmstadt, Germany) and incubated at 37°C for 24 hours. The number of colonies formed was calculated using the following formula: TGN = number of colonies x dilution factor x 1/volume plated. Qualitative analysis: For bacterial identification, samples from the final dilutions were inoculated on MacConkey agar (BioMaxima S.A., Lublin, Poland) and UriSelect medium (Bio-Rad Laboratories Inc., Hercules, CA, USA) URI chromogenic medium. Colonies that

could not be identified based on morphological or cultural characteristics were further analyzed using the Vitek® 2 Compact device (BioMerieux, Marcy l'Etoile, France), which determines 64 biochemical characteristics of bacteria. Mycotoxin Analysis: for the mycotoxicological examination, the test of RIDASCREEN®FAST Aflatoxin, RIDASCREEN®FAST Ochratoxin A and RIDASCREEN®FAST Deoxynivalenol were used, competitive enzyme immunoassay tests for the quantitative determination of total aflatoxin, ochratoxin A and deoxynivalenol in cereals and food. The basis of the test is the antigen-antibody reaction. The measurement was performed photometrically at 450 nm using Awareness Technology Model 4300 ChroMate Microplate Reader.

3. Results

The chemical composition analysis of *Hermetia illucens* larvae was conducted to evaluate their nutritional potential, focusing on key parameters such as water content, dry matter, crude protein, crude fat, NFE (nitrogen-free extract), and ash content. The laboratory analysis revealed a water content of 9,70%, contributing to a dry matter (DM) content of 90,30%. This dry matter was composed predominantly of organic matter (82.76% of the sample, or 91.65% of DM), while ash content, representing mineral salts, accounted for 7.54% of the sample (8.35% of DM). Protein analysis demonstrated a substantial nitrogenous content, with crude protein making up 43.22% of the sample, equivalent to 47.86% of DM. Furthermore, the fat content measured 19.99% of the total sample (22.14% of DM), underscoring the larvae's suitability as a source of essential lipids. The fiber content of 12.05% (13.34% of dry matter), is substantial for promoting gastro-intestinal health in dogs. A significant portion of this fiber is represented by chitin, a natural polysaccharide found in the exoskeletons of insects. Chitin is known for its prebiotic properties, contributing to the development of beneficial gut bacteria, and promoting overall digestive health in dogs [15]. However, chitin is not as easily digestible as other fibers, meaning that while it has nutritional benefits, it must be complemented with other easily digestible ingredients to ensure a well-rounded diet. The nitrogen-free extract (NFE), at 7.50%, consists mainly of digestible carbohydrates, providing an important source of energy. Despite its nutritional value, the moderate levels of NFE and fiber indicate that this insect powder should be included as a supplementary ingredient in dog food formulations, rather than being used as a sole dietary component. *Hermetia illucens* larvae reared on wheat bran exhibit a high protein and fat content, along with moderate levels of ash, indicating a rich mineral composition, as well as moderate amounts of fiber and nitrogen-free extract (carbohydrates). While these nutritional properties make them a promising ingredient for dog food, particularly for fulfilling protein and essential fatty acid requirements, it is recommended that the larvae powder be used as a supplementary ingredient rather than a singular, complete meal for dogs.

Table 1. Values obtained from the performed determinations (chemical composition)

GROSS CHEMICAL COMPOSITION	% of sample	% of DM
Water	9,70	-
Dry matter	90,30	100
Ash	7,54	8,35
Total organic matter	82,76	91,65
Protein	43,22	47,86
Fat	19,99	22,14
Fibres	12,05	13,34
NFE	7,50	8,31

NFE- nitrogen-free extract; DM-dry matter

The detected concentrations of various mycotoxins in the analyzed samples are presented in Table 2. Aflatoxin B1 was measured at 1.29 μg/kg, while Deoxynivalenol was found at 6.0 μg/kg. Ochratoxin A levels were undetectable in the samples.

Table 2. Mycotoxin Levels in BSF Larvae

Mycotoxin	Detected Level (µg/kg)
Aflatoxin B1	1.29
Deoxynivalenol	6.0
Ochratoxin A	0.0

The microbial load and species diversity across different life stages and processing conditions of wheat bran-fed insects were assessed through quantitative and qualitative tests (Table 3). The highest microbial load was observed in the adults, with a concentration of 2.72×10^7 CFU/mL. The alive larvae (first/second instar) showed a microbial load of 9.36×10^6 CFU/mL while for the third/fourth instar, we managed to detect 1.12×10^6 CFU/mL. For the alive eggs, microbial counts were found at 9.0×10^3 CFU/mL and 1.0×10^4 CFU/mL. Alive pupae expressed a microbial load of 1.32×10^7 CFU/mL. Microwave-dried larvae showed lower microbial levels, with 2.1×10^5 CFU/mL and 1.6×10^4 CFU/mL (Table 3). The microbial load in black soldier flies increases progressively from eggs to adults, with the highest concentration observed in the adult stage (2.72×10^7 CFU/mL), compared to lower levels in eggs (9.0×10^3 to 1.0×10^4 CFU/mL) and other developmental stages (Figure 1).

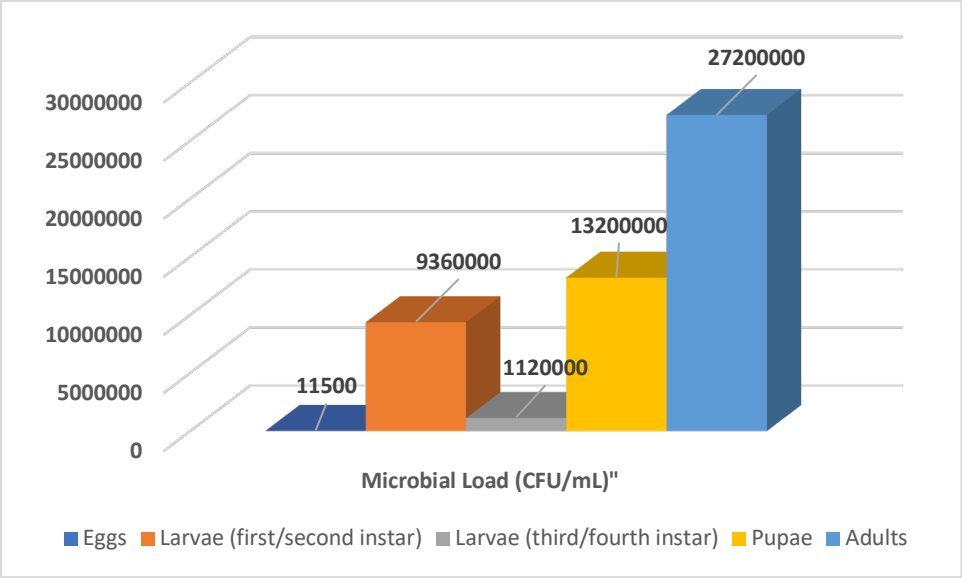

Figure 1. Quantitative test - Microbiology essay

A qualitative microbiological analysis was conducted to evaluate the microbial presence at various developmental stages of *Hermetia illucens* (Black Soldier Fly). The results showed that in adults, the microbial species included *Enterococcus* spp., *Proteus mirabilis*, *Myroides* spp., and *Providencia rettgeri* was detected. For the eggs, *Enterococcus* spp. was the only isolated genus. In the first and second instar larvae cultivation showed the presence of *Klebsiella aerogenes, Escherichia coli, Myroides* spp., *Enterococcus* spp., *Klebsiella pneumoniae ssp. pneumoniae*. In the third and fourth instar larvae, *Escherichia. coli, Enterococcus* spp., *Klebsiella aerogenes, Myroides* spp. and members of *the Enterobacter* cloacae complex were present. The analysis of the pupae indicated the presence of *Enterococcus* spp. and *Klebsiella aerogenes* .

Furthermore, in microwave-dried larvae, only *Enterococcus* spp. showed any growth. These results demonstrate that the microbial diversity changes across developmental stages of *Hermetia illucens.* In particular, larvae exhibit a more diverse microbiota compared to pupae and adults, with a predominance *of Enterococcus* spp. and *Klebsiella* spp.. Thermal treatment, such as microwave drying, reduces bacterial contamination significantly but does not eliminate it, as evidenced by the persistence of *Enterococcus* spp. in the dried larvae.

Table 3. Results of qualitative and quantitative examination of Black Soldier Fly larvae /adults/pupae/ eggs fed

Product name			Adults	Alive eggs	Alive larvae – First/Second instar	Alive larvae – Third/Fourth instar	Alive pupae	Microwave-dried wheat barn-fed larvae
Quantitative test (TNG-CFU/mL)	10^{-1}		-	-	-	-	-	-
	10^{-2}		-	9000	-	-	-	-
	10^{-3}		-	10000	-	11200000	-	210000
	10^{-4}		-	-	-	-	132120000	160000
	10^{-5}		272800000	-	93600000	-	-	-
Quality review	URI	10^{-1} / 10^{-2} / 10^{-3}	*Enterococcus* spp., *Proteus mirabilis*, *Myroides* spp.	*Enterococcus* spp.	*Klebsiella aerogenes*, *E. coli*,*Myroides* spp., *Enterococcus* spp.	*E. coli*, *Enterococcus* spp., *Klebsiella aerogenes*, *Myroides* spp	*Enterococcus* spp., *Klebsiella aerogenes*	*Enterococcus* spp.
	MAC	10^{-1} / 10^{-2} / 10^{-3}	*Providencia rettgeri*	0	*E. coli*, *Klebsiella pneumoniae ssp. Pneumoniae*	*E. coli*, *Enterobacter cloacae complex*	0	0

4. Discussion

The gross chemical composition of *Hermetia illucens* larvae in our study aligns with previously reported findings, particularly for dry matter content, supporting the consistency of the larvae's moisture retention and nutrient concentration [3]. Our protein analysis showed levels comparable to those cited in the literature, reinforcing the larvae's role as a protein-rich resource for both animal and human nutrition [16]. The stable protein content, even with minor substrate variations, suggests that wheat bran is a viable alternative for maintaining nutritional value in pet diets.

In addition to protein, the larvae powder is rich in lipids and minerals, while providing moderate amounts of fiber and carbohydrates. Although the measured fat and ash content were slightly below typical ranges [3, 20], they still confirm the larvae's potential as a significant lipid source with a valuable mineral profile, particularly for calcium and phosphorus, which are essential for canine health [17]. Moreover, the presence of chitin, known for its prebiotic properties, highlights the potential gut health benefits of incorporating *Hermetia illucens* larvae into dog diets [18].

Moreover, *Hermetia illucens* larvae powder is already used as an ingredient in several commercial recipes for extruded dry dog food due to its balanced nutrient profile and environmental sustainability [19]. Beyond its industrial applications, the powder could also serve as an ingredient in homemade dog feed or treats, offering pet owners a sustainable and nutrient-dense alternative [20]. However, despite the impressive nutritional qualities of *Hermetia illucens* larvae powder, it is not suited to be the sole ingredient for balanced canine nutrition. While it provides high levels of protein, fat, and minerals, a complete and balanced diet requires a more diverse range of nutrients that cannot be met by insect powder alone. Therefore, this ingredient should be used as part of a broader nutritional strategy to meet all of a dog's dietary needs [21]. Our study aimed to assess the quality of insect meal, taking *Hermetia illucens* larvae in their dried form, grinding them ourselves, and evaluating the nutritional properties of the resulting powder. Specifically, we

sought to determine whether the substrate used to feed the larvae influences the nutritional composition of the insect powder and assess its potential for inclusion in dog nutrition. Through this analysis, we have contributed to understanding the versatility and limitations of *Hermetia illucens* powder as a functional ingredient in canine diets.

The analysis of mycotoxins in *Hermetia illucens* (Black Soldier Fly) larvae revealed relatively low contamination levels compared to the maximum allowable limits set by the European Union for dogs [22]. Specifically, Aflatoxin B1 was measured at 1.29 μg/kg, Deoxynivalenol (DON) at 6.0 μg/kg, while Ochratoxin A was below detectable levels. These findings are significant as they suggest that BSF larvae raised on wheat bran present a low risk of acute mycotoxin toxicity for animals, including dogs. When compared to the maximum admitted levels, which are 20 μg/kg for Aflatoxin B1(C, 2006), 5,000 μg/kg for Deoxynivalenol [22], and 10 μg/kg for Ochratoxin A [22], the detected concentrations in our study are far below the thresholds. This suggests that feeding BSF larvae to dogs would not likely lead to acute mycotoxicosis, a condition that typically occurs following the ingestion of high mycotoxin concentrations. However, while the acute risk is minimal, the potential for chronic exposure should not be ignored. Prolonged consumption of feed containing low levels of mycotoxins could predispose dogs to long-term health issues, such as liver disease or cancer, particularly in the case of Aflatoxin B1 [23]. Chronic aflatoxin exposure has been linked to hepatotoxicity and hepatocellular carcinoma in various animal species, including dogs, even at subclinical levels. This raises concerns about the potential cumulative effects of long-term, low-level mycotoxin ingestion in pet diets [24]. To mitigate chronic exposure to mycotoxins in animal diets, particularly for dogs, it is crucial to implement effective monitoring systems and establish clear thresholds for mycotoxin levels in feed ingredients such as Black Soldier Fly larvae. While commercial dry dog food typically shows mycotoxin contamination levels below regulatory limits, the risk of chronic exposure from consistent, low-level intake remains a concern. Dogs may be exposed daily to small quantities of mycotoxins, which, over time, could pose health risks. Regular testing of both the larvae and their substrates, as well as periodic monitoring of mycotoxins in dog feed, is essential to ensure safety [25]. Further processing methods, including thermal treatments and the use of detoxifying additives, can help significantly reduce mycotoxin concentrations. Additionally, high-quality substrate management is vital, as it directly affects the nutritional value and safety of the larvae as a feed source. Prioritizing these strategies will enhance the health and well-being of pets and livestock, minimizing the long-term risks associated with mycotoxin exposure [26]. Despite the low levels of mycotoxins detected in BSF larvae, the literature suggests that BSF larvae have a unique ability to degrade or tolerate certain contaminants, including mycotoxins. Previous studies have highlighted that Black Soldier Fly larvae can degrade aflatoxins and other harmful compounds during digestion, reducing the risk of contamination in the final product [27]. However, the efficacy of this bioconversion varies depending on the type of mycotoxin and the concentration present in the feed substrate [28]. For instance, some research has shown that BSF larvae can significantly reduce Aflatoxin B1 levels in contaminated substrates, although complete degradation may not always occur [29]. This ability to partially detoxify their feed could explain the low mycotoxin concentrations observed in our study, despite the presence of wheat bran, a substrate that can be prone to fungal contamination. Our findings align with this body of research, as the larvae's low mycotoxin levels indicate that wheat bran is a suitable substrate for BSF rearing without introducing significant risks of contamination. Nevertheless, further research is required to better understand the long-term effects of chronic exposure to low mycotoxin levels in both BSF larvae and the animals consuming them. For now, the results are promising in terms of the safety and sustainability of using BSF larvae as a protein source for dog nutrition, particularly given their low mycotoxin content and the larvae's inherent detoxification capabilities.

Our study explored the microbial load and species diversity across different developmental stages of *Hermetia illucens* (Black Soldier Fly) fed on wheat bran, without dissecting the larvae, pupae, or adult gut. Instead, we aimed to observe the bacterial dynamics throughout their development in a commonly used substrate, assessing microbial presence on the external body surface and internal gut as total. The results highlight significant changes in both microbial load and bacterial species composition as the larvae progressed through their life cycle, as well as the effect of thermal processing (microwave drying) on bacterial contamination. Quantitatively, the microbial load increased progressively from eggs to adults, with the highest load detected in the adults (2.72×10^7 CFU/mL at a dilution of 10^{-5}), while the lowest levels were recorded in the eggs (9.0×10^3 to 1.0×10^4 CFU/mL). Notably, the microbial load in the larvae also varied with developmental stage; first/second instar larvae exhibited a load of 9.36×10^6 CFU/mL, while the third/fourth instar larvae

showed a reduced load of 1.12×10^6 CFU/mL. These fluctuations in microbial load are consistent with findings in the literature [7], which emphasize that as insects grow, they change the microbiota due to shifts in their diet, metabolic activity, and immune system responses [30]. Qualitatively, our analysis revealed diverse microbial communities at different life stages. For example, first/second instar larvae hosted a more varied microbiota, including *Klebsiella aerogenes*, *Escherichia coli*, *Myroides* spp., and *Enterococcus* spp., while third/fourth instar larvae displayed similar species with the addition of members from the *Enterobacter cloacae* complex. This aligns with previous research [31] indicating that BSF larvae possess a dynamic microbiota, which can help degrade organic matter and enhance nutrient recycling. The decrease in microbial diversity observed as the larvae progressed to the pupal and adult stages, particularly the predominance of *Enterococcus* spp. and *Klebsiella* spp., has also been reported in other studies [32], suggesting that microbial diversity tends to narrow as insects undergo metamorphosis. The results highlight significant changes in both microbial load and bacterial species composition as the larvae progressed through their life cycle, as well as the effect of thermal processing (microwave drying) on bacterial contamination. This variation in microbial load across different developmental stages is crucial for optimizing thermal treatment methods, as certain life stages may require more stringent processing to ensure microbial safety. Practical solutions could involve tailoring the duration and intensity of heat treatment based on the larvae's developmental stage. For instance, larvae in later stages, which may harbour more resilient bacterial species, could benefit from longer microwave drying times or higher temperatures to ensure a more comprehensive microbial reduction [33]. Furthermore, our study aimed to assess whether thermal treatment via microwave drying could effectively reduce bacterial contamination in BSF byproducts to make them safer for consumption, particularly in animal feed. The results indicate that while microwave drying significantly reduced the microbial load—showing 2.1×10^5 CFU/mL and 1.6×10^4 CFU/—it did not eliminate microbial presence. *Enterococcus* spp. was still detected in dried larvae, a finding that aligns with the notion that while heat treatment is effective [34], complete sterility is difficult to achieve. These results are critical when considering the potential use of BSF larvae as a feed ingredient for dogs. Although thermal processing substantially reduces microbial contamination, the persistence *of Enterococcus* spp. is noteworthy. In the context of canine nutrition, this species, while commonly present in the environment, could pose a risk to immunocompromised dogs [34,35]. Chronic exposure to low levels of potentially pathogenic bacteria could increase the likelihood of infection or contribute to the development of other conditions in susceptible animals [36]. To mitigate this risk, future studies could explore combining microwave drying with other sterilization techniques, such as pressure-based methods (e.g., high-pressure processing), which have been shown to inactivate heat-resistant bacterial species. [37]

In comparison to findings in the literature [38], our study supports the general trend that microbial load and diversity fluctuate across the life stages of BSF, influenced by both the insect's developmental biology and the substrate composition. Additionally, the literature suggests that BSF larvae can reduce certain pathogens in their environment due to their microbial communities [39], yet some bacteria, such *as Enterococcus* spp., are resilient and can persist despite thermal processing. This highlights the practical importance of refining thermal treatment methods, such as adjusting drying times or temperatures, to target resilient bacteria more effectively. Solutions such as incorporating temperature gradients—where the drying process begins at lower temperatures to avoid nutritional degradation and gradually increases to ensure bacterial inactivation—could optimize both safety and quality. Additionally, the use of microbial inhibitors (e.g., organic acids) during the processing phase could further suppress bacterial survival while maintaining the larvae's nutritional integrity.

Therefore, our findings contribute to the ongoing understanding of microbial dynamics in BSF and underscore the need for further investigation into optimizing processing methods to ensure microbial safety without compromising the larvae's nutritional integrity. These insights could inform future processing standards, where variations in microbial load across different life stages are accounted for. Establishing specific protocols for each larval stage could lead to more precise drying times and temperatures, minimizing nutrient loss while maximizing microbial safety. A combination of tailored heat treatment, surface cleaning, and supplementary sterilization methods could be integrated into industrial processes to achieve the highest safety standards for BSF-derived products.

5. Conclusions

Our study highlights the nutritional viability of *Hermetia illucens* larvae as a protein-rich, sustainable ingredient for dog nutrition. The larvae's gross composition, particularly their high protein content, aligns well with the dietary needs of most animals. Despite slight deviations in fat and ash content compared to previous research, the larvae maintain a robust nutritional profile suitable for pet diets, supporting both energy needs and bone health. The low levels of mycotoxins detected, far below the EU-admitted thresholds, indicate minimal risk for acute toxicity in dogs. However, the potential long-term effects of low-level Aflatoxin B1 exposure warrant further investigation, particularly given its link to chronic liver disease. Additionally, our microbial analysis underscores the need for rigorous safety protocols in processing larvae for dog food, as *Enterococcus* spp. can pose a risk to immunocompromised individuals.

Black soldier fly larvae reared on wheat bran demonstrate great potential as a sustainable, nutrient-dense ingredient for dog food and animal feedings in general, offering high protein content with manageable mycotoxin contamination. While thermal processing effectively reduces bacterial load, optimizing this process for microbial safety remains crucial, especially for sensitive pets. However, while *Hermetia illucens* larvae powder has many desirable nutritional qualities, it cannot serve as a sole ingredient for a balanced canine diet. A complete and balanced diet for dogs requires a broader range of nutrients that cannot be provided by insect powder alone. Therefore, its role should be considered as a complementary ingredient within a more comprehensive dietary framework. Overall, our findings support the further investigation of *Hermetia illucens* larvae as a cost-effective, eco-friendly alternative protein source for not only pet nutrition but also for various animal feed industries. Its excellent nutritional profile, combined with its sustainable production, offers a promising solution for advancing animal feed formulations without compromising nutritional quality or safety across different species."

Supplementary Materials: Not applicable.

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Anaesthetic Efficacies of Epidurally Administered Lignocaine-HCl and Bupivacaine-HCl Alone and Their Combination in Rabbits

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Abstract: The present study aimed to evaluate the anaesthetic efficacy of epidurally administered lignocaine-HCl and bupivacaine-HCl, both individually and in combinations, in rabbits. A total of 15 rabbits was equally divided into three groups. Group A received an epidural injection of 4 mg/kg of 2% lignocaine-HCl, while Group B received 1 mg/kg of 0.5% bupivacaine-HCl. Group C was administered a combined solution of 2 mg/kg of 2% lignocaine-HCl and 0.5 mg/kg of 0.5% bupivacaine-HCl. Physiological parameters such as heart rate, respiratory rate, and rectal temperature were recorded 10 minutes before and then at 10-minute intervals for 120 minutes post-epidural anaesthesia. Additionally, the onset and duration of anaesthesia, onset and duration of loss of weight-bearing ability, and onset and duration of flaccid paralysis were observed following epidural administration. The anaesthesia onset times were 8.0 ± 0.354 minutes, 12.5 ± 0.224 minutes, and 10.1 ± 0.272 minutes in rabbits receiving lignocaine, bupivacaine, and the lignocaine + bupivacaine combination, respectively. The duration of anaesthesia was significantly higher ($P < 0.01$) in Group B (138.00 ± 5.15 minutes) than in Group A (50.60 ± 1.60 minutes) and Group C (87.20 ± 5.05 minutes). The onset and duration of loss of weight-bearing ability and flaccid paralysis were significantly higher ($P < 0.05$) in Group B (17.60 ± 0.245 minutes) compared to Groups A and C. The lumbosacral epidural administration of the combined lignocaine and bupivacaine solution provided enhanced anaesthetic effects compared to lignocaine or bupivacaine alone.

Keywords: Analgesia; Regional anesthetics; Lumbosacral; Rabbits

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1. Introduction

Lumbosacral regional anesthesia results in effective analgesia due to its proximity to the spinal cord receptors responsible for the regulation and transmission of nociceptive signals [1], [2]. The epidural administration of regional anesthetic is relatively safe and provides effective anesthesia and post-operative analgesia. The drugs and their combinations have been used to induce epidural analgesia in dogs and cats [3], [4],[5]. The ideal epidural local anesthetic should have a rapid onset of action, a long duration of action, good analgesia, and muscular relaxation [6]. There is no single anesthetic that possesses all of these qualities. Although lignocaine has a short latency period for epidural anesthesia, its effectiveness becomes limited for longer surgical procedures [7]. Bupivacaine lasts longer but it takes more time to onset and its muscular relaxation is also *poor* [6]–[8]. Therefore, an agent with a rapid onset and a sufficient duration of action would be ideal and could be obtained with regional anesthetic mixtures that combine the

desirable properties of each component drug. To the knowledge of the authors, there are no studies of epidural regional anesthesia using a combination of lignocaine and bupivacaine in rabbits.

The present study aimed to compare the effects of lignocaine and bupivacaine alone and their combinations for epidural anesthesia considering the onset and duration of analgesia in rabbits.

2. Materials and Methods

Study Location

This study was done from August- 2023 to October- 2023 in the NPI college research unit

Experimental Rabbits

Fifteen clinically normal rabbits *(Oryctolagus cuniculus)* weighing 1.8 ± 2.3 kg were used in this study. They were purchased from a local rabbit market in Bharatpur. The rabbits were housed in a 12-h light-dark photoperiod in 3 different cages of the research laboratory of NPI College. They were fed seasonal grasses, concentrates, and water *ad libitum*. The rabbits were kept for 2 weeks for acclimatization in in laboratory environment. The experimental protocol was approved by the Nepal Veterinary Council on animal research (Ref. no. 35/2080/81).

Study design

Food was withheld from the rabbits for 8 hours (i.e. overnight) prior to the experiments, while water was allowed freely. The body weight of each rabbit was determined using a weighing balance. The rabbits were restrained to facilitate the epidural anesthesia. The injection site was located and the hair of the lumbosacral region was shaved and the skin was disinfected. Then after, 2% lignocaine gel was applied topically on site to reduce pain and to prevent unnecessary suffering and the epidural puncture was performed using 26 gauge*5/8" needle. A total of fifteen rabbits were divided into three groups, with each group containing five different rabbits: the LIG group, the BUP group, and the LIG+BUP group. The rabbits of LIG, BUP, and LIG+BUP groups received the lumbosacral epidural administration of 2% lignocaine (4 mg/kg) 0.5% bupivacaine (1mg/kg) and 1:1 mixture of 2% lignocaine and 0.5% bupivacaine (2 mg/kg and 0.5 mg/kg). The dose rate were used in this study following the doses of prior studies [9]. Physiological parameters were recorded 10 minutes before the epidural anesthesia and then after anesthesia at every 10 min intervals over a period of 120 min.

Assessment of Epidural Anesthesia

The onset of anesthesia was assessed by observing the reflexes to needle pinprick stimulation to the hind paddle or the web. It was performed at one-minute intervals until surgical anaesthesia. However, the duration of anesthesia was determined by the return of response to a bipedal pinprick stimulus. Loss of weight-bearing was detected when rabbits did not stand on its hind limbs. The recovery was confirmed when the rabbits became stable. Flaccid paralysis was confirmed when no measurable tone in both hind limbs. The heart rate (HR), respiratory rate (RR) and rectal temperature (RT) were recorded appropriately 10 minutes before and then after every 10 minutes interval over a period of 120 min of epidural injections of regional anesthetics.

Statistical analysis

The data are expressed as Mean ± Standard Error of Mean. The data was analyzed in Minitab version 17 software manufactured in Pennsylvania State University. Data were analyzed using one-way ANOVA followed by post hoc Tukey's test. However, the physiological variables were compared using for repeated measures in each group. The P value less than 0.05 was considered significant.

3. Results

The onset of anesthesia with LIG-Group (8.0 ± 0.354 min), BUP-Group (12.5 ± 0.224 min) and LIG-BUP combination- Group (10.1 ± 0.272 min) was significantly different (P<0.05) from each other among the three groups. The mean duration of anesthesia of LIG group (50.60 ± 1.60), BUP group (138.00 ± 5.15), and LIG+BUP group (87.20 ± 5.05) differs highly significantly (P<0.01) from each other. The mean onset of loss of weight-bearing ability was significant (P<0.05) in the BUP group with (17.6000 ± 0.245). The mean duration of loss of weight-bearing ability was highly significant (P<0.05) in the BUP group (76.80 ± 3.57) compared to LIG group and LIG+BUP which also differs from each other significantly (P<0.05) with values of (24.000 ± 0.949) and (49.00 ± 2.74). The mean onset of flaccid paresis in BUP group (19.600 ± 0.927) was highly significant (P<0.05) as compared to LIG group and LIG+BUP group with values of (23.900 ± 0.400) and (24.600 ± 0.927) respectively. The mean duration of flaccid paresis was highly significant (P<0.05) in LIG group (10.900 ± 0.332) compared to the BUP group and LIG+BUP group which also differs from each other significantly (P<0.05) with values of (50.20 ± 2.22) and (19.00 ± 1.52) respectively. There were no significant changes in heart rate, respiration rate and temperature.

Table 1: The mean ± SEM of onset time of anesthesia (min), duration of anesthesia (min), onset and duration of loss of weight bearing ability (min), onset and duration of flaccid paresis (min).

Parameter	LIG	BUP	LIG+BUP
Time of onset of anesthesia	8 ± 0.354 [a]	12.5 ± 0.224 [b]	10.1 ± 0.272 [c]
Duration of anesthetic effect	50.60 ± 1.60 [a]	138.00 ± 5.15 [b]	87.20 ± 5.05 [c]
Onset of loss of weight-bearing ability	16.00 ± 0.707 [a]	17.60 ± 0.245 [b]	16.00 ± 0.274 [a]
Duration of loss of weight bearing ability	24.00 ± 0.949 [a]	76.80 ± 3.57 [b]	49.00 ± 2.74 [c]
Onset of flaccid paralysis	23.90 ± 0.400 [a]	19.60 ± 0.927 [b]	24.60 ± 0.927 [a]
Duration of flaccid paresis	10.90 ± 0.332 [a]	50.20 ± 2.22 [b]	19.00 ± 1.52 [c]

Figures with similar symbols in the same row indicate non- significant

Table 2: The mean ± SEM of HR (beat/min) measured at different times 10 minutes prior and then after anesthesia at every 10 minute intervals over a period of 120 min post injection.

Time	LIG	BUP	LIG+BUP
-10	232.60 ± 9.79	225.4 ± 10.3	222.80 ± 4.79
10	218.60 ± 7.61	242.8 ± 15.2	215.60 ± 6.18

20	200.40 ± 8.18	213.80 ± 9.68	200.00 ± 5.67
30	208.40 ± 7.28	206.2 ± 10.8	208.40 ± 5.61
40	234.40 ± 6.76	203.2 ± 13.8	210.00 ± 7.13
50	225.2 ± 11.9	197.4 ± 12.1	208.40 ± 3.82
60	203.6 ± 12.3	204.60 ± 9.91	204.40 ± 2.99
70	203.4 ± 13.2	205.00 ± 7.63	221.60 ± 2.64
80	218.80 ± 7.71	209.80 ± 8.32	212.2 ± 10.1
90	216.40 ± 4.30	210.00 ± 6.88	218.40 ± 2.66
100	215.80 ± 4.61	203.60 ± 8.19	221.60 ± 3.06
110	219.80 ± 6.00	207.6 ± 13.7	223.20 ± 1.53
120	214.00 ± 6.03	211.4 ± 11.1	225.20 ± 2.71

Table 3. The mean ± SEM of RR (breaths/min) measured at different times 10 minutes prior and then after anesthesia at every 10 minute intervals over a period of 120 min post injection

Time	LIG	BUP	LIG+BUP
-10	129.60 ± 3.83	148.6 ± 11.8	143.2 ± 12.6
10	127.40 ± 4.49	159.6 ± 7.46	135.20 ± 6.21
20	123.40 ± 7.70	147.40 ± 7.40	141.40 ± 6.66
30	125.00 ± 7.30	138.4 ± 10.2	144.20 ± 6.33
40	122.6 ± 13.2	142.6 ± 10.9	145.20 ± 9.09
50	131.2 ± 14.6	134.0 ± 12.0	142.80 ± 8.55
60	142.4 ± 16.0	137.8 ± 12.6	149.60 ± 9.10
70	149.8 ± 15.5	136.8 ± 10.8	138.40 ± 7.09
80	140.0 ± 12.7	143.0 ± 12.3	146.60 ± 7.24
90	138.4 ± 11.0	146.6 ± 15.3	135.60 ± 7.30
100	134.20 ± 8.01	144.0 ± 17.2	135.60 ± 6.56
110	131.60 ± 9.05	146.4 ± 13.3	139.60 ± 8.42
120	129.40 ± 6.46	149.0 ± 10.8	137.00 ± 6.71

Table 4. . The mean ± SEM of RT (°F) measured at different times 10 minutes prior and then after anesthesia at every 10 minutes intervals over a period of 120 min post injection

Time	LIG	BUP	LIG+BUP
-10	101.76 ± 0.129	101.68 ± 0.0583	101.84 ± 0.211
10	101.56 ± 0.0510	101.46 ± 0.0872	101.60 ± 0.0600
20	101.54 ± 0.0510	101.32 ± 0.0735	101.42 ± 0.121
30	101.56 ± 0.103	101.40 ± 0.268	101.52 ± 0.0583
40	101.60 ± 0.0707	101.30 ± 0.103	101.36 ± 0.354
50	101.72 ± 0.116	101.34 ± 0.103	101.42 ± 0.0840

60	101.64 ± 0.0748	101.38 ± 0.0800	101.32 ± 0.371
70	101.50 ± 0.0707	101.42 ± 0.180	101.46 ± 0.0812
80	101.40 ± 0.0949	101.34 ± 0.121	101.34 ± 0.206
90	101.70 ± 0.210	101.38 ± 0.185	101.38 ± 0.273
100	101.7 ± 0.169	101.40 ± 0.0949	101.70 ± 0.0949
110	101.65 ± 0.150	101.48 ± 0.107	101.52 ± 0.0663
120	101.68 ± 0.133	101.48 ± 0.0374	101.50 ± 0.134

4. Discussion

None of the rabbits died or showed any side effects during and after anesthesia for a week period. In this study, the rabbits were gently restrained for injection of epidural anesthesia with short needles which produced no apparent discomforts. The lumbosacral epidural anesthesia technique in rabbits and ferrets *(Mustela furo)* is identical to dogs and cats, with the exception of the rarely definitive popping sensation when the intervertebral ligament is punctured [10]. The spinal cord continues caudally into the sacral vertebrae in rabbits and thereby increased the risk of puncture of both the dura and arachnoid membranes during lumbosacral epidural injection [11]. To overcome this situation, the anesthesia was administered once cerebrospinal fluid was seen in the hub of the needle. In this study, solutions of lignocaine and bupivacaine were observed to be dispersed in the syringe, showing pharmacological compatibility which is similar to previous studies [12]. The doses of LIG 0.2 ml/kg i.e. (4 mg/kg) and BUP 0.2 ml/kg i.e. (1 mg/kg) were used in this study following the doses of prior studies [9]. The combination of lignocaine and bupivacaine exhibited a longer onset of action ($P<0.05$) than lignocaine but shorter ($P<0.05$) than that of bupivacaine. This result is similar findings by [13]. Onset of action of regional anesthetics differs due to pka (acid dissociation constant or pH at which the non-ionized and ionized fractions are at equilibrium) values when pH value of tissue remains constant. Regional anesthetics with a low pKa of 7.6-7.9 have a rapid onset of action because 30% to 40% of these drugs exist in the unionized state at pH 7.4 of which lignocaine has pKa value of 7.9. Lignocaine has a faster onset time than drugs with a high pka because its pka is closer to tissue pH [7]. Conversely, regional anesthetics with high pKa of 8.0 - 8.9 are slow acting agents because only 15% or less of these drugs are unionized at pH 7.4 of which bupivacaine holds value of 8.1.

Moreover, the duration of analgesia was significantly longer with lignocaine and bupivacaine combination ($P<0.01$) than lignocaine alone and significantly shorter than bupivacaine alone ($P<0.01$), indicating that adding bupivacaine to lignocaine accelerates and prolongs the duration of anesthesia. Similar findings were reported in a previous study conducted on dogs [13]. The mean duration of loss of weight bearing ability was significantly high in lignocaine- bupivacaine combination group ($P<0.05$) than in lignocaine alone indicating that adding bupivacaine to lignocaine accelerates and prolongs the quality of analgesia. Onset of flaccid paresis was significantly rapid ($P<0.05$) in bupivacaine group. The mean duration of flaccid paresis was significantly longer in the lignocaine bupivacaine combination group ($P<0.01$) than in lignocaine alone. This finding can be attributed to a synergistic effect between lignocaine and bupivacaine. The combination of the two drugs can decrease the side effects of each drug and increase the duration of flaccid paresis and analgesia [14]. No significant differences were observed in heart rate, respiration rate and temperature between three groups.

5. Conclusions

The combination of lignocaine and bupivacaine showed an ideal anesthetic effect over lignocaine or bupivacaine alone having shorter onset and prolonged duration of action with good analgesia. None of the treatments induced significant changes in heart rate, respiration rate and temperature. Further research is needed to investigate the analgesic effect of lignocaine and bupivacaine combination in rabbit under surgical conditions.

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CVJ CLUJ VETERINARY JOURNAL

Case Report

Endovascular single-chamber pacemaker implantation using active fixation in a 8 years old Dogue de Bordeaux with presumed Persistent Atrial Standstill: the first case documented in Romania

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Abstract: Background: Symptomatic bradyarrhythmias in dogs can lead to sever hemodynamical compromise due to impaired oxygen and nutrients delivery and even sudden cardiac death. In cases refractory to medical therapy the heart rate and therefore the cardiac output are maintained in physiological ranges using artificial external, internal and temporary (emergency situations) or internal and permanent cardiac pacing.

Methods: This case report documents the implantation of a permanent transvenous Pacemaker using active fixation, for the first time in Romania in a 8 years old canine Dogue de Bordeaux with a suspicion (on surface electrocardiography) of Persistent Atrial Standstill. The diagnostic in this atrial muscular dystrophy requires differentiation from Atrial Fibrillation with Third Degree Atrioventricular Block using Cardiac Mapping, but in emergency situations or progressive Congestive Heart Failure the decision to implant the pacemaker should be based on the hemodynamical effects of the dysrhythmia and the definitive diagnostic should be made during the implantation of the device.

Results: We succesfully implanted in this case a transvenous ventricular Solia S60 lead at the level of the 1/2 distance between the mid-septal and right ventricular apex that was connected to the subcutaneously placed Pulse Generator (Enitra 6 SR).

The survival after the procedure was 202 days (6 months and 18 days) and the patient's death was not related to a cardiac cause. The necropsy showed that the active fixation in this case was preserved and there were not identified any local rejections of the lead or at the site of the pulse generator.

Conclusions: The outcome of the patient was positive regarding the described cardiac function in this case and the clinical signs went into remission shortly after the pacing.

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1. Introduction

Symptomatic bradyarrhythmias in dogs are a class of primary or secondary cardiac conditions that can lead to temporary loss of consciousness - TLOC, Transient Loss of Consciousness 23%-77% (in dogs) (3), Cardiogenic Shock, Multiple Organ Dysfunction System (MODS) (6), or sudden death. The main bradyarrhythmias that usually require therapeutic intervention are advanced Second Degree Atrioventricular Block (3.8%-9%), Third Degree Atrioventricular Block (53%-64%), Sinus Node Disease (14.8%-29 %) (5), 4permanent pacemakers is the most effective method of treatment in symptomatic cases,

but the medical decision must take into account the severity of the hemodynamic disturbances, the coexistence of other heart diseases (valvular disease, neoplasia, etc.) or extracardiac (systemic diseases). The type of pacing, pacemaker programming, the

method of pacemaker implantation and the paced anatomical region require electrophysiologic evaluations and studies prior to the procedure (Atropine response test, Holter Electrocardiography, Cardiac Mapping) or during the procedure (by examining myocardial response to Temporary Pacing). Transvenous implantation is the most common technique in dogs but is dependent on the size of the patient (2). In cats (and dogs under 2 kg), epicardial implantation by thoracotomy or laparotomy (5) is preferred for permanent pacing or transesophageal implantation for temporary pacing (4). The pacing mode frequently preferred is VVI (Ventricular Sensing and Pacing, Sensing and Inhibited) (1) and can be used as a first intention method followed by adjustment of the pacing type according to myocardial response, condition or hemodynamic evolution. In those situations in which the type of electrical disturbance responsible for the induction of bradyarrhythmia cannot be accurately determined, the decision whether to place a pacemaker is based on the hemodynamic effect of the dysrhythmia.

This case report documents the implantation of a VVI Transvenous Pacemaker, active fixation, for the first time in Romania in a case of suspected Persistent Atrial Standstill in a dog (absence of P waves, presence of idioventricular escape rhythm in the absence of hyperkalemia) diagnosed by surface electrocardiography (diagnosis requiring differentiation from Atrial Fibrillation with Third Degree AV Block by cardiac mapping).

2. Case presentation

The patient (8 years old Dogue de Bordeaux, intact male) was referred to our clinic for cardiac exam due to bradyarrhythmia and ascites. Owner's complaints were fatigability, weight loss, "swollen belly", "dizziness". On presentation the dog was responsive, with severe abdominal distension, marked cachexia with a heart rate of approximately 40 bpm (beats per minute), synchronous pulses and a respiratory rate of 43 rpm (respirations per minute). The mucous membranes were moist and pink, with a CRT (capillary refill time) of approximately 2 seconds and a rectal temperature of 38,7 C.

Cardiac Examination

The cardiac examination consisted of electrocardiography, 24 hours Holter examination, echocardiography, blood pressure measurement, blood tests including cardiac biomarkers.

Electrocardiography

The severe bradycardia was documented with a 5 minutes (short-term) 6-lead ECG with the patient positioned in right lateral recumbency using Poly-Spectrum 8-v ECG Machine. The ECG measurements obtained were: a heart rate of 40 bpm with regular R-R Intervals, absent P-waves, Atrioventricular conduction and an Idioventricular Escape Rhythm with wide QRS complexes (137 ms) (Fig. 1).

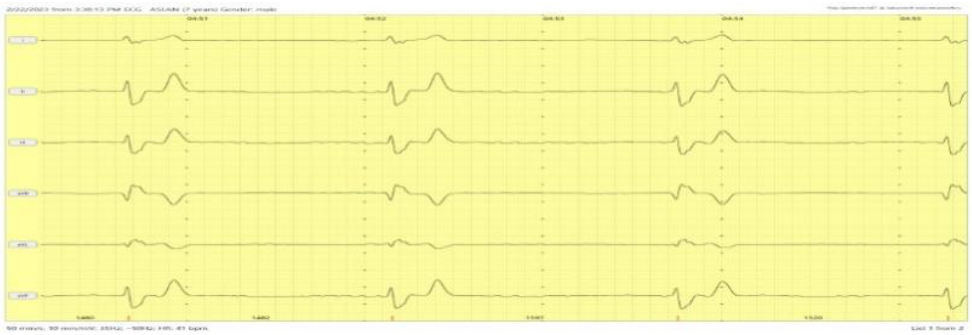

Fig. 1. ECG trace at the first visit documenting the dysrrhytmia (original photo)

Blood tests

Complementary blood tests, electrolytes and cardiac biomarkers (Cardiac Troponin I, Pro-BNP) were performed with no remarkable findings at biochemistry panel and increased cardiac biomarkers (Cardiac Troponin I 0,46 ng/ml and NT-proBNP 2845,3 pmol/l).

Echocardiography

The echocardiography was performed in a standing position using the right parasternal short axis, long axis and left apical views before the patient was positioned in a right and left lateral recumbency (in order to avoid any procedural complications related to sympathetic stress response to mechanical contention, since the patient was not compliant). The measurements were made using a Siemens Acuson Juniper Ultrasound Machine equipped with a 5P1 Phased Array and a 8V4 Phased Array probe.

Echocardiographic morphological examination

Right atrium and right ventricle enlargement, enlarged Cranial and Caudal Vena Cava, flattening of the interventricular septum, systolic pulmonary hypertension with TR Vmax (tricuspid regurgitation maximum velocity) 3,20 m/s, TR PG max (tricuspid regurgitation pressure gradient) 40,96 mmHg.

Echocardiographic functional examination

Absent A-waves on tricuspid inflow tract, mild aortic and mitral valve regurgitation, severe tricuspid valve regurgitation (Fig. 2).

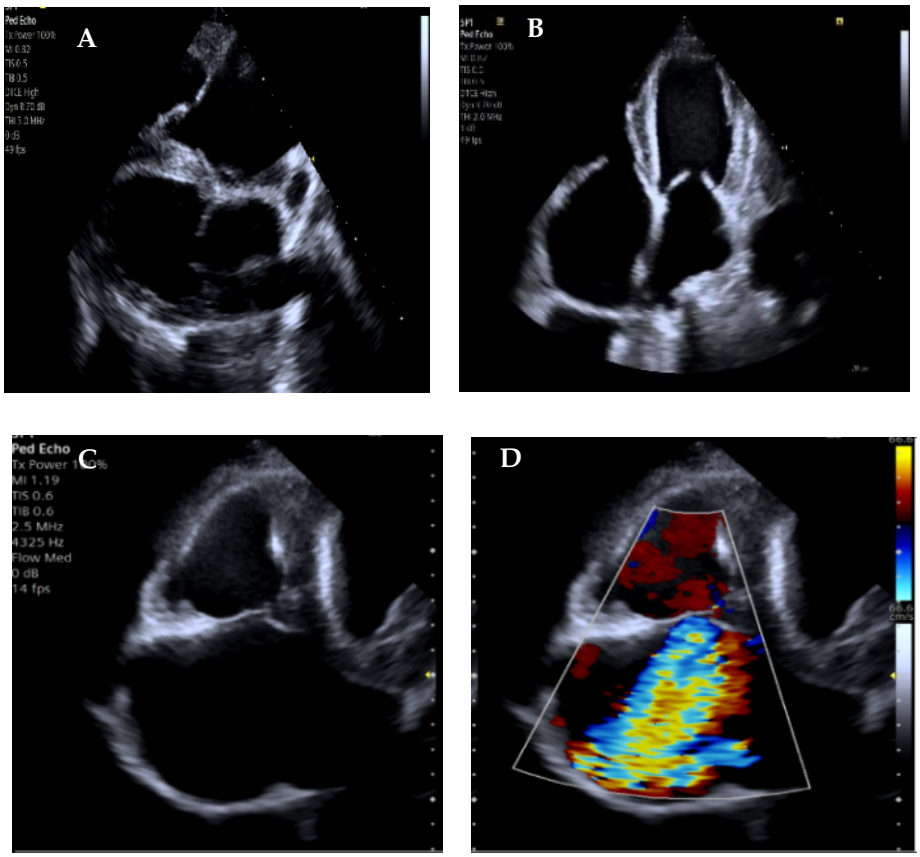

Fig. 2. (A, B, C) Right ventricle and right atrium enlargement - A. Right parasternal long axis 4 chambers view, B. Apical 4 chambers view, C, D. Apical view optimized for right heart side assessment. (D) Severe tricuspid regurgitation – D. CFM (Color Flow Mode) at tricuspid valve level (original photos)

24 hours Holter Examination

On a Dynamic Electrocardiography represented by a Holter (24h ECG) examination an Idioventricular Escape Rhythm was observed, with regular R-R interval and a 34 bpm heart rate (Fig. 3).

Fig. 3. Original ECG trace during Holter examination (original photos)

Therapeutic intervention

The patient was started on Sildenafil 2,5 mg/kg BID (to exclude precapillary pulmonary hypertension and to evaluate response to therapy) and Furosemide 1,5 mg/kg BID, Taurine and Carnitine supplementation by the first cardiologist who performed the examination.

At the one month follow-up the clinical signs were not remitted (as we expected). Ascites, fatigability, exercise intolerance and the patient's clinical condition were not improved.

At this point pacing therapy was recommended as election therapy for chronotropic insufficiency (PAS/Third Degree AVB with Afib.). Apical or Right Mid septal Single Chamber Endovascular Pacing VVI Mode – active fixation is normally recommended in this type of arrhythmia (1)

Pacing Therapy

The procedure was performed by the AvantGard CardioTeam in the "Interventional Veterinary Radiology Laboratory Doctor's Vet Univers", Bucharest, Romania.

Anaesthesia protocol and monitoring

The anaesthesic protocol included premedication with Butorhanol 0,2 mg/kg IV and Midazolam 0,25 mg/kg IV; induction with Ketamine 2 mg/kg iv and Propofol 4 mg/kg IV; maintaining using Isoflurane 1% and local anesthetic blocks with Lidocaine 1 mg/kg

Anaesthesia monitoring measured heart rate (20-24 bpm before pacing, 70 bpm after pacing), Breathing rate (10-14 rpm), Electrocadiograhy (PVCs during the lead fixation, spontaneously remitted after the procedure).

Surgical technique

After optimum anaesthetic depth was obtained, the surgical area was prepared antiseptically (clorhexidine gluconate 4%, povidone-iodine 100mg/ml, etc). The patient was placed in left lateral recumbency to expose the right external jugular vein (EJV). The neck was extended dorsally and the anterior right leg was pulled caudally for a better exposure of the right EJV and also for assuring a straight passage of the pacemaker lead at the level of the confluence of Brachial vein and the Cephalic vein communication with EJV. The skin was pulled dorsally from the projection of the EJV and the cutaneous layer was incised, the EJV was exposed and lifted using two multifilament wires placed cranially and caudally to the incision with a Mixter Hemostatic Forceps. After the blunt dissection of the perivascular layer (using the same clamp and the surgical blade), the vessel was punctured and the 6 F "Peel-away" introducer sheath was prepared for inserting the lead. Using the Modified Seldinger Technique, the sheath was introduced through the EJV and secured with a mutifilament wire to the skin (Fig. 4).

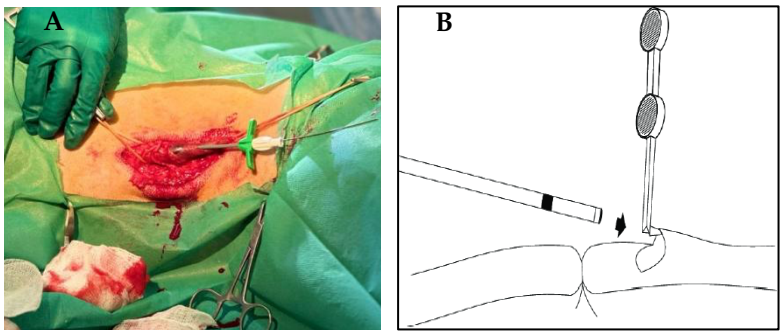

Fig. 4. A. Placing the introducer sheath in the EJV (original photo); B. Another technique is inserting the lead directly into the EJV using the vein picker (Biotronik original photo)

Imaging guidance

Fluoroscopic Image Guidance was provided during the procedure (using a Siemens Cios Select mobile C-Arm). Angiography was performed using a Floating Angiography Catheter (Pulmonary Capillary Wedge Pressure, PCWP) which was positioned using the venous access port (represented by the Peel Away introducer sheath) in the Cranial Vena Cava. Subsequently, by manual injection of a solution of Iohexol (diluted 1:1 with 0.9 % saline solution), the path of the Cranial Vena Cava and Right Atrium were visualized. (Fig. 5)

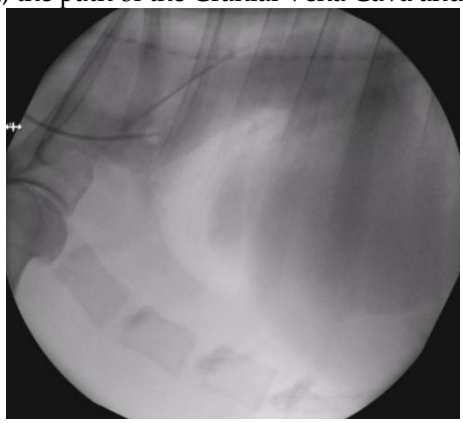

Fig. 5. Positioning the PCWP catheter in the Cranial Vena Cava and contrast administration (original photos)

Placing and securing the pacemaker lead

The pacemaker lead Solia S60 (Fig. 6) used had a diameter of 1.9 mm (5.9 F) at its tip, 1.8 mm (5.6 F) at its body and a steroid (dexamethasone acetate) reservoir at its tip, in the form of a silicone rubber ring, for anti-inflammatory effect. The body of the lead is formed by two coaxial coils made of several wires placed in parallel and the coils are the conductors to the tip and ring. The lead has a coaxially predesigned stylet which has the purpose of facilitating the desired position. Stimulation and detection take place between the distal pole and the annular electrode.

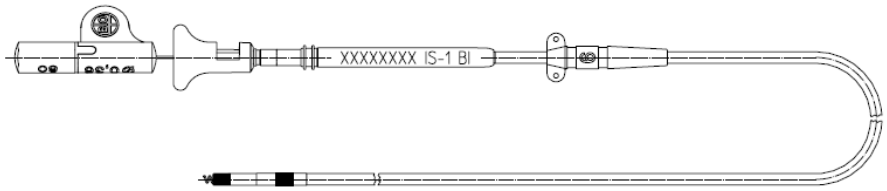

Fig. 6. Solia S lead, active fixation (Biotronik)

At the tip of the lead is a screw (Fig. 7) that can be inserted or withdrawn to actively fix it in the myocardium thickness.

The mechanism is operated by rotating the contact stiff of the probe connector using a plastic clamp (delivered with the lead by the manufacturer). The fixing screw, which is electrically active and forms the distal pole of the probe, is made of platinium-iridium alloy, has a maximum penetration depth of 1.8mm, an electrically active surface of 4.5mm2. The typical number of turns for insertion (and withdrawal) is 5 to 10 turns (maximum/optimum 23).

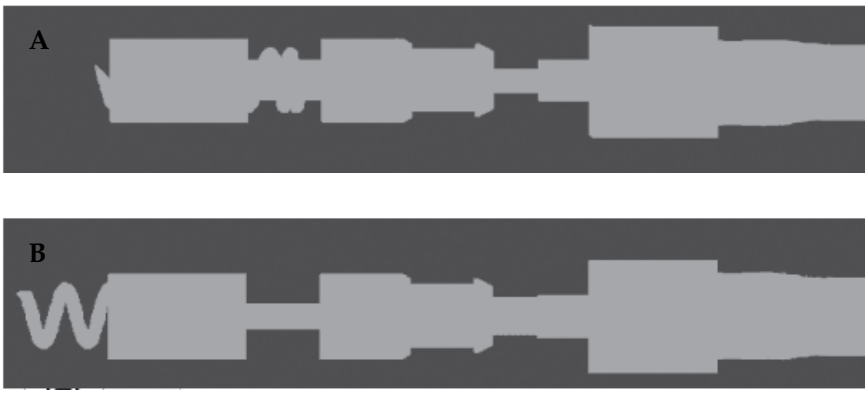

Fig. 7. Rontgen aspect of the unarmed (A) and armed (B) lead's screw (Biotronik original photo)

Inserting the lead through the peel-away introducer sheath and for a proper position the stylet was preshaped (S-shape) in order to obtain a better approach of the mid septal location or for the most perpendicular approach of the interventricular septum. At this point the neck of the patient was extended dorsally to exclude any tension applied to the lead when it will be connected to the pulse generator.

Since the site of this particular arrhythmia (supraventricular) was considered to be at the level of the Atrioventricular Junction, the lead position suited in this case was ideally the mid septal or at least right apical ventricular myocardium. Our team used the right ventricular apical positioning of the lead and secured it into the myocardial layer (Fig. 8). We investigated the response to 2.5 mV stimulation of the myocardium by connecting the lead to the pulse generator and observed no response on the surface ECG; afterwards we increased the voltage in order to obtain a normal response. At this point (due to the high level of electrical stimulation) we decided to reposition the lead, considering the possibility of previously pacing a non-responsive myocardial area (maybe due to fibrosis or other morphological changes of the myocardial tissue). Therefore the screw was withdrawn (23 turns clockwise) and the lead was repositioned targeting

the half distance between mid-septum and right ventricular apex. We reinvestigated the pacing effect over the myocardium and we observed a positive response, represented by a ventricular rate of 70 beats per minute.

Considering that, in this case, this site was proper for pacing therapy (based on ECG surface) our team decided to leave the pacemaker lead in this position.

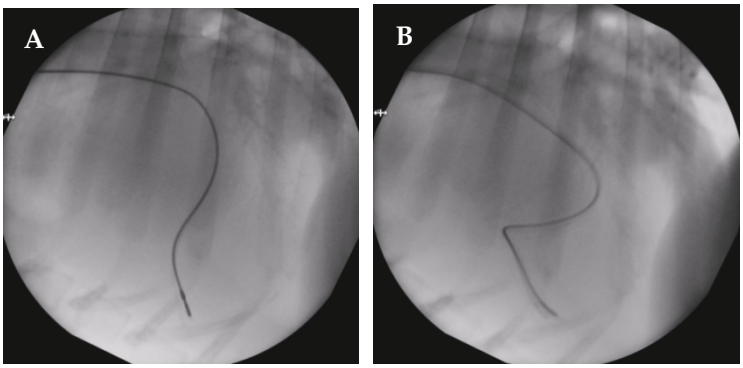

Fig. 8. Positioning the lead at A. the level of right ventricular apex; B. the half distance between mid-septum and right ventricular apex Pulse Amplitude 2.5 V with pacing response (original photos)

Pacemaker programming

In an emergency situation, normally the pulse generator (that is made for human use) comes with pre-defined programming, the heart rate being usually set at 60 beats per minute. Considering we had a large breed canine patient, the heart rate was set at 70 beats per minute (with the possibility to further adjust the settings).

The threshold (minimum amount of energy required to evoke an action potential) reaches its highest level in 2-6 weeks after the surgery, followed by attaining a stable level at approximately 2-3 times the acute level. Intensity of the electrical stimulus is described by its amplitude and duration. Heart Rate was fixed at 70 bpm, the lead impedance increased at 604 Ω, refractory period was set at 250 ms (miliseconds) with the possibility to be increased up to 280-300 ms in the next 8 weeks. After 8 weeks the target was to decrease the pulse amplitude (initially set at 5.0 V, 1.0 ms) and possibly to set the heart rate at a higher value during the day and at effort (70 bpm during the night, approximately 140 bpm during exercise).

Securing the lead to the adjacent connective tissue

After removing the peel-away introducer sheath the next step is to secure the lead in a proper position.

At this point we fixed the external connector of the lead with a monofilament suture material to the EJV and with a second wire we secured the lead to the perivascular connective tissue in order to achieve a fixed position of the external part of the lead (Fig. 9).

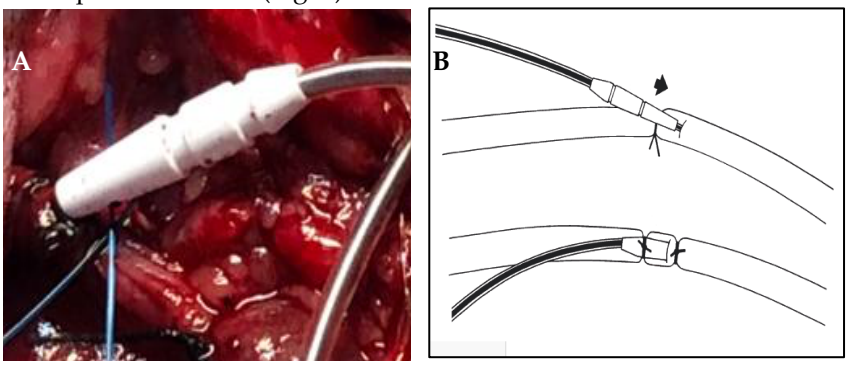

Fig. 9. A. Close-up aspect of lead fixation at the level of the jugular vein, by using the plastic fixation tool provided with the lead (original photo) and B. Biotronik original photo of the same aspects

Surgical placement of the pacemaker

Once the lead was fixed and connected, a small pocket was made by blunt dissection in the caudo-lateral area of the neck, in order to place the pulse generator and afterwards the pocket was ligated routinely.

Pacemaker evaluation

We evaluated the response of the external pacing 24 hours after the surgery by performing 5 minutes (short time) 6 leads ECG (Fig. 10) with the patient positioned in right lateral recumbency. The ventricular rate was in accordance with the intra operatory settings of the pacemaker (70 bpm) (Fig. 11), which produced a secondary improvement of the cardiac output. We also interrogated the 48 hours heart rate trend using the Biotronik programmer and we observed a normal and constant heart rate response to pacing therapy (Fig. 12).

Fig. 10. 6 leads ECG performed 24 hours after pacing, showing R-R intervals and 70 bpm HR (original photos)

Parameters - Overview		(1st interrog.)
Mode		VVI
Basic rate/Night rate [bpm]		70/OFF
Sensor/Rate fading [bpm]		-----/OFF
Upper rate response [bpm]		
Pulse amplitude [V]		5.0
Pulse width [ms]		1.0
Capture control		OFF
Sensitivity [mV]		AUTO
Refract. period [ms]		250
Sensing polarity	↕	BIPL
Pacing polarity	↕	BIPL

Fig. 11. Pacemaker parameters interogation using pacemaker programmer (original photo)

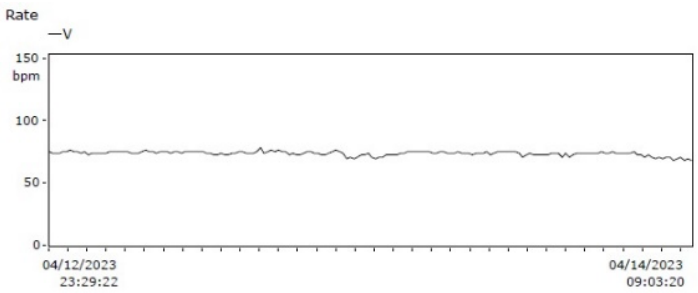

Fig. 12. 48 hours HR trend provided by the programmer (original photo)

The patient was reassessed monthly and no changes from the baseline settings were noted. Six months postoperatively the patient was hospitalized for Acute Abdominal Syndrome and later died. A necropsy

was performed and showed small foci of intestinal necrosis, considered not related to the cardiac disorder. The pulse generator and the lead were in proper initial position confirming that the surgical technique and right ventricular apical fixation were optimal (Fig. 13).

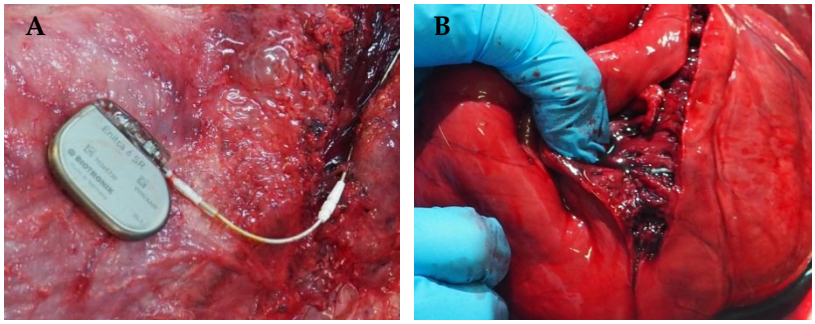

Fig. 13. A, Necropsic images of the Pulse generator and B, Fixed lead into the right ventricular myocardium

3. Results

The suspicion of Persistent Atrial Standstill can be easily made using a 6-lead surface ECG, but there is a possibility that a regular Idioventricular Rhythm, similar to that of PAS, may be found in patients with Atrial Fibrillation and Complete Atrioventricular Block.

The anchoring of the active-fixation pacemaker lead can be easily accomplished by fluoroscopic imaging, where even the corkscrew-like fixation loops at the tip of the lead can be visualized.

The follow up of this procedure showed a stable heart rate and a secondary improvement of the cardiac output which were considered normal for assuring an optimal hemodynamic stability.

4. Conclusions

Using a programmer to detect myocardial viability during implantation can improve outcome

The anchoring of the active-fixation pacemaker lead can be easily accomplished by fluoroscopic imaging guidance, where the corkscrew-like fixation loops at the tip of the lead can be visualized.

The outcome of the patient was positive regarding the cardiac function and the clinical signs went into remission shortly after the pacing.

The survival after the procedure was 202 (6 months and 18 days) days and the patient's death was not due to a cardiac cause.

5. Patents

Funding: This research received no external funding

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

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CVJ CLUJ VETERINARY JOURNAL

Case report

Unusual Fatal Gastric Sand Impaction in an Argentine Polo Pony in Nigeria: A Case Report

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Abstract: Equine colic remains a significant health concern with complex, multifactorial aetiologies. Gastric sand impaction, while less common than large colon sand accumulation, can lead to severe complications including gastric rupture if left untreated. This report documents a case of fatal gastric sand impaction in a 12-year-old Argentine Polo Pony mare from Nigeria. The mare, maintained on ground feeding with hay and commercial concentrate feed, developed progressive colic signs over three days. Despite severe clinical deterioration, including reduced appetite, abdominal discomfort, and frequent rolling, no veterinary examination was pursued. Post-mortem examination revealed a 15 cm gastric rupture with approximately 8 kg of accumulated sand, accompanied by severe peritonitis and gastrointestinal inflammation. This case emphasizes the importance of proper feeding practices, regular veterinary monitoring, and prompt intervention in equine colic cases. It also demonstrates that significant sand accumulation can occur in the equine stomach, not just the large colon, potentially leading to gastric rupture. Immediate veterinary intervention for colic signs and use of elevated feeding systems to prevent fatal sand impaction are recommended.

Keywords: Gastric sand impaction, equine colic, gastric rupture, Argentine Polo Pony, equine critical care

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1. Introduction

Equine colic continues to be one of the most important issues in the entire equine industry, not only because of the morbidity and mortality but also due to great economic loss worldwide [2, 8]. Colic is a broad general term for various gastrointestinal conditions. On the other hand, sand-induced gastrointestinal disease has its own problems in particular geographic areas and management conditions [5].

Historically, sand accumulation has been mainly reported in the large intestine, leading to impaction, irritation of the mucosal lining, and alterations in motility [7]. However, gastric sand impaction, although less frequently reported, generally represents a more serious and life-threatening condition because of the possibility of gastric rupture [3]. The restricted distension capacity of the stomach is further compounded by the inability of horses to vomit upon the severity of gastric impactions.

Management considerations are important in the development of sand-induced gastrointestinal problems. Ground-feeding practices, insufficient pasture, and low-quality feed are all examples of management issues that contribute to increased sand ingestion [6]. Sand consumption may be common in nations like Nigeria due to sandy soil and significantly varying management strategies.

Recent literature has emphasised the need for early identification and management of colic in horses [1, 4]. Clinical manifestations can quickly worsen into significant problems, particularly if there is stomach distention. Understanding its process and the factors that drive it is critical for achieving better therapeutic results.

This case report aims at documenting the clinical course of events and pathological findings in a case of fatal gastric sand impaction in an Argentine Polo Pony in Nigeria. We tend to present this unusual case with the hope that it would add to the growing interest in the field of equine gastro-intestinal medicine and emphasize the need for good management practices and timely veterinary intervention.

2. Case Report

2.1. Patient Information and History

A 12-year-old Argentine Polo Pony mare (600 kg) from Kaduna State, Nigeria, presented with progressive colic signs on August 5, 2024. The horse, used primarily for recreational polo, was kept in a sandy paddock with three other horses. The feeding regimen consisted of grass pasture and hay supplemented with commercial concentrate feed, which was fed directly on the ground. Fresh water was provided *ad libitum.*

2.2. Clinical Progression

The progression of clinical signs occurred over a three-day period. On the first day, the mare presented with reduced appetite and mild signs of abdominal discomfort, though she continued to drink water normally. No vital signs were assessed at this time, establishing a pattern of insufficient monitoring that would continue throughout the case.

By the second day, the clinical picture deteriorated significantly. The mare showed increased signs of discomfort, characterized by frequent pawing at the ground and repeatedly looking at her flanks. The owner noted reduced faecal output, a significant clinical sign that warranted veterinary attention. However, no veterinary examination was pursued at this stage.

The third day marked a severe deterioration in the mare's condition. She made frequent attempts to lie down and roll, accompanied by profuse sweating - classic signs of severe abdominal pain. In response to these concerning signs, the owner administered ketoprofen (2.2 mg/kg IM, Ketofen® 10%, Merial). Despite the severity of clinical signs, no veterinary examination was performed. The mare died approximately six hours after the ketoprofen administration.

2.3. Post-mortem Examination

A complete necropsy was performed within four hours of death, revealing multiple significant findings. External examination showed clear evidence of rolling behaviour, with dirt and abrasions present on the dorsal surfaces of the body. The mare exhibited moderate dehydration, estimated at 8-10% based on skin turgor assessment. No external traumatic injuries were identified beyond the abrasions associated with rolling.

Examination of the abdominal cavity revealed approximately 12 litres of serosanguineous peritoneal fluid. The peritoneal fluid appeared cloudy with a pH of 7.8, and contained visible feed material, indicating gastrointestinal rupture. Diffuse peritoneal inflammation was evident throughout the cavity, consistent with acute peritonitis.

The gastrointestinal tract showed multiple pathological changes, with the most severe findings in the stomach. A 15 cm longitudinal tear was present in the cardial region (Fig. 1), accompanied by severe gastric distention. The ventral portion of the stomach contained approximately 8 kg of accumulated sand, mixed with partially digested feed material. The gastric mucosa appeared haemorrhagic, particularly in the glandular region, indicating significant inflammation and compromised blood flow prior to rupture.

The small intestine showed moderate gaseous distention and congested mucosa, though notably without evidence of displacement or obstruction. These changes were likely secondary to the primary gastric pathology. The large intestine exhibited significant abnormalities, including caecal distention with gas (Fig. 2) and haemorrhagic areas throughout the caecal and colonic mucosa.

2.4. Diagnosis

Based on the post-mortem findings, the primary diagnosis was gastric rupture secondary to severe gastric sand impaction. Contributing factors include ground feeding practices and delayed veterinary intervention.

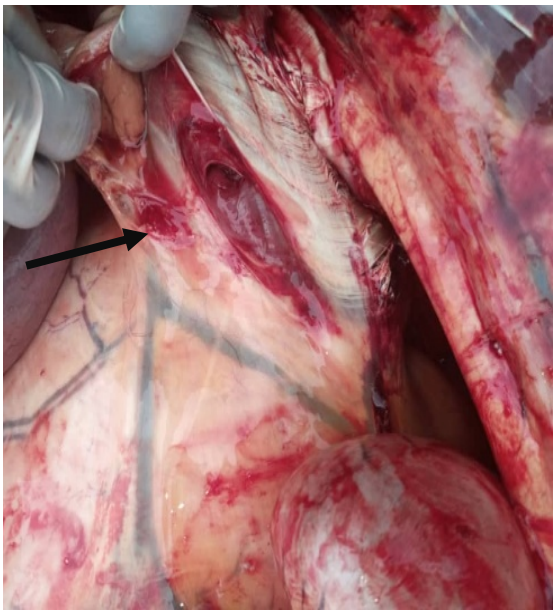

Fig. 1. Ruptured cardia of the stomach (black arow)

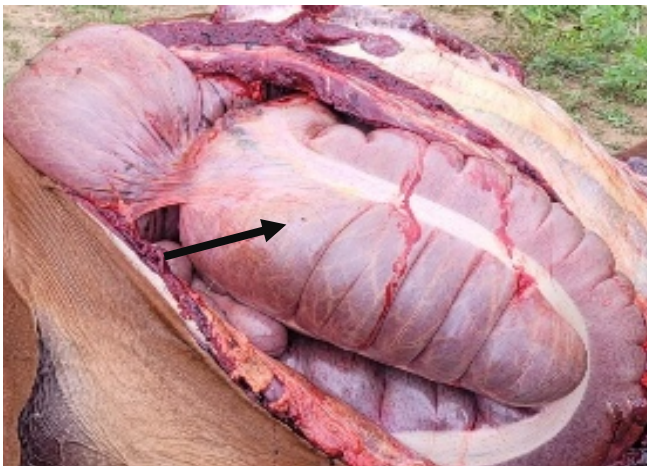

Fig. 2. Engorged caecum due to accumulated gas (black arrow)

3. Discussion

This case of fatal gastric sand impaction provides valuable insights into the consequences of poor management practices in equine care, particularly in regions like Nigeria where environmental and management factors may increase the risk of sand ingestion. The progression from initial clinical signs to gastric rupture over approximately 72 hours aligns with previous reports of equine gastric rupture [1, 3].

The development of gastric sand impaction in this case can be primarily attributed to poor management practices. Ground feeding of concentrate and hay significantly increased the risk of sand ingestion, a practice that has been associated with increased sand accumulation in horses [9].

The absence of regular veterinary monitoring and delayed intervention significantly impacted the case outcome. Early recognition and treatment of sand accumulation can prevent the development of severe complications such as gastric rupture [5]. The administration of ketoprofen without veterinary examination represents a critical missed opportunity for proper intervention. While non-steroidal anti-inflammatory drugs can provide temporary pain relief in colic cases, their use without proper diagnosis may mask deteriorating conditions and delay essential treatment [4].

Of particular interest is the unusual accumulation of 8 kg of sand in the stomach, rather than the more commonly reported large colon impaction. While gastric sand impaction has been reported in the literature, it is relatively rare compared to large colon sand accumulation [9]. This case demonstrates that significant gastric sand impaction can occur and may progress rapidly to fatal complications, emphasizing the need for

veterinarians to consider this possibility when diagnosing horses with colic symptoms, particularly when poor management practices are identified.

The pathophysiology of the gastric rupture likely involved progressive gastric distention from both sand accumulation and gas production, leading to stretching and eventual rupture of the cardiac region. The haemorrhagic changes observed in the gastric mucosa suggest significant inflammation and compromised blood flow preceded the rupture, consistent with findings reported in other cases of severe gastric distention [1, 3].

Limitations of this case study include the lack of ante-mortem clinical data and diagnostic imaging, which could have provided valuable information about the progression of the condition.

4. Conclusion

This case report highlights a fatal instance of gastric sand impaction in a horse, emphasizing the critical need for early veterinary intervention in colic cases and comprehensive preventive measures. The unusual presentation of sand accumulation in the stomach, rather than the typical large colon impaction, expands current understanding of sand-related gastrointestinal disease in horses and suggests that veterinarians should consider gastric sand impaction when diagnosing horses with colic signs. The case underscores the importance of feeding practices and comprehensive health programs to prevent sand ingestion. We recommend immediate veterinary intervention at the first signs of equine colic, along with implementation of elevated feeding systems to prevent sand ingestion, as delayed treatment and ground feeding practices can lead to gastric rupture.

Ethics Statement: Informed consent was obtained from the owner for the publication of this case report and accompanying images. All procedures and examinations were conducted in accordance with relevant guidelines and regulations.

Authors contribution: Conceptualization, P.W.M. and S.A.; investigation, R.E.E. and O.O.A.; writing—original draft preparation, P.W.M.; writing—review and editing, S.A., R.E.E. and O.O.A.; supervision, S.A.; All authors have read and agreed to the published version of the manuscript.

Data Availability Statement: For further information, please contact the corresponding author via email

Conflict of Interest Statement: The authors declare no conflict of interest.

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