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CONTENTS

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ORIGINAL ARTICLE

THE EFFECT OF 2.4 GHZ ELECTROMAGNETIC RADIATION ON THE GROWTH AND VIABILITY OF *ESCHERICHIA COLI*

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

The impact of radiofrequency electromagnetic field (RF-EMF) on living organisms is an important and current topic of scientific research, especially in the context of the growing use of wireless technologies. The aim of this study was to investigate the effects of non-ionizing electromagnetic radiation with a frequency of 2.4 GHz on the growth and viability of a selected model organism – *Escherichia coli*. The study was aimed at analyzing how exposure to this frequency affects the growth and viability of bacteria. The samples were exposed to RF-EMF for 2, 4, and 24 hours, then stained and analyzed by flow cytometry. The results showed a statistically significant inhibition of bacterial growth during the first 2 hours of exposure compared to the control sample. However, no significant differences were observed at the 4- and 24-hour time points. This effect is likely due to the selection of resistant bacterial subpopulations, reflecting the rapid life cycle and high adaptability of *Escherichia coli* to environmental stress. These findings suggest that short-term exposure to 2.4 GHz RF-EMF can temporarily suppress bacterial growth but also demonstrate the role of adaptive responses during long-term exposure.

Key words: *Escherichia coli*; exposure; growth; RF-EMF; viability

INTRODUCTION

Electromagnetic radiation

Electromagnetic radiation can be divided into ionizing and non-ionizing based on wavelength, frequency, source, and also photon energy. The limit for ionization of an atom or molecule is generally accepted as a photon energy of 10 electron volts (eV), which after conversion corresponds to 124 nm of wavelength. Electromagnetic radiation (RF-EMF) includes the electromagnetic spectrum: gamma rays, X-rays, ultraviolet radiation, visible radiation, infrared radiation, microwave radiation, and radio waves [1] (Fig.1).

Living organisms have adapted exclusively to natural electromagnetic radiation from natural sources during

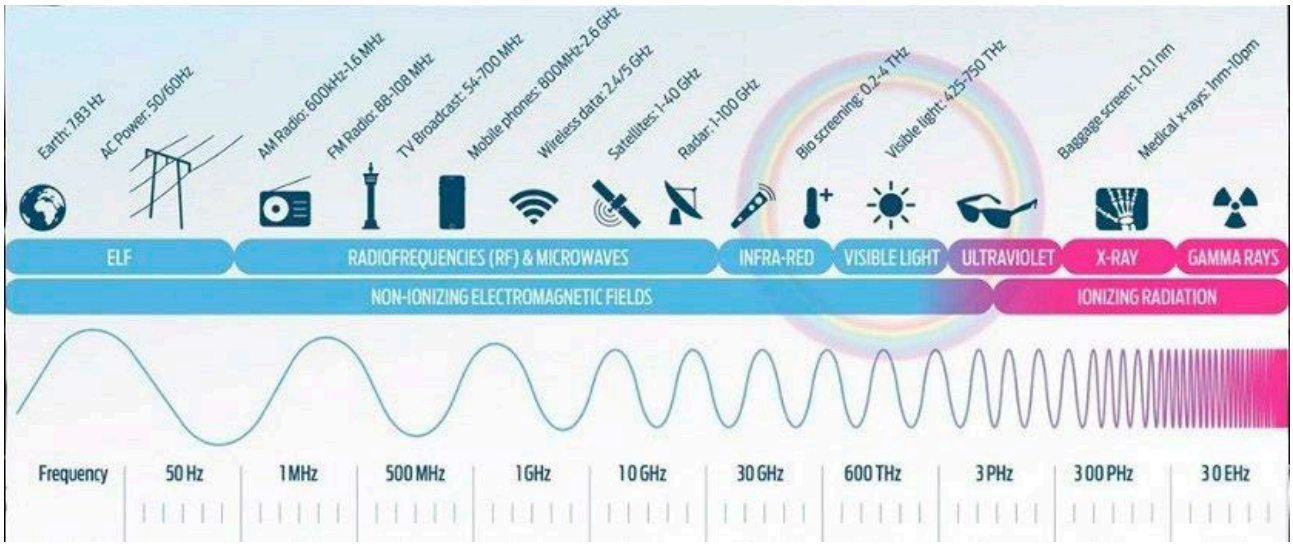

Fig. 1. Distribution of electromagnetic radiation from several aspects

evolution, which suggests that artificial RF-EMF are foreign to biological systems and may have a beneficial effect in some cases, but more often an adverse effect [2].

Thermal and non-thermal effects of electromagnetic radiation

The effects of electromagnetic radiation on living organisms can be divided into two categories: thermal and non-thermal. Thermal effect is caused by an increase in temperature upon absorption of energy, while non-thermal effect occurs without this heating and is more complex, including the induction of electrical currents, cell stimulation, and various interactions at the molecular level. Although the division is somewhat artificial, thermal effects are better understood and documented, while non-thermal effects are the subject of ongoing research and debate, as their mechanisms and health consequences are not fully understood [3, 4, 5].

Wi-Fi and routers

The term "Wi-Fi" refers to a wireless local area network that allows devices to be connected wirelessly using non-ionizing radiation at various frequencies, mainly 2.4 GHz and 5 GHz. Wi-Fi is used to exchange data and access the Internet in various environments from homes to public spaces. A Wi-Fi router is an electronic device that functions as a router and a wireless access point. Depending on the manufacturer and model, it can work in a wired local area network, in a wireless network, or in a mixed network. The most important parameter of a router is the frequency band and the number of channels. The difference between the 2.4 GHz and 5 GHz frequencies lies in the number of channels that a Wi-Fi router uses in its operation, which affects the stability and speed of the network connection [6, 7]. The 2.4 GHz frequency band is 70 MHz wide, and devices are usually limited to three 20 MHz channels. The advantage is a longer range and better overcoming obstacles (walls); the disadvantages include slower data throughput under heavy load and fewer available channels. The 5 GHz Wi-Fi spectrum is approximately 500 MHz wide, and devices can use up to six larger 80 MHz channels. The advantage is the high speed and reliability of the connection, suitable for e.g. streaming; the disadvantages include shorter range and worse overcoming obstacles (walls) [7]. Exposure limits for Wi-Fi radiation are set by international organizations such as ICNIRP in order to protect people from potentially harmful effects [8].

Effects of Wi-Fi on living organisms

Wi-Fi uses electromagnetic radiation at frequencies of 2.4 GHz and 5 GHz to transmit data. Although it is non-ionizing radiation, which does not have enough energy to directly damage DNA like ionizing radiation (e.g., X-rays), its potential effects on living organisms are the subject of scientific research. The potential health effects of wireless technologies such as WiFi and 3G/4G signals are the subject of various research studies [9, 10, 11].

Current findings indicate negative effects of acute and long-term exposure in animal models, including changes in the cardiovascular system, behavioral changes, the oc-

currence of oxidative stress, and cell damage [12]. Regarding the effects in humans, systematic reviews of studies do not confirm harmful effects of WiFi at levels below the regulatory limits applicable to protect human health. The current state of knowledge on this issue presents diverse and not always consistent research results [13,14].

Several studies have shown that Wi-Fi radiation can affect different types of bacteria. It has been found that shorter exposures can stimulate the growth and production of lactic acid in probiotic bacteria, while longer and continuous exposure can increase antibiotic resistance, as well as motility and biofilm formation in some pathogenic bacteria. The overall effects depend on the type of bacteria and the duration or frequency of radiation [15, 16, 17].

In our study, we set out to find out how 2.4 GHz Wi-Fi affects the growth and viability of the bacterium *Escherichia coli*.

MATERIALS AND METHODS

The experiment used the Network Flood software in a setup consisting of a Mikrotik hAP ac3 router and two HP ProBook laptops. Due to the precisely set transmission speed between the two laptops, a constant data transfer was established between these devices. (Fig. 2A). This setting resulted in the router being forced to operate in constant transmission conditions. This achieved a precisely defined electromagnetic radiation between the two antennas of the router, which operated at a frequency of 2.4 GHz and a radiated power density of 0.062 W. In the experiment, we installed the router in a closed space of the thermostat and placed bacterial cultures between the antennas of the router (Fig. 2B).

To determine the lifespan, number of bacteria, and oxidative stress after exposure to RF-EMF, a suspension of *Escherichia coli* (CCM3954) was used, which was cultured for 24 hours at 37 °C on Endoagar (Himedia, India) (Fig. 2C). After collecting the colonies into tubes with sterile physiological solution and filtered PBS (phosphate buffered saline) (MP Biomedicals, France) through a 0.2 µm filter, the optical density was adjusted to 0.5 McFarland (DEN-1 McFarland Densitometer, Biosan). Subsequently, a control sample (KVz) and a sample exposed to RF-EMF (EVz) were created by adding 100 µl of the sample to 900 µl of BHI broth (Brain-Heart Infusion broth; Himedia, India). The control and exposed samples were created in triplicate to determine the number of bacteria. The numbers were determined before the experiment (0 h) and then after 2 h, 4 h, and 24 h of RF-EMF exposure by the standard 10-fold dilution plating method on Endoagar. The agar plates were cultured at 37 °C for 24 h, and *E. coli* counts are expressed as colony-forming units per milliliter (CFU/ml). Subsequently, we determined the viability of the bacteria and oxidative damage, which we analyzed before exposure to 0 h and then after 2 h and 4 h of exposure.

Bacterial viability was determined by staining with carboxyfluorescein diacetate (cFDA; Sigma Aldrich, USA) [18, 19]. Staining procedure: 2.5 µl of 1 mM cFDA and 222.5 µl of PBS (phosphate-buffered saline; MP Biomedicals, France) were added to 25 µl of bacterial samples diluted 1:100 in PBS. The sample was incubated for 30 minutes at 37 °C in a water bath. Oxidative damage to bacterial membranes was assessed using the fluorescent dye BODIPY C11 [20]. BODIPY C11 is an analogue of fatty acids, which can be incorporated into the cell membranes. In its unoxidized form, it produces red fluorescence, which shifts to bright green upon undergoing peroxidation. Ten µl of a 20 µM BODIPY 581/591 C11 solution of 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid (Molecular Probes, ThermoFisher Scientific, Scientific) was added to 25 µl of bacterial samples (diluted 1:100 in PBS). After 15 min incubation at 37 °C, 1.F5 µl of PI (1 mg/ml) was added and incubated for another 15 min. After incubation, 200 µl of PBS was added, and the samples were analyzed. Analysis was performed on a BD FACSCantoTM flow cytometer using BD FACS DivaTM software (Fig. 3). The location of the bacteria was delineated on a dot plot of forward scatter (FSC-A) versus side scatter (SSC-A) (Fig. 4), and the bacterial viability as well as oxidative damage of the membranes were determined on the histograms for green fluorescence using fluorescein isothiocyanate (FITC-A) versus counts (Fig. 5). Statistical analysis of cell viability was performed using an unpaired T-test within individual samples in the statistical program GraphPad Prism version 3.00.

Fig. 2. A- Set of equipment used in the experiment, B- Placement of the exposed sample in the thermostat between the router antennas, C- *E. coli* cultured on Endoagar

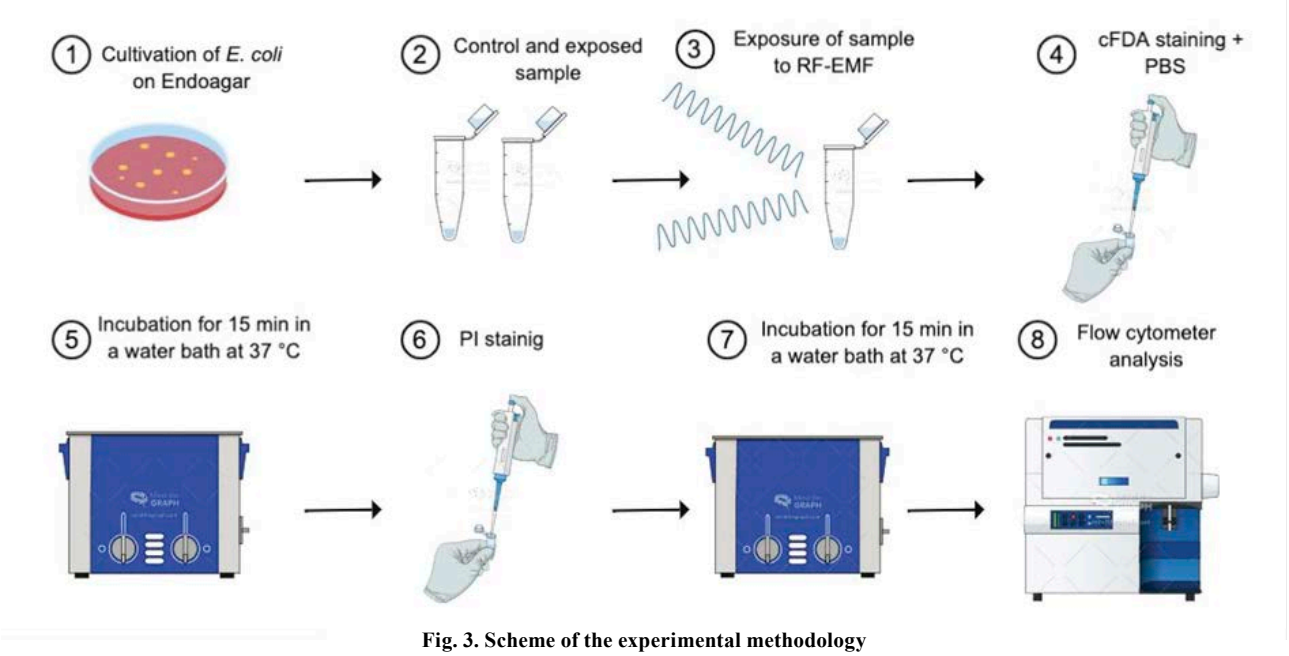

Fig. 3. Scheme of the experimental methodology

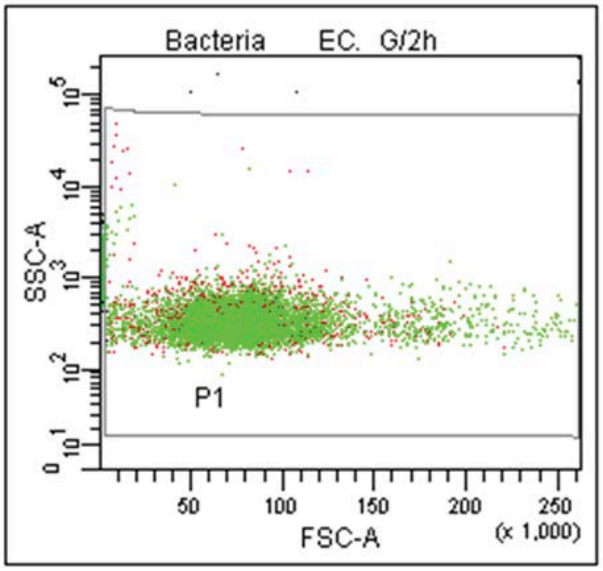

Fig. 4. Delineation of the position of bacteria (P1) on a basic dot plot showing size (FSC-A) versus granularity (SSC-A)

RESULTS

Based on the above-described experiment, the samples were analyzed on a flow cytometer. Before RF-EMF exposure, we measured the bacterial counts: control sample (KVz) 1.97 x 10^6 /ml and exposed sample (EVz) 1.97 x 10^6 /ml. After two hours of RF-EMF exposure, there was a significant decrease in the number of *E. coli*: control sample 1.1 x 10^7 /ml and exposed sample 4.73 x 10^6 /ml. After four hours of exposure, the values of the number of *E. coli* ranged from 1.0 x 10^7 /ml in the control sample to 1.85 x 10^7 /ml in the exposed sample. Then after twenty-four hours of exposure, we measured the value of 2.0 x 10^9 / ml in the control sample and 6.15 x 10^8 /ml in the exposed sample (Tab. 1, Fig. 6). So after two hours of exposure, we

Table 1. *E. coli* counts expressed as cfu/ml before and after exposure to 2.4 GHz radiation

E. coli	0 h	2 h	4 h	24 h
KVz	1.97 x 10^6 /ml	1.1 x 10^7 /ml	1.0 x 10^7 /ml	2.0 x 10^9 /ml
EVz	1.97 x 10^6 /ml	4.73 x 10^6 /ml	1.85 x 10^7 /ml	6.15 x 10^8 /ml

KVz – control sample, EVz – exposed sample

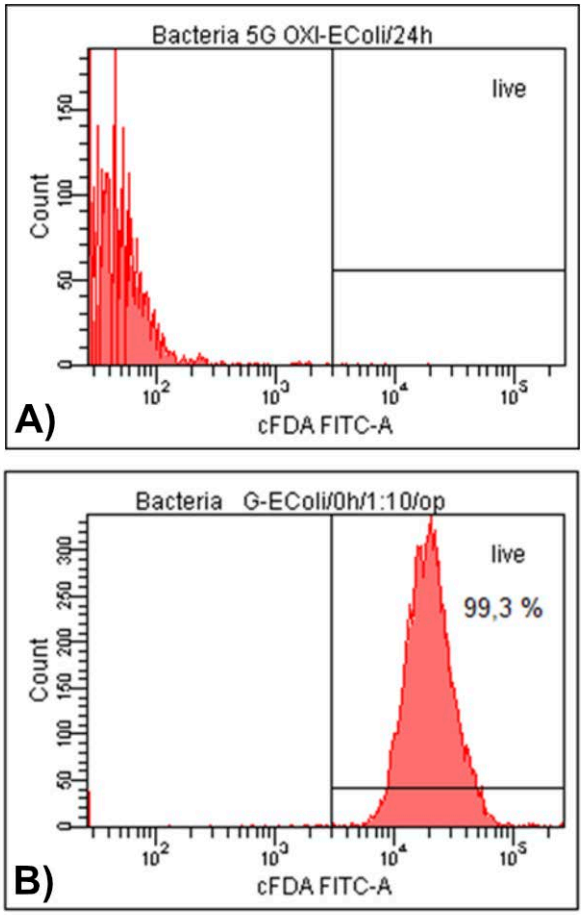

Fig. 5. Bacterial viability determined on the histograms for green fluorescence (FITC-A) versus count, A) negative sample – unstained; B) live bacteria – 0 h

observed a significant negative effect on *E. coli*, but then after four hours of exposure, we did not see a significant difference in the number. The result showed that resistant *E. coli* were selected, and in the following multiplication cycles (the rate of cell division of *E. coli* is very high – on average once every 30 min), their number began to increase again. After twenty-four hours of RF-EMF exposure, the exposed sample began to overtake the control sample in number.

Analysis of oxidative damage in *E. coli* recorded the largest increase after 2 h of RF-EMF exposure, up to 7.5%, but after 4 h of exposure it decreased again to 1.1% (Tab. 2, Fig. 7).

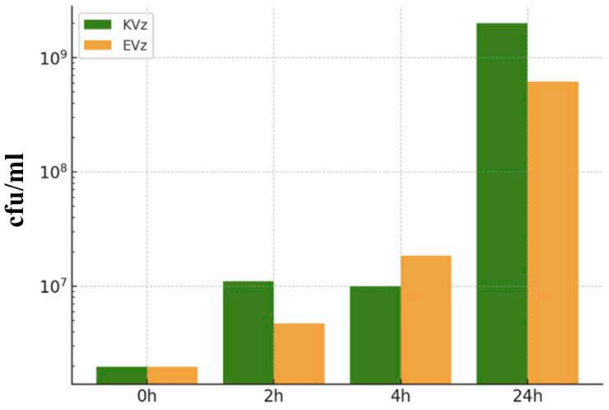

Fig. 6. *E. coli* counts expressed as cfu/ml before and after exposure to 2.4 GHz radiation, KVz – control sample, EVz – exposed sample

Table 2. Percentage expression of oxidative stress in *E. coli* before and after exposure to 2.4 GHz radiation

E. coli	0 h	2 h	4 h
KVz	0.2%	2%	1.6%
EVz	0.2%	7.5%.	1.1%

KVz – control sample, EVz – exposed sample

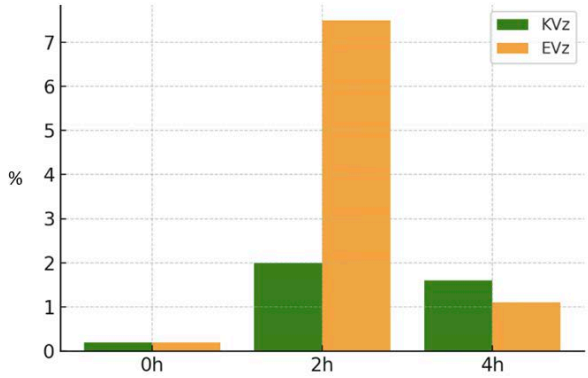

Fig. 7. Percentage expression of oxidative stress in *E.coli* before and after exposure to 2.4 GHz radiation, KVz – control sample, EVz – exposed sample

Table 3. Percentage expression of *E. coli* viability before and after 2.4 GHz exposure

E. coli	0 h	2 h	4 h
KVz	99.3 %	72.0 ± 1.35	51.9 ± 2.16
EVz	99.3 %	59.4 ± 2.16	27.5 ± 0.33

KVz – control sample, EVz – exposed sample

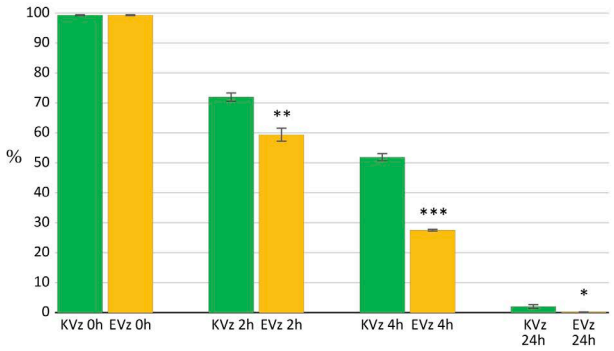

Fig. 8. Percentage of viable *E. coli* before and after exposure to 2.4 GHz radiation
* significantly different from control (** p<0.01, * p<0.05, *** p<0.001)

When analyzing the viability of E. coli, we observed a decrease after 2 h of RF-EMF exposure of approximately 12.6% and after 4 h of exposure of approximately 24.4% (Tab. 3, Fig. 8, 9, 10, 11, 12, 13).

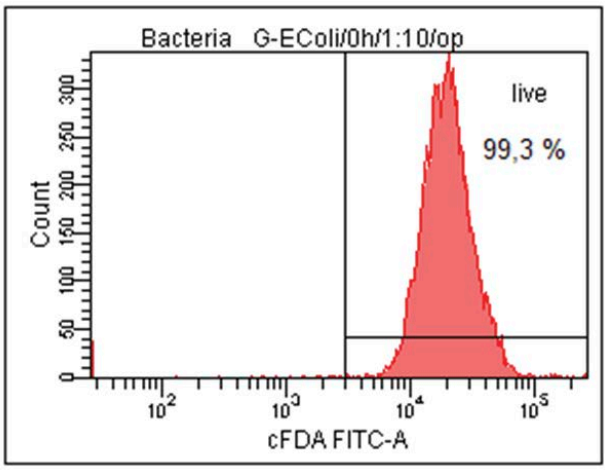

Fig. 9. Histogram of the proportion of live bacteria emitting green fluorescence after staining with cFDA (FITC-A), at initial values (0 h)

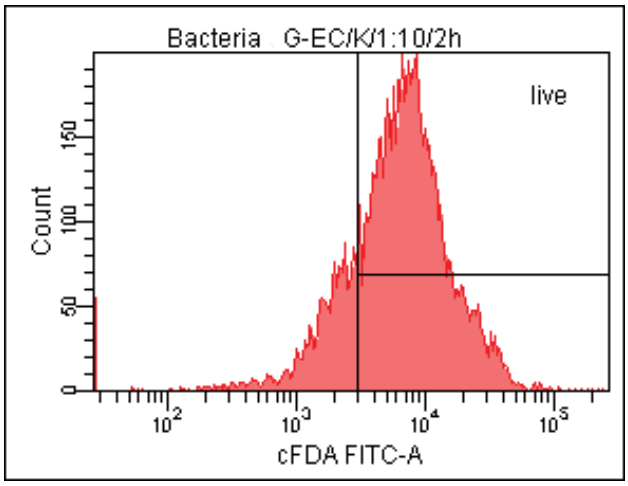

Fig. 10. Histogram of the proportion of live bacteria emitting green fluorescence after staining with cFDA (FITC-A), in the control sample after 2 h

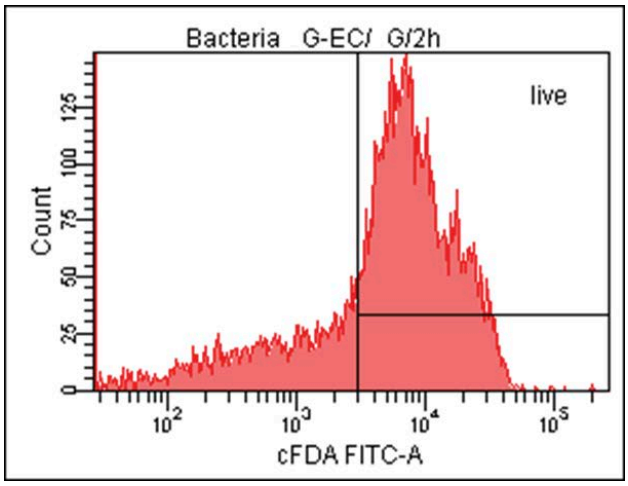

Fig. 11. Histogram of the proportion of live bacteria emitting green fluorescence after staining with cFDA (FITC-A), in the exposed sample after 2 h

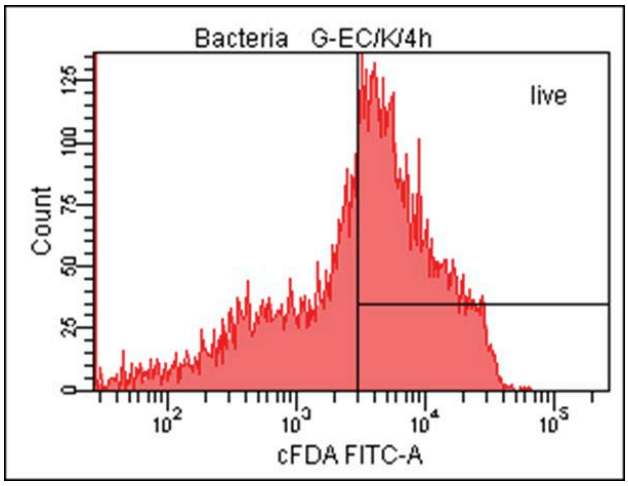

Fig. 12. Histogram of the proportion of live bacteria emitting green fluorescence after staining with cFDA (FITC-A), in the control sample after 4 h

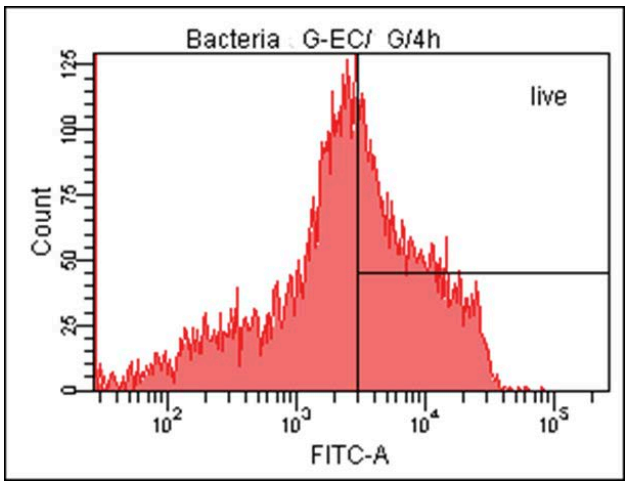

Fig. 13. Histogram of the proportion of live bacteria emitting green fluorescence after staining with cFDA (FITC-A), in the exposed sample after 4 h

DISCUSSION

The router works by constantly emitting electromagnetic radiation, but only in standby mode, which is characterized by low radiation power. Only when it receives a request from a device that wants to automatically or manually connect to the Wi-Fi network is this connection established, and the router then switches to the standard transmission power, which, however, depends on the speed of the transmitted data, i.e., it is not constant. However, these posed a problem in carrying out the experiment, because in order to assess the impact of electromagnetic radiation on bacteria, it was necessary for the radiation to be constant, at a standard wavelength. This problem was solved in cooperation with experts from the Faculty of Electrical Engineering and Informatics (FEI) of the Technical University of Košice (TUKE), who developed the Network Flood software.

RF-EMF can have different effects on bacteria depending on the frequency, intensity, length of exposure, and type of bacteria. The main effects of RF-EMF on bacteria include changes in growth and proliferation, with some studies suggesting that RF-EMF can decelerate bacterial growth [21], while others show its acceleration [22].

In several studies, changes in structure and morphology have been observed, where it has been shown that RF-EMF can disrupt the cell wall and membrane of bacteria, which can lead to cell weakening or death [23, 24]. In other cases, changes in the size and shape of bacteria, oxidative stress, and the formation of reactive oxygen species (ROS) were registered, where RF-EMF can induce the formation of ROS, which causes oxidative stress and can lead to damage to DNA, proteins, and lipids [25]. Some research suggests that RF-EMF can affect the expression of genes associated with bacterial resistance to antibiotics. RF-EMF has been shown to alter the sensitivity of some microorganisms to various antibiotics [26]. An effect on biofilm formation has also been observed, where RF-EMF can affect the ability of bacteria to form biofilms that provide them with protection from adverse conditions. In some cases, a weakening of biofilms has been noted, while in others, their formation has increased [15, 17, 27]. The effects of RF-EMF on bacteria are therefore complex and depend on many factors. Some studies point to negative effects such as cell damage and growth inhibition, while others suggest the possibility of bacterial adaptation and increased resistance. The results of research in this area are still ambiguous and require further detailed studies.

Key exposure parameters include exposure duration, frequency band (Hz), and power density (Watt). These variables determine the reaction of bacteria to RF-EMF, and, in general, whether the thermal or non-thermal effect of this type of radiation will be the priority depends primarily on the power density and water content in the living organism [28]. The most commonly used Wi-Fi transmitters (routers) currently use the permitted frequency bands of 2.4 GHz and 5 GHz, which are characterized by a very low level of power density. For example, the router used in our experiment is capable of operating in two frequency bands, either simultaneously or only in one of them. The first frequency band at 2.4 GHz has a radiated power density of 0.062 W and the second frequency band at 5 GHz has a density of 0.077 W [6].

From the above, it follows that in our study we can with great probability exclude the thermal effect of RF-EMF, for which many times higher power density values are required. When comparing the effect of RM-EMF exposure on *E. coli* in our experiment with the results of other authors, we could only work with a limited number of relevant professional publications, both due to the absence of extensive research in this area and because other authors had different exposure parameters set in their experiments.

When setting up the experiment, we tried to determine the so-called exposure window, with statistically significant growth inhibition compared to the control. For this reason, we also performed measurements after 2 and 4 hours, and subsequently, after evaluation, we found that the exposure window was located somewhere in the time interval from the beginning of exposure to 4 hours of exposure. Only in measurements after 2 hours of exposure did we observe a significant difference in the number of bacteria (about 0.5 log) between the experimental and control samples, while in measurements after 4 hours and 24 hours of exposure, the numbers between the experimental and control samples were approximately the same. This result can be explained by the fact that resistant bacteria were probably selected in the further course of the experiment, which is related to the fast life cycle of *E. coli* [29, 30]. This property allows for rapid adaptation to the environment, and therefore, in subsequent multiplication cycles, the numbers in the control and experimental samples equalized and after 24 hours were even slightly higher

than in the control sample. The exposure windows where specific RF-EMF parameters cause maximum biological effects are also mentioned in Pall's work [14]. There are several mechanisms that could have contributed to the inhibition of *E. coli* growth, and given the aforementioned inconsistency of the study results, we cannot clearly state which one was decisive. However, they were most likely related to changes in cell membrane morphology, where the study results are best documented [31, 32, 33].

Our observations also confirm the results of similar experiments with *E. coli* after exposure to RF-EMF, which revealed that 88% of the cells survived [23]. The authors conclude that one of the effects of exposing *E. coli* cells to RF-EMF under sublethal temperature conditions is that the cell surface undergoes a modification that is electrokinetic in nature, leading to reversible RF-EMF-induced porosity of the cell membrane. Scanning electron microscopy (SEM) analysis performed immediately after RF-EMF exposure revealed that *E. coli* cells displayed a cell morphology significantly different from that of negative controls. However, this effect appeared to be temporary, as after another 10 minutes, the cell morphology appeared to return to a state that was identical to that of the control sample. Confocal laser scanning microscopy (CLSM) revealed that dextran (150 kDa) conjugated with fluorescein isothiocyanate (FITC) was taken up by *E. coli* cells after exposure to RF-EMF, indicating that pores were formed in the cell membrane [23].

The effect of RF-EMF on cell membrane integrity was also demonstrated by the results of another study conducted on *E. coli*. The aim of this study was to investigate the effects of RF-EMF at a frequency of 2.45 GHz on *Escherichia coli* cell membranes under different conditions with the average temperature of the cell suspension maintained at 37 °C in order to investigate possible thermal versus non-thermal effects of short-term exposure. For this purpose, exposure of bacteria to RF-EMF was carried out under well-defined and controlled parameters, such that the average temperature of the bacterial cell suspensions was maintained at 37 °C. *Escherichia coli* cells were exposed to discontinuous radiation for different periods of time. For each experiment, conventional heating in a water bath at 37 °C was performed as a control. The effects of RF-EMF exposure on cell membranes were investigated using flow cytometry after staining the cells with propidium iodide and also by assessing intracellular protein release in bacterial suspensions. Thermal characterization indicated that the temperature reached by the samples exposed to RF-EMF during the specified contact time was not high enough to explain the measured modifications in cell membrane integrity [24]. In another study with *E. coli*, where a mobile phone simulator emitting radiation at a frequency of 900 MHz and a router emitting radiofrequency radiation at a frequency of 2.4 GHz were used as sources of electromagnetic radiation, RF-EMF was shown to alter the sensitivity of microorganisms to various antibiotics. Pure cultures of *Listeria monocytogenes* and *Escherichia coli* were exposed to RF-EMF, and the results showed that exposure to RF-EMF within a narrow exposure level (exposure window) renders microorganisms resistant to antibiotics. The findings of this study demonstrated that exposure to RF-EMF can significantly alter the inhibition zone diameters and growth rates for *L. monocytogenes* and *E. coli*. These findings may have implications for the management of serious infectious diseases [26]. Therefore, this adaptive phenomenon and its potential threats to human health should be further investigated in future experiments.

CONCLUSIONS

The study investigates the various biological effects of Wi-Fi radiation (RF-EMF) on microorganisms, emphasizing that these effects depend on the type of bacteria and exposure parameters such as duration, frequency, and power density. In our experiment, we investigated the effect of Wi-Fi radiation on *Escherichia coli*. The experiment with measurements after 2, 4, and 24 hours showed that after 2 hours of exposure there was a growth inhibition (0.5 log), which was not detected in later measurements, probably due to the selection of resistant bacteria. Our results showed the existence of an exposure window at low power densities and excluding the thermal effect, in which RF-EMF causes maximum biological effects.

The exact mechanisms of action of radiofrequency electromagnetic fields on microorganisms are not yet fully understood, and the results of studies are sometimes inconsistent. Research in this area is continuously improving our understanding of adaptive responses of microorganisms and potential health risks in the form of increased bacterial resistance to antibiotics, which can have a significant impact on healthcare.

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Conflict of Interest

We declare that all listed authors are without a Conflict of Interest.

Data Availability Statement

The raw data of this article will be made available by the authors, without undue reservation.

Ethical Statement

This study did not require any Ethical approval (Directive 2010/63/EU).

Authors' Contributions

All authors contributed to the study revision. Material preparation, data collection and analysis were performed by R.G., D.M., L.S., M.M. and J.M. All authors read and approved the final manuscript.

Generative AI Statement

The authors declare that no generative AI was used in the creation of this manuscript.

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ORIGINAL ARTICLE

OVICIDAL AND LARVICIDAL ACTIVITIES OF *PROSOPIS AFRICANA* LEAF AND FRACTIONS ON *HAEMONCHUS CONTORTUS*

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

This study was carried out to investigate the anthelmintic efficacy of the crude extract and fractions of *Prosopis africana* leaves against the gastrointestinal parasitic ova and larvae of *Haemonchus contortus*. The methanol crude extract of *P. africana* was partitioned into four fractions: N-butanol, petroleum ether, ethyl acetate, and aqueous. The concentrations of the extracts and fractions used in the treatment groups were 0.78 mg/ml, 1.56 mg/ml, 3.125 mg/ml, 6.25 mg/ml, and 12.5 mg/ml. At the concentration of 12.5 mg/ml the result showed that the egg hatch and larval development inhibition were 99.5% and 96%, respectively. This shows a significant difference ($p < 0.05$) when compared with the 100% inhibitory effect of albendazole. In the assay for ovicidal activity, all the fractions showed no significant difference ($P > 0.05$) when compared with albendazole. However, a significant difference ($P < 0.05$) was observed with all the fractions against larvae of *H. contortus*. This indicates a dose-dependent increase in the ovicidal and larvicidal activity of the crude extract and the fractions of *P. africana* leaves. However, this study confirms that the crude extract and fractions of *P. africana* possess anthelmintic compounds against *H. contortus*.

Keywords: crude; fractions; *Haemonchus contortus*; larvicidal; ovicidal; *Prosopis africana*

INTRODUCTION

Ruminant (sheep and goat) production is one of the main economic and farming activities for rural people in both the tropics and subtropic regions. Their challenges in ruminant production are due to gastrointestinal (GI) nematodes infections, caused by *Haemonchus contortus*, which are some of the numerous GI parasites causing great economic losses and threats to the livestock sector [1, 2, 3, 4]. In Nigeria, helminthosis, indiscriminate or irregular use of synthetic anthelmintics, and high cost of anthelmintic production is one of the major problems in ruminant produc-

tion, leading to the development of resistance by GI parasites to anthelmintic control. This has led to investigations and developments of new anthelmintics from medicinal plants using *Prosopis africana* as an alternative source to combat these problems [5, 6, 7, 8].

Prosopis africana (*P. africana*) (Guill and Perr) Taub (syn: *P. oblonga* Benth) grows wildly in the Middle Belt and Northern parts of Nigeria and some other African countries (Aremu et al., [9]; Ken, [10]). *P. africana* leaves, stem barks, and roots are used in the treatment of headache, toothache, rheumatism, naso-pharyngeal infections, cutaneous and subcutaneous parasitic infections (parasitic Loranthaceae) [10, 11].

MATERIALS AND METHODS

Plant collection, identification and extraction

The plant part used for this study was collected in the field from Kwali Area Council of Abuja, Federal Capital Territory, in months of March and April, 2024. Plants were identified by Mr. Alfred Ozioko, a botanist with the Bio-resources Development and Conservation Program (BDCP), Nsukka, and voucher specimens of each plant species were deposited at the Department of Pharmacology, Faculty of Veterinary Medicine, University of Abuja. The plant materials were air-dried, weighed, and pulverized. The extraction process was carried out according to the method described by Olayemi et al. [12] and Simon et al. [13].

Extraction of plant material

The pulverized plant material was weighed and extracted according to the method described by Olayemi et al. [12] and Simon et al. [13]. Three hundred grams of each plant material were weighed and wrapped in a tumble sack, placed in the Soxhlet apparatus and extracted with one litre of 80% methanol. The plant crude extract was collected in a flask attached to the Soxhlet apparatus, and poured into an evapourating dish and concentrated to dryness over water baths at 50–64 ˚C.

Solvent partitioning of the crude extract

The crude extract was then subjected to column fractionation and partitioning with the solvents petroleum ether, ethyl acetate, and N-butanol in order of increasing polarity, using 150 ml of each solvent. The whole process was repeated three times for each solvent, and the methanol-water portion was concentrated to dryness as described by Olayemi et al. [14] and Simon et al. [13].

Fig. 1. *Prosopis africana* leaves

In vitro assay

The Egg Hatch Assay (EHA) was performed using the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines [14]. Adult *Haemonchus contortus* were obtained from the abomasum of naturally infected sheep slaughtered at the Dogarawa abattoir, Zaria. Female *H. contortus* were identified, individually picked, separated, and suspended in distilled water and later crushed with mortar and pestle to liberate the parasite eggs (Simon et al. [15] and Taylor et al. [16]

Approximately 200 (*H. contortus*) eggs contained in 0.08 ml were pipetted into a 96-flat-bottomed microtitre plate. While 0.5 ml at different concentrations of 0.78 mg/ml, 1.56 mg/ml, 3.125 mg/ml, 6.25 mg/ml, and 12.5 mg/ml of the crude extract and the fractions were added. Similarly, the same process was repeated for the positive control albendazole, 0.5 ml at different concentrations of 0.78 mg/ml, 1.56 mg/ml, 3.125 mg/ml, 6.25 mg/ml, and 12.5 mg/ml, whereas 0.5 ml of the distilled water was used for the negative control. The concentrations were then incubated for 48 hours at 27°C. Each concentration was done in triplicate as described by Cala et al. [17].

$$\text{Eggs (\%)} = \frac{\text{Eggs + Larvae 1 – Larvae 1 x 100}}{\text{(Eggs + Larvae 1)}}$$ (Cala et al. [17]).

Evaluation of larvicidal activity of the extracts

The evaluation of the larvicidal activities of the different portions of the extracts and fractions was done according to methods described by Ameen et al. [18]. One hundred (100) larvae of *H. contortus* contained in 0.1 ml of suspension were added into each of a labeled 96-well flat-bottom microtitre plate. Thereafter, 0.5 ml of the different concentrations of 0.78 mg/ml, 1.56 mg/ml, 3.125 mg/ml, 6.25 mg/ml, and 12.5 mg/ml of the extracts and fractions were added. For albendazole 0.5 ml at different concentrations of 0.78 mg/ml, 1.56 mg/ml, 3.125 mg/ml, 6.25 mg/ml, and 12.5 mg/ml were added. 0.5 ml of the distilled water was used for the negative control. Each concentration was carried out in triplicate. Thereafter, the content of each well was stirred and pipetted onto a clean glass slide and then examined under the microscope to determine for mortality.

$$\text{Mortality (\%)} = \frac{\text{Number of dead larvae X 100}}{\text{Number of larvae in culture}}$$

(Ameen et al. [18])

Table 1. Mean (±SD) percentage of ovicidal inhibition of crude extracts at different concentrations (mg/ml)

Treatment groups	0.78 mg/ml	1.56 mg/ml	3.125 mg/ml	6.25 mg/ml	12.5 mg/ml
Prosopis africana	94 ± 1.00^{a}	96 ± 1.00^{a}	97.33 ± 0.76^{a}	98.33 ± 0.76^{a}	99.5 ± 0.50^{a}
Albendazole	96.8 ± 1.04^{b}	98 ± 0.50^{b}	99 ± 0.00^{b}	99.8 ± 0.29^{b}	100 ± 0.00^{b}
DW	6.2 ± 2.33				

Means with different superscript letters (a, b, c) differ significantly ($p < 0.05$) from the positive control group (albendazole). Negative control: distilled water (DW); n = 3.

Table 2. Mean (±SD) percentage of larvicidal inhibition of crude extract at different concentrations (mg/ml)

Treatment groups	0.78 mg/ml	1.56 mg/ml	3.125 mg/ml	6.25 mg/ml	12.5 mg/ml
Prosopis africana	62 ± 3.00^{a}	72 ± 2.00^{a}	77.6 ± 2.52^{a}	84 ± 2.00^{a}	96 ± 1.00^{a}
Albendazole	73.47 ± 2.25^{b}	77 ± 1.73^{b}	91.3 ± 3.2^{b}	100 ± 0.00^{b}	100 ± 0.00^{b}
DW	13 ± 1.15				

Means with different superscript letters (a, b, c) differ significantly ($p < 0.05$) from the positive control group (albendazole). Negative control: distilled water (DW); n = 3.

Table 3. Mean (±SD) percentage of ovicidal inhibition by fractions at different concentrations (mg/ml)

Fractions	Treatment group	0.78 mg/ml	1.56 mg/ml	3.125 mg/ml	6.25 mg/ml	12.5 mg/ml
N-Butanol	*P. africana*	86.17 ± 1.76^{a}	88.50 ± 1.50^{a}	90.17 ± 3.75^{a}	94.5 ± 1.00^{a}	97.17 ± 0.76^{b}
Ethyl acetate	*P. africana*	88.3 ± 5.11^{c}	90.6 ± 4.93^{c}	93.3 ± 4.37^{c}	94.6 ± 4.30^{c}	96.6 ± 3.80^{b}
Petroleum ether	*P. africana*	90.33 ± 1.53^{d}	92.83 ± 1.44^{d}	93 ± 2.18^{d}	95 ± 2.18^{d}	97.6 ± 2.05^{b}
Aqueous	*P. africana*	88 ± 1.00^{e}	90.5 ± 0.87^{e}	92.5 ± 0.87^{e}	94.5 ± 1.32^{e}	97.6 ± 1.76^{b}
	Albendazole	96.8 ± 1.04^{b}	98 ± 0.5^{b}	99 ± 0.00^{b}	99.8 ± 0.28^{b}	100 ± 0.00^{b}
	DW	6.2 ± 2.33				

Means with different superscript letters (a, b, c) differ significantly ($p < 0.05$) from the positive control group (albendazole). Negative control: distilled water (DW); n = 3.

Table 4. Mean (±SD) percentage of larvicidal inhibition of fractions at different concentrations (mg/ml)

Fractions	Treatment group	0.78 mg/ml	1.56 mg/ml	3.125 mg/ml	6.25 mg/ml	12.5 mg/ml
N-Butanol	*P. african*	57.0 ± 2.00^{a}	55 ± 2.00^{a}	65 ± 4.58^{a}	74 ± 2.60^{a}	82.0 ± 2.00^{a}
Ethyl acetate	*P. africana*	58.6 ± 6.59^{c}	59.3 ± 2.03^{c}	63.6 ± 10.7^{c}	80.2 ± 2.01^{c}	94.2 ± 2.00^{c}
Petroleum ether	*P. africana*	59.2 ± 3.00^{d}	69.9 ± 1.00^{d}	76 ± 2.00^{d}	80 ± 2.00^{d}	96.6 ± 3.0^{d}
Aqueous residue	*P. africana*	59.3 ± 2.00^{e}	61.2 ± 2.00^{e}	60.5 ± 0.00^{e}	84.5 ± 2.00^{e}	95 ± 3.00^{e}
	Albendazole	73.47 ± 2.25^{b}	77 ± 1.73^{b}	91.3 ± 3.20^{b}	100 ± 0.00^{b}	100 ± 0.00^{b}
	DW	13 ± 1.15				

Means with different superscript letters (a, b, c) differ significantly ($p < 0.05$) from the positive control group (albendazole). Negative control: distilled water (DW); n = 3.

Data management and statistical analysis

Comparison of mean percentages of egg hatch and larval development inhibition assays, at different concentrations with the controls, was performed by one-way ANOVA. The Post hoc statistical significance test employed was least square difference (LSD), the difference between the means is considered significant at $p < 0.05$.

RESULTS

The results observed for qualitative phytochemical analysis showed that saponins, alkaloids, carbohydrates, steroids, triterpines, cardiac glycosides, condensed tannins, flavonoids and phenols were positive metabolites contained in *P. africana* leaves except for anthraquinones which were negative. The quantitative phytochemical analysis showed the contents of the flavonoids, alkaloids, tannins, phenols and saponins to be 10.9%, 4.50%, 10.7%, 19.7%, and 1.3%, respectively.

DISCUSSION

The selection of *P. africana* plant was based on a literature survey and the use of these plants in traditional medicine in some parts of Nigeria. This study showed that phenols had the highest percentage (19.7%) of the metabolites, while saponins had the lowest (1.3%) The results observed in this study revealed that the crude extracts and fractions of *P. africana* significantly ($p < 0.05$) had ovicidal and larvicidal inhibitory effects on the parasite in a concentration-dependent manner.

The crude extract and fractions of *P. africana* leaves showed a more significant ($p < 0.05$) inhibitory effect on the hatching of *H. contortus* eggs than on the larvicidal development. Even though there were differences in the activity between the extracts of the plant, that was shown to be statistically non-significant ($p > 0.05$) with the effect observed with albendazole. In this regard, this study agrees with the previous study investigated by Enejoh [20]. *P. africana* shows no significant difference ($p > 0.05$) in ovicidal activity on the egg hatching of *H. contortus,* while the fractions of *P. africana* showed significant difference ($p < 0.05$) in larvicidal activity when compared to the larvicidal activity of albendazole against the *H. contortus* parasite.

This study observed that the ovicidal inhibitory effects of crude extract and fractions of *P. africana* against *H. contortus* were more effective than larvicidal inhibitory activity. The higher ovicidal inhibitory effect produced by these crude extracts and fractions could be attributed to the penetration of the phytochemicals, such as tannins, flavonoids, and saponins, into the parasite's eggshell which completely affects the biology of the egg's composition, as previously revealed by Enejoh [19]; Soetan et al. [20]; Dotto and Abihudi, [21]; and Kollins et al. [22]. The larvicidal inhibitory effect produced by *P. africana* extracts was likely due to the penetration of the active chemical constituents of the extracts across the cuticle of the larvae into their circulatory system when the larvae had contact with the extracts, as reported earlier by Payne et al., [23].

The present study on *P. africana* is in agreement with previous studies carried out by Aliyu et al. [24] and Kipyegon [25], who observed that as the treatment concentration increases, the anthelmintic activity of *P. africana* crude extract and fractions increases in a dose-dependent manner. Suteky and Dwatmadji [26] also studied the *in vitro* anthelmintic activity of *Melastoma malabatricum* extract against *H. contortus*, which showed significantly increased anthelmintic activity as the concentration dose increased. Therefore, this study showed that there was a significant increase in the mean percentage inhibition of egg hatch and larval development against the *H. contortu*s at different concentrations.

CONCLUSION

The results from this study suggested that the crude and fraction extracts of *P. africana* had significant anthelmintic activities on ovicidal and larvicidal inhibitory effects against *H. contortus*. The anthelmintic activities of the plants selected for this study showed promising novel drug potential. Therefore, further *in vivo* studies are required to profile the toxicity level and safety of this plant for use as an anthelmintic drug model against the *H. contortu*s parasite.

Data Availability Statement

The raw data of this article will be made available by the authors, without undue reservation.

Conflict of Interest

The authors hereby wish to declare to the editor of this journal, that there is no conflict of interest between the authors of this manuscript.

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ORIGINAL ARTICLE

BEHAVIOURAL DETERMINANTS OF LIVESTOCK FARMERS AND SERO-PREVALENCE OF BRUCELLOSIS IN SMALL RUMINANTS IN THE SUB-PREFECTURE OF DOUNET (MAMOU), REPUBLIC OF GUINEA

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

This study assessed the brucellosis seroprevalence of small ruminants and hazardous practices of contamination in the sub-prefecture of Dounet in Guinea. A survey was carried out among 60 people, mainly livestock farmers, and 192 small ruminants were clinically examined. Ninety serum samples were subjected to the Rose Bengal Test (RBT) and Indirect Enzyme-Linked Immunosorbent Assay (I-ELISA). Abortion and sterility were the most commonly observed symptoms, with 73.33% and 18.18%, respectively, for goats, and 81.82% and 26.67% for sheep. Farmers' knowledge about brucellosis in small ruminants was significantly lower (18.33% [9.9%–30.85%]). Regarding sanitary measures to prevent disease occurrence, 31.67% [20.60%–45.09%] of the farmers did not apply any specific measures, compared to 55% who applied sanitary measures and 8% who applied quarantine. The results of RBT showed that the overall seroprevalence of brucellosis was 27.77% (25/90) compared to 11.11% (10/90) using I-ELISA (p = 0.008). Female goats were significantly more affected, with a prevalence of 85% (17/20) compared with 20% (3/15) in males (p < 0.001). Bacterial infection did not vary significantly between study sites or age groups. The results of this study confirm that brucellosis is widespread among small ruminants and underline the urgent need for increased surveillance of the disease in all regions of the country.

Key word: Brucellosis; goats, Guinea; I-ELISA; RBT; sheep

INTRODUCTION

Small ruminant farming is a highly profitable activity that contributes significantly to Guinea's food self-sufficiency and economy. Factors affecting the productivity of the country's livestock include reproductive problems, poor nutrition, and various bacterial, viral, and parasitic diseases. One of the diseases responsible for the high number of abortions, stillbirths, prolonged calving intervals, infertility, and reduced milk production on most farms is brucellosis. It is a widespread zoonotic disease that is a public health problem in most parts of the world, including Guinea [1]. It affects both animal and human populations but is classified by the World Health Organization (WHO) as one of the seven most neglected diseases, with 500,000 new cases worldwide each year [2, 3]. This zoonosis is caused by Gram-negative bacteria of the genus *Brucella*. *Brucella abortus* affects cattle, *Brucella melitensis* is found in small ruminants, and *Brucella suis* in pigs [4]. Transmission between animals is generally achieved through direct and indirect contact. Fluids such as infected birth fluids, placental mucosa, skin cuts, and aborted fetuses can all be sources of infection [5]. Human infection is caused by direct contact with sick animals and consumption of raw milk and other unpasteurized dairy products contaminated with *Brucella* [6]. *Brucella* species can cause fever, arthritis, and neurological symptoms in humans [7, 2]. The risk of transmission from animals to humans appears to be increased by certain factors such as recent climate change and its impact on farming practices, dietary habits, and human activities [8, 9].

In sub-Saharan Africa, brucellosis remains endemic, with seroprevalence in humans and cattle ranging from 0 to 40%, depending on geographical location [10, 11, 12]. A survey carried out in different regions of Guinea showed a prevalence of brucellosis in sheep/goats of 12.5%, compared with 30% in humans [1].

Overall, data on the true prevalence of brucellosis in Africa are patchy. In Guinea, the first epidemiological survey to assess the presence of this disease in cattle was carried out in 19 herds in different regions. The baseline data showed that seroprevalence varied according to the geographical location of the country, with an average of 6.9% [0–27%] [13]. A few years later, Diallo [9] showed that the coastal region of Guinea appeared to be the most affected. Since then, several studies have confirmed the influence of geographical location on the overall seroprevalence of bovine brucellosis, ranging from 8.7% [5.3–12%] in 2010 to 11.8% in 2020 [14, 15]. In western Guinea, brucellosis seroprevalence in cattle was estimated at 11%. In sheep and goats, it was 0.4% and zero, respectively [16]. A recent study on priority zoonoses showed a high risk of brucellosis in Kankan but less in Conakry and N'Zérékoré [17]. Although there is some data on this pathology, it remains poorly documented.

In Dounet, a location in the Mamou region, more than 80% of the population is involved in agro-pastoral activities. Unfortunately, the livestock sector is currently experiencing enormous difficulties. The climatic conditions (low temperatures and waterways used as drinking troughs for migratory animals) are conducive to the occurrence of infectious diseases, such as brucellosis, in small ruminants. However, the clinical and epidemiological diagnosis of brucellosis is variable and not very specific. Brucellosis is associated with a variety of symptoms: abortion, retained placenta, mastitis, sterility, orchitis, and epididymitis. However, confirmation by a laboratory diagnostic test is absolute proof of infection and has a specificity of 100% [18]. Nevertheless, as no serological test is 100% accurate, the simultaneous use of at least two different serological techniques is strongly recommended to assess the brucellosis status of herds, with combinations of tests varying according to the epidemiological situation [19].

This study was carried out to provide updated epidemiological data on brucellosis in small ruminants (goats and sheep) in Dounet, located in the Mamou region of Guinea. This information on the seroprevalence and factors associated with this zoonosis will be useful in assessing the risks to human health and animal production and in defining appropriate prevention and control strategies.

MATERIALS AND METHODS

Study sites

This study was carried out from October to December 2023 in the sub-prefecture of Dounet, Mamou prefecture (Fig. 1). It covers an area of 570 km^2 and has a population of 32,656, giving a density of 57 inhabitants per km^2. It has a tropical climate with two alternating seasons: a dry season (November to May) and a rainy season (June to October). The area is characterized by a steep rainfall gradient,

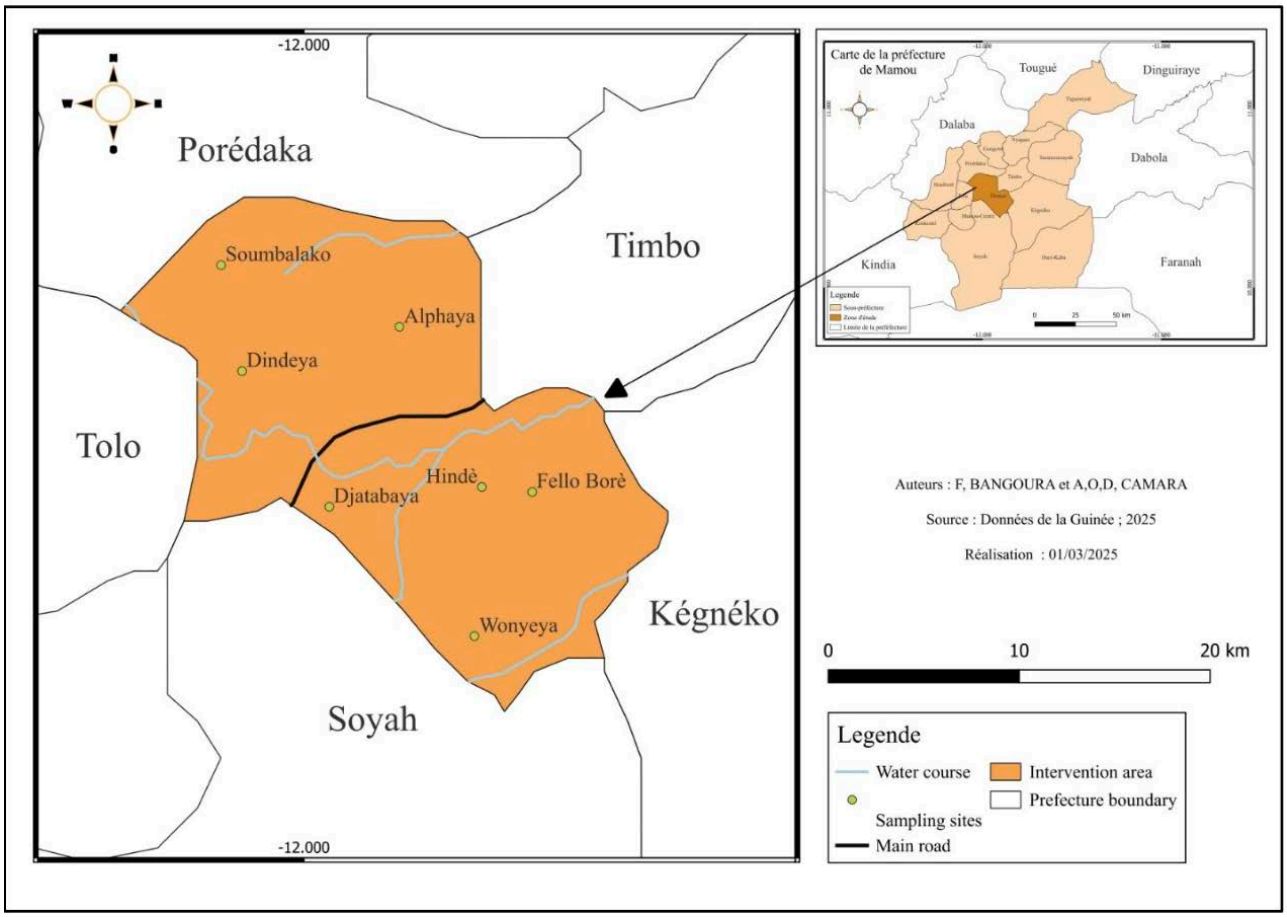

Fig. 1. Study area

ranging from 2,400 to 650 mm per year. The average annual rainfall can be as high as 2,400 mm. Average temperatures range from 15°C to 36°C and average relative humidity from 23% to 45% [20]. The dominant winds are the Harmattan, which blows from east to west during the dry season, and the Monsoon, which blows from south-west to north-west. Wind speeds vary between 9.3 km/h and 11.7 km/h. These climatic conditions (temperature, humidity, wind, rainfall) favor the development and multiplication of bacteria (*Brucella*) and, above all, their persistence in the external environment. The relief of the area is rugged, with plateaus that form the beginning of the Fouta Djallon. The average altitude varies from 400 m in the south to 800 m in the north of the prefecture.

The vegetation consists mainly of open forests, wooded savannahs, and galleries. The most common species are *Erythropheum guineensis*, *Khaya senegalensis,* and *Cassia siberiana*. The area is irrigated by the Konkouré river, which has its source in the Mamou region at an altitude of almost 1,000 meters.

Agriculture, trade, and livestock are the main income-generating activities. Livestock rearing is essentially family-based, and most animals are on the move throughout the year, except during the growing season. However, the mixture of different species, the lack of identification of pregnant females, the extensive nature of livestock farming, and poor feeding are all factors in the occurrence and spread of brucellosis. Similarly, the use of manure from brucellosis-infected animals in horticulture and the consumption of vegetables grown on soil treated with this manure are risk factors for brucellosis contamination in humans. Finally, the presence of a market for animal products and a livestock market is a factor in the spread of this zoonosis [20].

Type and period of survey

The present survey, carried out from October to December 2023, was cross-sectional and descriptive, based on a quantitative and qualitative questionnaire. The surveys were carried out specifically among farmers/shepherds. In addition to the quantitative surveys, a focus group was organized with livestock management staff, followed by an analysis of health records for diseases affecting small ruminants.

The survey process

The survey was conducted in the field in several stages. The first stage was to contact the local chiefs to explain the importance of the study and to invite them to participate and to involve their peers. Once we had the agreement of the chiefs, a member of the community was appointed to accompany us to the pastoralists, where the importance and scope of our study were explained as well. After their consent, the survey has been carried out. Farmers were selected using the snowball method (non-probabilistic). Sixty (60) farmers were interviewed by qualified interviewers. This survey allowed to collect information on the animal characteristics of each breeding, their management, and their movements. The questionnaire covered the type of farming practiced, the farmers' knowledge of brucellosis and other small ruminant diseases, their attitudes and practices regarding brucellosis, preventive measures, and the socio-economic impact of brucellosis on the farms.

In addition to the individual information gathered through the questionnaire, a focus group was held with ten (10) executives of the Mamou Prefectural Department of Agriculture and Livestock and from the Dounet Livestock Section to get a better understanding of the existence, evolution, and impact of small ruminant brucellosis in the sub-prefecture. An analysis of the archives was also carried out to obtain information on the various diseases prevalent in the area, in particular abortive diseases such as small ruminant brucellosis.

This approach also made it possible to identify the links between breeding: communal drinking troughs for stray animals, the existence of livestock markets, nocturnal gatherings, the exchange of animals, and the management of abortions, with a view to identifying the epidemiological risks that could favor the circulation of pathogens and the creation of outbreaks.

Sampling and blood collection

Herd sampling was snowball sampling (non-probabilistic). Herds were thus selected in a non-random manner on the basis of recommendations and with the consent of the farmers. A total of 101 goats and 91 sheep were included in this study. All of these animals underwent a clinical examination to identify those with the main symptoms of brucellosis (abortions, infertility, orchitis, and reproductive problems).

All animals with at least one of these symptoms had blood samples taken. When the number of symptomatic animals was less than ninety (90), asymptomatic animals from the seven (07) target localities in the sub-prefecture of Dounet were selected and added to reach this sample size. These samples were subjected to serological tests.

After each animal was restrained, a total volume of 5 ml of blood was collected by venipuncture into an EDTA tube and stored tilted in carboglass before being transported to the laboratory. Then, the tubes were centrifuged at 5,000 rpm for 10 to 15 minutes. After centrifugation, the clear serum from each sample was transferred to sterile, labelled tubes and immediately stored at -20°C for serological testing.

Serological tests

Two serological tests were used to detect brucellosis in animal serum samples in this study: the Rose Bengal Test (RBT) and the Indirect Enzyme-Linked Immunosorbent Assay (I-ELISA).

Rose Bengal Test (RBT)

The RBT was used to test for anti-*Brucella* antibodies according to the manufacturer's instructions (ID vet, Rose Bengal antigen for RSA test, ID vet 310, rue Louis Pasteur, Grabels, France). Thereby, 30 µl of *Brucella* antigen was mixed with an equal volume of serum on a single-ended plate and spread on complete circles of approximately 2 cm diameter according to the manufacturer's instructions. The plates were manually rotated for four minutes according to the standard procedure described by the International Office of Epizootics (OIE) [21]. Any agglutination is considered positive, while the absence of agglutination is considered negative. The Rose Bengal Test has a sensitivity of 96.10% and a specificity of 99.30% [22].

Indirect Enzyme-Linked Immunosorbent Assay (I-ELISA)

The indirect enzyme-linked immunosorbent assay (I-ELISA) was used to detect antibodies to *Brucella* species. The I-ELISA kit (ID vet France, kit reference BRUSMS-5P) was used to test sera for multi-species smooth lipopolysaccharide (S-LPS) antibodies of *Brucella*, including *Brucella melitensis*, *Brucella abortus,* and *Brucella suis*, according to the standard protocol of the International Office of Epizootics (OIE) [21]. For this purpose, all serum

samples, including controls and reagents, were brought to room temperature (18–25°C). Diluted (1:20) test sera and controls were incubated with Brucella lipopolysaccharide (LPS)-coated plates for 45 minutes at room temperature. After washing, 100 µl of multi-species horseradish peroxidase (HRP) conjugate was added to each microplate well and stored at room temperature for 30 minutes. After washing in order to remove excess conjugate, a tetramethylbenzidine (TMB) substrate solution was added and stored in the dark for 15 minutes. The color developed depended on the amount of specific antibody present in the test sample. The plates were then read using an ELISA microplate reader (Lab system, USA) at a wavelength of 450 nm. The percentage value (S/P%) was calculated using the formula recommended by the kit manufacturer (ID Vet, France) for the BRUS-MS ver 1014GB batch kit protocol.

Statistical analysis

Survey data were collected using the KoboCollect mobile application, then transferred to KoboToolbox for processing. Microsoft Excel spreadsheet software was used for data storage, and R statistical software, version 4.1.2 (R CoreTeam, 2021), was used for analysis. Seroprevalence in sheep and goats was calculated by dividing the number of RBT and I-ELISA positive animals by the total number of animal sera tested. The Chi2 test for comparison of proportions was used to compare the proportions of farmers and the seroprevalence of brucellosis in goats and sheep. A p-value less than or equal to 0.05 was considered statistically significant.

RESULTS

Farmers' knowledge of brucellosis and identification of hazardous contamination practices

Extensive livestock production is the main type of farming practised in Dounet. In fact, 86.67% (n = 58) of the 60 farmers surveyed practised it (Table 1). Overall, a small proportion (18.33% [9.9%–30.85%], 8/60) of farmers were aware of the existence of brucellosis in small ruminants. The proportion of farmers who were aware of peste of small ruminants (60% [46.54%–72.17%]; n = 36) was significantly higher than those who reported being aware of brucellosis (18.33% [9.9%–30.85%]), Q fever (3.33% [0.57%–12.54%]), ovine abortive salmonellosis (6.67% [2.15%–17.00%]), and fasciolosis (11.67% [5.21%–23.17%]) (Table 1).

Regarding hygiene measures on the farm, 50% [37.73%–62.26%] of farmers did not use any hygiene measures, whereas 15% [7.50%–27.07%] cleaned it regularly, 11.67% [5.21%–23.17%] disinfected the premises, and 23.33% [13.77%–36.33%] isolated sick animals. Overall, 55% ([41.69%–67.66%]; n = 33) of the farmers took sanitary measures to prevent the outbreak of zoonotic diseases, whereas only 13.33% ([6.33%–25.14%], n = 8) quarantined animals. On the other hand, 31.67% [20.60%–45.09%] of farmers did not apply any specific measures (Table 1).

Overall, 50% [37.73%–62.26%] of the farmers surveyed did not take any measures to deal with abortion cases, and 30% [19.20%–43.37%] discarded the fetus and placenta in the wild (Table 1). On the other hand, a few minor measures, such as burial (13.33% [6.33%–25.14%]) and incineration (6.67% [2.15%–17.00%]) of the fetus and placenta, were used in the management of abortion cases.

The socio-economic impact of brucellosis on farms varied. According to the farmers interviewed, abortions due to brucellosis and neonatal deaths resulted in economic losses of 43.33% [30.81%–56.70%] and 38.33% [26.35%–51.80%], respectively. Other economic losses were due to the reduced market value of diseased animals (13.33% [6.33%–25.14%], n = 8) and treatment costs (5% [1.30%–14.82%], n = 3).

Statement on brucellosis by the executives of the Mamou Prefectural Livestock Directorate

The focus group with managers from the Prefectural Livestock Directorate of Mamou and the sub-prefecture of Dounet showed that they were aware of the existence of brucellosis in small ruminants. According to them, brucellosis is a recurrent disease that generally occurs at the end of the rainy season. It is most commonly characterized by abortions in the last months of pregnancy, with females more susceptible than males. In some cases, the pregnancy can be completed, but the resulting young (lambs and kids) are very weak or die within a few hours of birth. If there is a series of abortions in several female ruminants, brucellosis may be suspected. However, serological tests are required to confirm or exclude this possibility.

A nosological analysis of infectious diseases in health records showed that brucellosis in small ruminants reduces

Table 1. People's knowledge, attitudes, and practices regarding brucellosis

Terms and conditions		Number of respondents	Proportions (%)	95% CI
Farmers' knowledge of brucellosis in small ruminants	Know	11	18.33	[9.9—30.85]
	Do not know	49	81.67	[69.14—90.06]
Types of livestock farming	Extensive	52	86.67	[74.85—93.66]
	Semi-extensive	8	13.33	[6.33—25.14]
Knowledge of the main pathologies	Ruminant plague	36	60.00	[46.54—72.17]
	Brucellosis	11	18.33	[9.9—30.85]
	Q fever	2	3.33	[0.57—12.54]
	Ovine abortive salmonellosis	4	6.67	[2.15—17.00]
	Fasciolosis	7	11.67	[5.21—23.17]
Health measures in place for the prevention of disease outbreaks	Health measures	33	55.00	[41.69—67.66]
	Quarantine	8	13.33	[6.33—25.14]
	No specific measures	19	31.67	[20.60—45.09]
Premises hygiene measures	Regular cleaning	9	15.00	[7.50—27.07]
	Disinfection	7	11.67	[5.21—23.17]
	Separation of sick animals	14	23.33	[13.77—36.33]
	No specific measures	30	50.00	[37.73—62.26]
Abortion case management measures	By burial	8	13.33	[6.33—25.14]
	By incineration	4	6.67	[2.15—17.00]
	Disposal in the natural environment	18	30.00	[19.20—43.37]
	No measures	30	50.00	[37.73—62.26]
Socio-economic impact of the disease on farms	Abortion	26	43.33	[30.81—56.70]
	Treatment costs	3	5.00	[1.30—14.82]
	Neonatal losses	23	38.33	[26.35—51.80]
	Loss of market value of animals	8	13.33	[6.33—25.14]

%: percentage; CI: Confidence Interval

the number of small ruminants because of the abortions or losses of lambs and kids that it causes. In addition to brucellosis in small ruminants, other abortifacient diseases such as chlamydiosis and toxoplasmosis are also widespread in the sub-prefecture of Dounet.

Pathological effects of the disease on the animals

Table 2 revealed that of the 101 goats and 91 sheep examined, 13.54% (n = 26) showed various symptoms (abortion and sterility) of brucellosis. Abortion was the most frequently observed symptom (73.33% in goats and 81.82% in sheep) compared to sterility (26.67% in goats and 18.18% in sheep), with a significant difference (p-value < 0.05) (Table 2).

Results of the serological test

The overall seroprevalence of brucellosis detected in small ruminants was 27.77% (25/90) using the RBT test

Table 2. Clinical examination of animals

Localities	Workforce surveyed		Number of animals with symptoms		Symptoms			
					Number of goats		Number of sheep	
	Goats	Sheep	Goats	Sheep	Abortion	Sterility	Abortion	Sterility
Alphaya	14	18	2	2	2	0	2	0
Soumbalako	13	10	2	1	2	0	0	1
Dindeya	10	10	1	0	1	0	0	0
Hinde	12	12	3	1	2	1	1	0
Djatabaya	15	20	2	2	1	1	2	0
Fello Borè	17	11	3	2	1	2	2	0
Wouyeya	20	10	2	3	2	0	2	1
TOTAL	101	91	15	11	11	4	9	2
%	-	-	14.85	12.09	73.33	26.67	81.82	18.18
P-value	-	-	0.728		0.0284		0.0105	

%: percentage

Table 3. Serology by test used (RBT and I-ELISA) in both species

Species	Serological tests	Number of samples tested	Number of positives	Seroprevalence (%)	P-value
Goats	RBT	45	14	31.11	
	Indirect ELISA	45	6	13.33	0.076
Sheep	RBT	45	11	24.44	
	Indirect ELISA	45	4	8.89	0.0896
Total	RBT	90	25	27.77	
	Indirect ELISA	90	10	11.11	0.008

%: percentage

Table 4. Seroprevalence of brucellosis in small ruminants by study site

Species	Localities	Numbers surveyed	Number of samples analysed	Number of positives	Seroprevalence (%)	95% CI
Goats	Alphaya	14	5	3	60.00	[17.04–92.74]
	Soumbalako	13	5	2	40.00	[7.25–82.95]
	Dindeya	10	6	2	33.33	[5.99–75.89]
	Hindè	12	5	1	20.00	[1.05–70.12]
	Djatabaya	15	6	1	16.67	[0.87–63.51]
	Fello Borè	17	10	9	90.00	[54.11–99.47]
	Wouyeya	20	8	2	25.00	[4.45–64.42]
	Total	**101**	**45**	**20**	**44.44**	**[29.95–59.87]**
Sheep	Alphaya	18	10	6	60.00	[27.36–86.30]
	Soumbalako	10	6	1	16.67	[0.87–63.51]
	Dindeya	10	5	0	0.00	[0.00–53.70]
	Hindè	12	7	2	28.57	[5.11–69.74]
	Djatabaya	20	5	1	20.00	[1.05–70.12]
	Fello Borè	11	7	5	71.43	[30.25–94.88]
	Wouyeya	10	5	0	0.00	[0.00–53.70]
	Total	**91**	**45**	**15**	**33.33**	**[20.43–49.05]**

%: percentage; CI: Confidence Interval

compared with 11.11% (10/90) using the indirect ELISA (p = 0.008). These two serological tests showed a higher prevalence of brucellosis in goats, but without a significant difference (p > 0.05). In fact, out of a total of 45 serum samples tested by RBT and I-ELISA in goats, a brucellosis seroprevalence of 31.11% (n = 14 positive) and 13.33% (n = 6 positive), respectively, was observed (p = 0.0759). The same trend was observed in sheep, with seroprevalences estimated at 24.44% and 8.89% for the RBT and I-ELISA, respectively (p = 0.0896) (Table 3). However, 100% of small ruminants (n = 26) showing symptoms of reproductive disorders tested positive for brucellosis bacteria.

The seroprevalence of brucellosis in small ruminants by site (Table 4) showed no significant difference in seroprevalence between sites (p > 0.05). In goats, the highest seroprevalence was observed in Fello Borè (90% [54.11–99.47]) and the lowest in Djatabaya (16.67% [0.87–63.51]), with an overall prevalence of 44.44% [29.95–59.87] for the whole study area (Table 4).

For sheep, it ranged from 71.43% [30.25–94.88] in Fello Borè to 16.67% [0.87–63.51] in Soumbalako, with an overall prevalence of 33.33% [20.43–49.05] for the whole study area (Table 4).

Figure 2 shows the seroprevalence of sheep and goats based on sex. Analysis of the figure revealed that female goats are more affected by brucellosis, with a prevalence of 85% (17/20) compared to 20% (3/15) for males (p < 0.001). In sheep, the seroprevalence was higher in females (39.39%: 13/33) than in males (16.67%: 2/12), but the difference was not significant (p > 0.05).

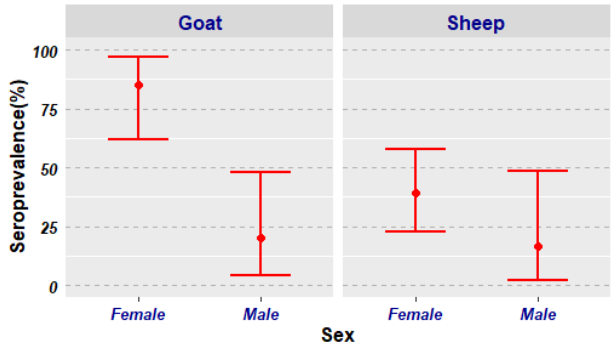

Fig. 2. Brucellosis seroprevalence in small ruminants by sex

Figure 3 examined the influence of age on the susceptibility of small ruminants to brucellosis. Analysis of this figure showed that infection with this bacterial disease in small ruminants did not vary according to age group. However, the most infected age group was between 4 and 6 years, but there was no significant difference with the other age groups (p > 0.05). In goats of this age group, the prevalence was 50% [27.99–72.00] compared to 43.75% [20.75–69.44] in sheep. The least affected age group was 6 months to 1 year, with a prevalence of 14.29% [0.75–57.99] in sheep and 40% [7.20–82.95] in goats.

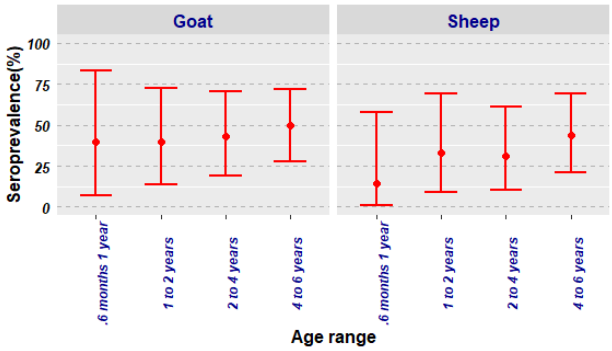

Fig. 3. Seroprevalence of brucellosis in small ruminants by age category

DISCUSSION

This study provided data on the seroprevalence of brucellosis in small ruminants in the sub-prefecture of Dounet, where livestock production is extensive.

Results showed that the seroprevalence of brucellosis in small ruminants in the study area was relatively high. It was 27.77% (25 positives) using the RBT test compared with 11.11% (11 positives) using the I-ELISA. This result is in contrast to that observed by Troupin et al. [16], who recorded a prevalence of *Brucella* bacteria of zero (0 out of 408) in goats and 0.4% (2 out of 486) in sheep between 2017 and 2019 in Guinea. According to this study, the prevalence of brucellosis was zero in the prefectures of Dabola, Dalaba, Faranah, Kouroussa, and Mandiana. However, high seroprevalence rates were only observed in cattle (51 positive; 11%) [16]. This increase in brucellosis infectivity in small ruminants may be due to the more active circulation of *Brucella melitensis* in Guinea [12]. Historically, *Brucella melitensis* has been associated with brucellosis epidemics in small ruminants, mainly in North, East, and Central Africa, while in West Africa almost 90% of isolates were *Brucella abortus* [23, 24]. This potential spread of *Brucella melitensis* would be due to the purchase of ruminants from other countries or regions, which would increase the likelihood of infected ruminants being

introduced into herds [25]. This hypothesis is plausible, as the results of the survey showed that farmers had little information about brucellosis in small ruminants, and their overall knowledge of the disease was low (18.33%). As a preventive measure, new animals should be quarantined and tested for *Brucella* infection before introduction into herds. However, it would be interesting to isolate and identify the *Brucella* species circulating among ruminants in Guinea. It would also be important to re-evaluate the seroprevalence in ruminants in the same areas in the coming years to determine whether this pattern of spread continues, with a view to recommending appropriate control measures. Brucellosis is generally transmitted by direct or indirect contact with diseased ruminants or their secretions [26]. Given the extensive nature of livestock farming in Dounet, this rapid spread of brucellosis in small ruminants could be due to contact with or ingestion of feed/drinking water contaminated with bacteria from calving products and uterine secretions [27]. This risk factor for *Brucella* infection is higher in dry areas [28]. Due to the scarcity of grazing land in arid areas, small ruminants are grazed over large areas, suggesting uncontrolled goat-cattle-bovine interaction with a potential risk of transmission. The relatively high prevalence rates observed among small ruminants in Dounet may also be due to the lack of hygiene and sanitation measures and the absence of screening for the disease. Our survey results showed that the majority of farmers do not use hygiene measures, and only 23% of them isolate sick animals. A significant proportion of farmers (31.67%) did not apply any specific measures. Most of the farmers surveyed (80%) had no significant measures to deal with cases of abortion in ruminants, and some even threw the fetus and placenta into the wild.

However, the seroprevalence observed in this study appears to corroborate the work of Akakpo et al. [1] in February 2009, where significant rates of 12.5% were observed in small ruminants in Guinea.

Our work showed that the seroprevalence of brucellosis was higher in goats (31.11% and 13.33%) than in sheep (24.44% and 8.89%) using the RBT and indirect ELISA tests, respectively. This result confirms the work of Djangwani et al. [29], who reported that the seroprevalence of brucellosis in small ruminants ranged from 0 to 20% in goats compared with 0 to 13.8% in sheep.

A similar prevalence (31%) was found in goats in another study conducted in the mountainous region of Libya between 2006 and 2008 [30]. This higher prevalence in goats is thought to be related to the higher sensitivity observed in goats compared to sheep [31]. In addition, the results showed that the RBT test was highly sensitive for detecting brucellosis in serum samples with high prevalence. As a result, the RBT test appeared to be the most suitable screening test to identify diseased individuals or to ensure the absence of infection in herds [22, 11].

Outbreaks of *Brucella* infection are influenced by a number of related factors, including age, sex, breed, size, housing system, and agro-ecology [12, 32, 33]. In this study, female goats were significantly more affected by brucellosis than males. This result confirms the high seroprevalence (100%) observed in animals showing signs of abortion and infertility. This finding had already been made in cattle, where females were more likely to be infected with *Brucella* than males [34, 35]. This could be explained by the fact that ruminant females generally lick their fetuses and newborns, which increases the level of infection with the bacteria [36]. Furthermore, although age is considered a factor influencing *Brucella* infection in cattle, seroprevalence did not vary significantly between age groups in small ruminants in this study. However, the most infected age group was between 4 and 6 years of age. Brucellosis infection was similar in the seven sites studied. The reasons for this similarity may be due to the similar environmental and geographical characteristics of the sites. Similarly, the small sample size may not have favored the detection of differences in seroprevalence between sites. However, according to one of the previous studies, the difference in the prevalence of *Brucella* could be linked to the different environments: the bacterium survives better in humid conditions but does not multiply very well in an environment that is too hot [16].

For this study, animals were selected on the basis of well-defined clinical signs and according to pre-established objectives: younger animals were of little concern, not all farmers were surveyed, not all farms were included, and serological analysis was carried out using two methods (RBT and indirect ELISA). This approach is a limitation of the study. Future studies will include all animals in the herd and evaluate the diagnostic capacity and discriminatory power of PCR, sequencing, fixing the complement (FC), I-ELISA, and RBT in detecting brucellosis.

CONCLUSION

This study revealed brucellosis spread in small ruminants with variable prevalence in the sub-prefecture of Dounet in Guinea. These animals may pose a potential risk of spreading brucellosis to humans and other animals due to their close association with the human population and the zoonotic nature of the disease. As part of a One Health approach, we recommend screening of human populations at risk (veterinarians, farmers, slaughterers) and public awareness programmes to reduce the risk of zoonotic disease in the human population.

Availability of Data and Materials

The datasets that were analyzed in this study are available from the lead author.

Ethics for Approval and Consent to Participate

Prior approval was obtained from the Mamou's Prefectural Department of Livestock before the study was carried out. After contacting breeders with the agreement of the local chiefs, the objectives and framework of the study were clearly explained. In this way, we were able to obtain or not the herders' consent to participate without any negative consequences for their activities. Only after obtaining their verbal consent were the questionnaires used and samples collected. Privacy of the participating farmers was respected, as other farmers prefer to hide what is happening in their herds to avoid being judged by their peers. To ensure privacy, the questionnaires did not include the names of the farmers but rather the names of the villages where they were run and numbered.

Competing Interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Generative AI Statement

The authors declare that no generative AI was used in the creation of this manuscript.

Authors' Contributions

The original study was conducted by AODC, ASS, LK, NK and AOSD who also supplied the data. The idea for the study was conceptualized and generated by AODC, ASS, LK, and AOSD. Data were collected by AODC, ASS, LWS, HMD, CDK, KC, MMD and MYB. AODC, ASS. LK and AOSD drafted the manuscript. Statistical data analysis by ASS and LK. GGP, SE and MCA provided intellectual criticism on the content of the manuscript. All authors have read and approved the final submitted manuscript.

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ORIGINAL ARTICLE

EVALUATION OF PREOPERATIVE GABAPENTIN ON CARDIOPULMONARY PARAMETERS AND POSTOPERATIVE PAIN IN DOGS UNDERGOING ELECTIVE OVARIOHYSTERECTOMY

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ABSTRACT

This study evaluated the efficacy and safety of oral gabapentin for perioperative pain control in dogs undergoing ovariohysterectomy (OVH). Ten female dogs were randomly assigned into two groups, and standard OVH was performed following anaesthesia with propofol injection (4 mg/kg). The treatment group was premedicated with 20 mg/kg oral gabapentin 8 hours before anaesthesia induction, while control dogs were administered a placebo. Adverse events associated with gabapentin were noted as they occurred. Changes in physiological and cardiopulmonary parameters were determined at 10-minutes intervals until recovery from anaesthesia. Pain severity was assessed using GCMPS-SF pain scales upon recovery from anaesthesia (time = 0) and at 12-hours intervals over 48 hours by independent observers. Physiological parameters and GCMPS-SF scores were compared using ANOVA. Diarrhoea (1/5) and swollen salivary glands (1/5) were the only adverse events associated with gabapentin administration. The rectal temperature, heart rate and pulse rate were not significantly ($p = 0.47$) different between the two groups. The postoperative pain score as well as respiratory rate and mean arterial pressure were significantly ($p < 0.05$) lower in gabapentin group. Premedication with gabapentin is safe and decreased postoperative pain scores in dogs presented for elective neutering.

Keywords: dogs; gabapentin; ovariohysterectomy; preoperative; pain

INTRODUCTION

Management of pain in animals is an essential welfare and ethical aspect of veterinary medicine. Perioperative pain can impact the well-being of the veterinary patient, while untreated acute pain can result in the development of maladaptive pain states [1]. Postoperative pain is associated with tissue damage and occurs as a result of activation of peripheral nociceptors or inflammation. Acute pain is traditionally managed using opioid analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), and local anaesthetics [2]. In spite of the effectiveness of opioid analgesic drugs in the treatment of acute pain, usage is limited because of attendant adverse effects [3]. The recent opioid epidemics in the USA and some other countries have also contributed to the poor availability of opioid analgesics for veterinary use. On the other hand, NSAID administrations are associated with gastrointestinal, hepatic, and renal toxicities in spite of their effectiveness in the control of mild to moderate nociceptive pain [2].

Xylazine is an alpha-2-adrenoreceptor agonist with analgesic and muscle relaxation properties commonly used as pre-anaesthetic and also for the control of acute pain in veterinary practice [4]. Incidence of xylazine involvement in drug-related human deaths in the US and other parts of the world has been on the increase in the last five years [5, 6]. This abuse might probably be responsible for the scarcity of xylazine, with concern that the drug may not be readily available for veterinary use, thus necessitating the need for alternative safe and effective drugs that can be used in the control of perioperative pain.

Gabapentin is a structural analogue of gamma-aminobutyric acid, formulated for treatment of convulsion and neuropathic pain [7]. The mechanism of action of gabapentin has been postulated to involve voltage-gated calcium channels binding to the alpha-2-delta subunit [8, 9]. Several clinical trials and meta-analyses showed that administration of gabapentin improves postoperative pain associated with abdominal hysterectomy and mastectomy in humans [10]. These findings led to the growing interest in the use of gabapentin for control of perioperative pain in veterinary medicine.

In veterinary medicine, gabapentin has gained popularity not only for control of acute and chronic pain but also to provide patient comfort by reducing fear and stress during transport and/or hospitalization [11]. Two recent surveys have shown widespread use of gabapentin for acute and chronic pain in veterinary medicine [7, 12] despite insufficient evidence available to support efficacy. In dogs, gabapentin has been used for postoperative pain control in dogs undergoing mastectomy with a significant reduction in the incidence of rescue medication [13]. Furthermore, the drug has been shown to decrease the isoflurane Minimum Alveolar Concentration (MAC) [14]. Pre-anesthetic oral administration of gabapentin has also been reported to prevent a rise in intraocular pressure following intubation in dogs [15].

There is a dearth of reports on the effectiveness of gabapentin for perioperative pain control in dogs undergoing reproductive surgery. A study in cats subjected to ovariohysterectomy (OVH) reported that preoperative administration of oral gabapentin significantly improved analgesia and mechanical nociception threshold [16]. With this promising efficacy result, there is the need for more clinical trials to validate the analgesic potentials of gabapentin in dogs undergoing reproductive surgery. This study evaluated the safety and efficacy of gabapentin in dogs subjected to elective OVH.

MATERIALS AND METHODS

Animals

Ten intact mongrel dogs between 4 and 6 months of age with a mean weight of 7.63 ± 0.63 kg were used. They were sourced from households around the university and housed at the Veterinary Teaching Hospital throughout the duration of the experiment. The dogs were fed on dog rations twice daily, while water was provided ad libitum. Prior to the commencement of the study, the dogs were acclimatized to their environment and the handlers for a minimum period of four weeks to get the dogs used to the new environment. During this period, they were dewormed with a broad-spectrum dewormer (Prazi-wormer, Barry Vet Animal Health, Nigeria). Ethical approval (FUNAAB/COLVET/CREC/2024/03/05) was received from COLVET Research and Ethics Committee (CREC), Federal University of Agriculture, Abeokuta.

Study Design

The study involved simple randomized design. Ten dogs were assigned randomly to two groups. The first

group (Gabapentin group) consisted of five dogs premedicated with oral gabapentin (Gabapentin-Teva^{R}, Teva Pharmaceuticals CR, Praha, Czech Republic) at 20 mg/kg eight hours before the start of OVH. The second group (Control group) consisted of five dogs administered a placebo as premedication eight hours before the start of OVH. The observers were unaware of the type of premedication received by each dog.

Anaesthetic Procedure

One hour prior to the start of OVH, each dog was treated with tramadol hydrochloride (Tramadol^{R}, Gland Pharmaceuticals, India) intravenously at the rate of 3 mg/kg body weight. After aseptic preparation of the surgical site, venous access was secured using the cephalic vein. Anaesthesia was induced using 1% propofol (Hyprovan 200®, Celon Laboratories PVT LTD, Telangana, India), administered intravenously at 4 mg/kg body weight. Venous access was maintained using normal saline infusion at the rate of 5 ml/kg/hr. Anaesthesia was maintained using a continuous rate infusion of tramadol (2.6 mg/kg/hr.) and propofol (1 mg/kg/hr.).

Once, the dogs were anaesthetized, they were positioned in dorsal recumbency and draped appropriately. Thereafter, a 2% lignocaine injection (Lignovit 20-AH^{R}, Vital Healthcare PVT LTD, Nashik, India) was infiltrated on the *linea alba* at the rate of 2mg/kg and laparotomy incision was made. This stage was followed by OVH using standard technique. Before closure of the laparotomy incision, 2% lignocaine was administered intraperitoneally, and the incision was closed routinely. The dogs were monitored until recovery and kept in individual cages within the recovery room for commencement of pain assessment.

Outcome Measurement

Rectal temperatures (RT), respiratory rates (RR), heart rates (HR), pulse rates (PR), and blood pressure of the dogs were determined following loss of righting reflex and every ten-minute interval until the end of OVH. RT was measured using a digital thermometer, while RR was counted manually from abdominal excursion. The HR was measured in beats per minute by auscultation of the heart with a stethoscope, while PR was counted in beats/min by applying digital pressure on the femoral artery. The blood pressures parameters including Sytolic Arterial Pressure (SAP), Mean Arterial Pressure (MAP), and Diastolic Arterial Pressure (DAP) were obtained through a non-invasive oscillometric blood pressure device.

Adverse Effects of Gabapentin

Each dog was monitored for adverse effects associated with gabapentin usage before, during, and after OVH. An adverse effect was defined as any undesirable change that occurred following gabapentin usage, whether considered or not considered to its use. Reported adverse reactions of gabapentin, such as sedation, depression, lethargy, incoordination, vomition and diarrhoea were looked out for. Sedation was considered as adverse effect if it became prolonged after OVH.

Postoperative Pain Assessment

The Glasgow Composite Pain Scale short form (GCMPS-SF) was used for pain assessment post OVH. Rescue analgesia (tramadol injection administered through the intramuscular route) was provided if GCMPS-SF scores were $\geq 6/20$. For postoperative pain assessment, the dogs were evaluated inside their cages without being disturbed. Assessment was done after recovery from anaesthesia and every 12 hours up to 48 hours post OVH.

Statistical Analysis

Data were tested for normality using a Shapiro-Wilk test. Physiological parameters and GCMPS-SF scores were compared using analysis of variance (ANOVA) for repeated measures at $p \leq 0.05$. Data analysis was performed using SPSS Statistics (V25, IBM, Armonk, NY, USA).

RESULTS

The adverse events reported in this study include vomition, diarrhoea and swollen salivary glands (Table 1). Vomition was observed in one (1) of the dogs in the control group, while diarrhoea was observed in one (1) of the dogs in the gabapentin group. The vomition was observed once and occurred before induction of anaesthesia, while the

diarrhoea occurred once after recovery from anaesthesia. In addition, one dog each from the gabapentin and control groups had swollen salivary glands. The swollen salivary glands were observed 12 hours post-surgery. All the adverse events observed resolved spontaneously without any medical intervention.

Table 1. Adverse events associated with oral administration of gabapentin in dogs undergoing elective OVH

Adverse Effects	Control Group	Gabapentin Group
Sedation	+(0/5)	+(0/5)
Depression	+(0/5)	+(0/5)
Lethargy	+(0/5)	+(0/5)
Incoordination	+(0/5)	+(0/5)
Vomition	+(1/5)	+(0/5)
Diarrhoea	+(0/5)	+(1/5)
Swollen salivary gland	+(1/5)	+(1/5)

Number of dogs in parentheses + = Present

The rectal temperature of the dogs decreased progressively throughout the surgery (Fig. 1). The rectal temperature did not differ significantly ($p > 0.05$) between gabapentin premedicated dogs and the control except at 70 minutes from commencement of anaesthesia when the rectal temperature was significantly ($p < 0.05$) higher in the gabapentin premedicated dogs than the control dogs (Fig. 1).

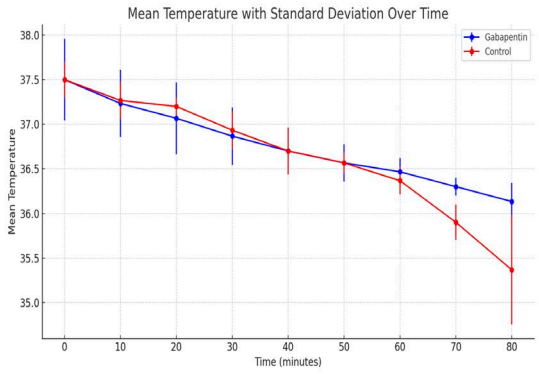

Fig. 1. Rectal temperature of dogs undergoing elective OVH following premedication with either gabapentin (20 mg/kg PO) or placebo (control group) and anaesthetized with propofol (4 mg/kg IV)

Changes in respiratory rates of dogs undergoing elective OVH following premedication with oral gabapentin or placebo is shown in Fig. 2. The respiratory rates in gabapentin premedicated dogs increased progressively throughout the duration of the surgery, while the respiratory rates of control dogs decreased progressively through the duration of the surgery. The respiratory rates were significantly ($p < 0.05$) lower in the gabapentin premedicated dogs up to 60 minutes of the surgery compared with the control dogs (Fig. 2). The heart rates of the dogs increased progressively throughout the surgery (Fig. 3). The heart rates did not differ significantly ($p > 0.05$) between gabapentin premedicated dogs and the control dogs throughout duration of the surgery. The pulse rates of the dogs increased progressively throughout the surgery (Fig. 4). The pulse rates did not differ significantly ($p > 0.05$) between gabapentin premedicated dogs and the control dogs throughout the duration of the surgery.

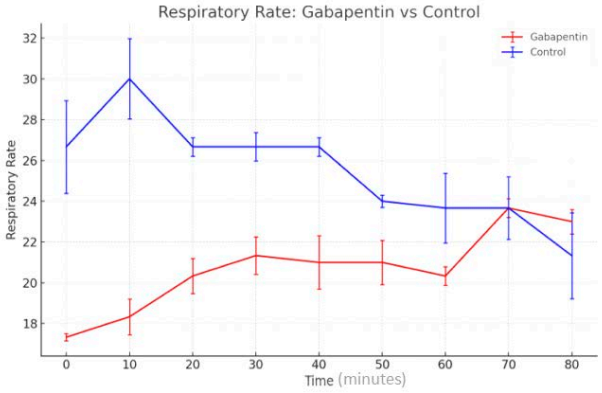

Fig. 2. Respiratory rates of dogs undergoing elective OVH following premedication with either gabapentin (20 mg/kg PO) or placebo (control group) and anaesthetized with propofol (4 mg/kg IV)

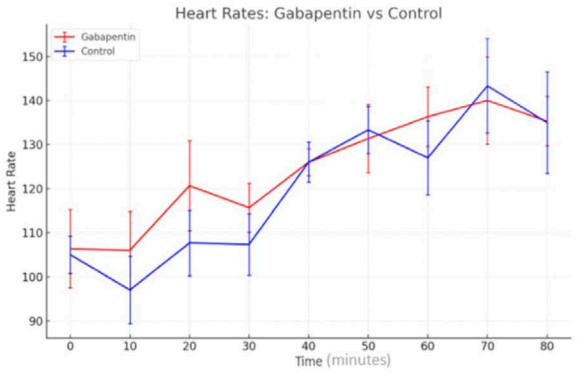

Fig. 3. Heart rates of dogs undergoing elective OVH following premedication with either gabapentin (20 mg/kg PO) or placebo (control group) and anaesthetized with propofol (4 mg/kg IV)

The systolic arterial pressure (SAP), diastolic arterial pressure (DAP), and mean arterial pressure (MAP) of dogs undergoing OVH and premedicated with either gabapentin or placebo are shown in figure 5a–c. The SAP, DAP, and MAP were significantly ($p < 0.05$) lower at 50- and

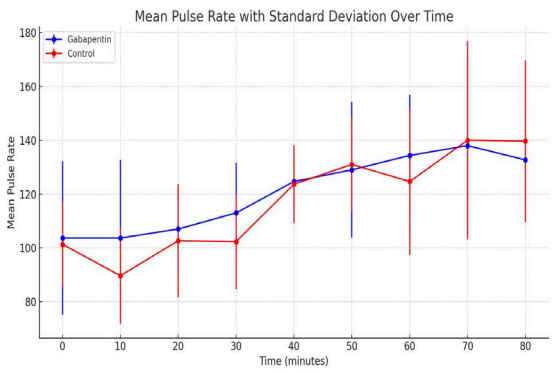

Fig. 4. Pulse rates of dogs undergoing elective OVH following premedication with either gabapentin (20 mg/kg PO) or placebo (control group) and anaesthetized with propofol (4 mg/kg IV)

60-minutes period of the OVH in gabapentin premedicated dogs than in the control dogs.

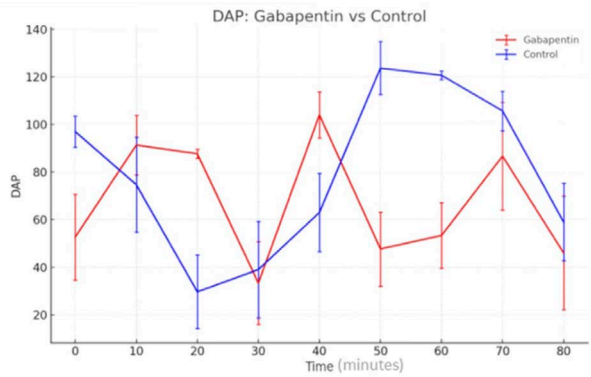

Fig. 5a. Diastolic blood pressure of dogs undergoing elective OVH following premedication with either gabapentin (20 mg/kg PO) or placebo (control group) and anaesthetized with propofol (4 mg/kg IV)

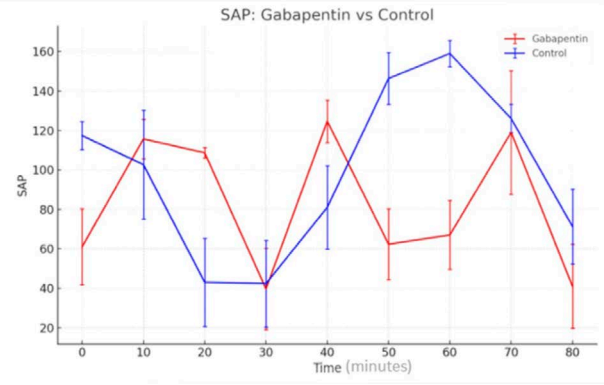

Fig. 5b. Systolic blood pressure of dogs undergoing elective OVH following premedication with either gabapentin (20 mg/kg PO) or placebo (control group) and anaesthetized with propofol (4 mg/kg IV)

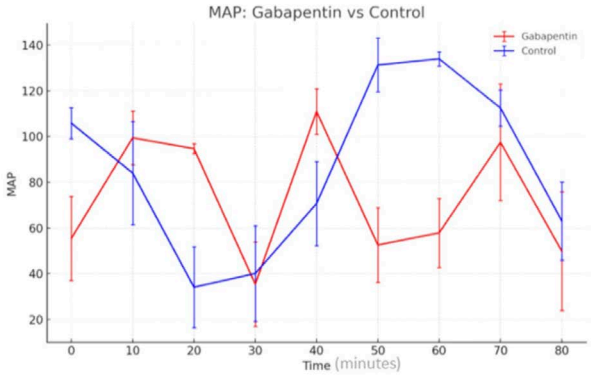

Fig. 5c. Mean arterial pressure of dogs undergoing elective OVH following premedication with either gabapentin (20 mg/kg PO) or placebo (control group) and anaesthetized with propofol (4 mg/kg IV)

The result of the postoperative pain score using the Glasgow Composite Pain Measure (GCMPS-SF) scale in dogs subjected to OVH is shown in Fig 6. The maximum pain score was 4, while the minimum pain score was 1. The pain score was significantly lower ($p < 0.05$) in dogs premedicated with gabapentin than in control dogs. Thereafter, there was no significant difference ($p > 0.05$) in the pain scores between dogs premedicated with gabapentin and the control group.

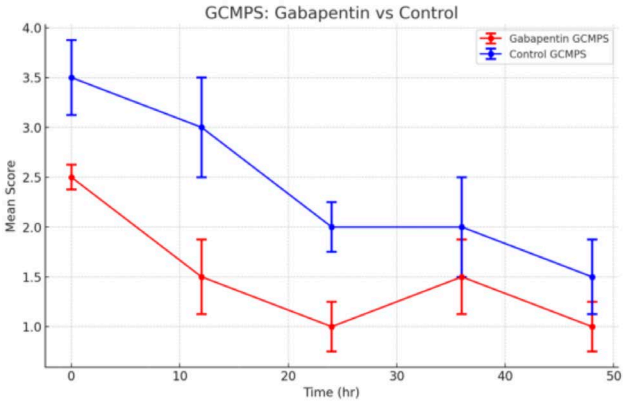

Fig. 6. Pain assessment scores of dogs subjected to elective OVH using the Glasgow Composite Pain Measure (GCMPS-SF) scale. Dogs were premedicated with either gabapentin (20 mg/kg PO) or placebo and anaesthetized with propofol (4 mg/kg IV).

DISCUSSION

The result of this study showed that preoperative administration of gabapentin in dogs undergoing elective OVH is characterized by minimal adverse events that require no medical intervention. In addition, premedication with oral tramadol did not significantly alter RT, HR, or

PR in dogs undergoing elective OVH. However, oral gabapentin significantly lowers respiratory rates and alters the blood pressures in dogs undergoing elective OVH. In addition, premedication with oral gabapentin in dogs undergoing OVH is associated with better postoperative pain scores compared with placebo.

Premedication with oral gabapentin was associated with fewer and milder adverse events that required no intervention. In a previous study involving evaluation of gabapentin and topiramate in dogs with Chiari-like malformation [17], oral gabapentin administration was reported to be associated with adverse events such as vomition, diarrhoea, sedation and polyphagia. None of the dogs in this current study were observed with prolonged sedation following recovery from anaesthesia and this may be related to the time of administration to the dog. In a similar study in healthy cats, sedation or other gabapentin-related adverse effects were not observed [18]. One dog each from the gabapentin premedicated and placebo groups was observed with a swollen salivary gland. The exact cause of this symptom is unknown and might be unrelated to the use of gabapentin since the same sign was also observed in the control groups. These findings suggested that premedication with oral gabapentin is safe in dogs.

The rectal temperatures of the dogs in this study progressively decreased throughout the duration of the surgery irrespective of the premedication type. This progressive decrease in rectal temperature might be associated with the surgery rather than the administration of gabapentin. A previous report in cats showed that gabapentin administration did not alter rectal temperature in cats [19]. Perioperative hypothermia is a common complication associated with anaesthesia and surgery and is being linked to redistribution of body heat and decreased metabolism [20]. Thus, it is logical to conclude that the progressive decrease in rectal temperature in the dogs is associated with the OVH. This explains why temperature control mechanisms such as the use of circulating hot water blankets should be used in dogs undergoing major surgeries.

Respiratory rates of dogs premedicated with oral gabapentin were significantly higher than the control dogs. This may be due to the anxiolytic effect of gabapentin. Stress, whether physiologic or surgical has been reported to increase sympathetic discharge with release of epinephrine, norepinephrine, and cortisol, resulting in elevation of respiratory rate and blood pressure [21].

In this study, the heart and pulse rates of dogs undergoing elective OVH and premedicated with either gabapentin or placebo increased progressively throughout the surgery. This progressive increase in heart and pulse rates might be associated with surgical stimulations rather than the type of premedication. Surgical stimulation has been reported to influence autonomic reflex regulation depending on the anaesthesia type [22]. Lack of changes in the heart rates between gabapentin premedicated and the control dogs suggests that oral gabapentin does not result in any significant change in haemodynamics as previously reported in cats [21].

Premedication with oral gabapentin has been reported to decrease intraocular pressure in dogs anaesthetized with propofol [15]. A similar blood pressure lowering effect was reported in cats receiving oral gabapentin [23]. The DBP, SBP, and MAP were significantly lower at 50 and 60 minutes during OVH in dogs premedicated with gabapentin. These findings may be associated with the inhibitory effect of gabapentin on membrane voltage-gated calcium channels [11].

Premedication with oral gabapentin in dogs undergoing OVH resulted in decreased postoperative pain scores compared to control dogs. This result is contrary to that reported in cats undergoing OVH, where premedication with gabapentin did not influence pain score [18]. Analgesic activities of gabapentin may be related to its anxiolytic effect, which mitigates pain experience or expression in the young dogs. The disparity in the results of this current study and the previous study might be related to the type of anaesthetic drugs used and the method of assessment of postoperative pain. In this study, the dogs were anaesthetized with propofol, an alkyl phenol hypnotic drug with an absence of analgesia [24], and tramadol, a weak mu opioid analgesic [25].

There are few limitations that should be taken into consideration when interpreting the result of this study. The sample size in this study is small when compared with similar studies. The relatively small sample size might have accounted for the large standard deviation obtained in the pain assessment score. The relatively young age of the dog might have influenced the pain correlates assessed by the pain scale. In addition, pain was assessed by final-year veterinary students who might not be experienced in recognizing the correlates for appropriate pain recognition. However, the observers were trained for two weeks before commencement of the study.

CONCLUSION

Premedication with oral gabapentin in healthy dogs undergoing elective OVH is relatively safe and did not alter the physiological parameters of the dogs. In addition, oral gabapentin decreased postoperative pain scores in dogs undergoing elective OVH. It is recommended that oral gabapentin can be used as a component of multimodal analgesic agents for surgical procedures of healthy dogs in poor resource settings where access to true opioid analgesics is difficult or in cases where non-steroidal anti-inflammatory drugs are contraindicated due to safety reasons.

Data Availability Statement

The raw data of this study will be made available by the authors upon request to the corresponding author.

Ethical Statement

Ethical approval (**FUNAAB/COLVET/CREC/2024/03/05**) was received from the COLVET Research and Ethics Committee (CREC), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Conflict of Interest

The authors declare no conflict of interest.

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Generative AI Statement

The authors declare that no generative AI or AI-assisted tools were used while writing the manuscript.

Authors' Contributions

Conceptualization, design, funding, supervision, and critical review of manuscript: R. A. Ajadi; Data collection, analysis, and processing; literature review and manuscript writing: I. O. Oyenekan, J. O. Osunlakin, K. Adeoyo, A. O. Makinde, and O. F. Kehinde

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FOLIA VETERINARIA, 69, 4: 37—48, 2025

REVIEW ARTICLE

PARVOVIRUS ENTERITIS IN NIGERIAN DOGS: A SYSTEMATIC REVIEW (2009–2025) AND A SIX-YEAR RETROSPECTIVE COHORT IN THE VETERINARY TEACHING HOSPITAL, UNIVERSITY OF IBADAN

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

Canine parvovirus (CPV) enteritis remains a major cause of morbidity and mortality among dogs in Nigeria. This study combined a systematic review (2009–2025) with a six-year retrospective cohort analysis of 415 laboratory-confirmed cases from the University of Ibadan Veterinary Teaching Hospital (2018–2024) to identify risk factors and quantify vaccination impact. Literature retrieved from Scopus, Web of Science, PubMed, and AJOL highlighted young age (<24 weeks), incomplete vaccination, breed predisposition (notably Boerboels and German Shepherds), and environmental exposure as key risk factors. Retrospective data showed a near-balanced sex ratio (52.1% female) and a mean age of 21.44 ± 22.77 weeks. Vaccination markedly improved survival (76.6% vs. 49.6%; $\chi^2 = 33.95$, $p < 0.001$), with the strongest benefit in Boerboels (93.3% vs. 46.2%; $p = 0.002$) and in dogs exhibiting >2 clinical signs (76.0% vs. 44.1%; $\chi^2 = 16.39$, $p = 0.003$). Literature evidence associated vaccine failures with poor handling and maternally derived antibody interference. Overall, vaccination conferred a significant survival advantage across breeds and severity categories. Strengthening early diagnosis, optimizing vaccine storage and administration, and ensuring completion of immunization schedules are essential to mitigate the persistent CPV burden in Nigerian dog populations.

Keywords: canine parvovirus; clinical signs; CPV enteritis; Nigeria; risk factors; vaccine efficacy

INTRODUCTION

Canine parvovirus (CPV) is a highly contagious viral disease that causes severe, often fatal gastroenteritis in dogs, particularly in unprotected puppies [1]. Since its first report in Nigeria in 1985 [2], all three CPV-2 variants (2a, 2b, and 2c) have been documented, with CPV-2c now predominating [1]. Despite widespread vaccination efforts, CPV remains endemic, exacting high morbidity and mortality in Nigerian canine populations. To generate both broad and locally grounded evidence, this paper combines a systematic meta-analysis of published Nigerian studies from 2009–2025 with an eight-year (2018–2024) retrospective cohort review of 415 CPV cases diagnosed by rapid antigen tests, haematology, PCR, and clinical signs. The meta-analysis collates risk factors (young age, incomplete vaccination, breed predisposition, environmental exposures), vaccine efficacy data, and commonly reported clinical signs (hemorrhagic diarrhea, vomiting, lethargy, dehydration) across the literature. The retrospective study then contextualizes these findings by evaluating actual demographic patterns, clinical severity, and treatment outcomes with detailed analyses of sex and age distributions (Table 1, Figure 3), vaccination status and survival (Figures 4 & 8), and the influence of clinical sign burden on mortality (Figures 6 & 7). By triangulating high-level evidence with real-world case data, this dual approach aims to provide robust, actionable insights for optimizing CPV prevention and management in Nigeria and evaluate the demographic patterns, clinical severity, and treatment outcomes of canine parvovirus infection across diverse dog breeds, with particular emphasis on sex and age distributions, vaccination status (complete, incomplete, or not done), and the influence of clinical signs on survival.

MATERIALS AND METHODS

A systematic review was conducted to evaluate published evidence on canine parvovirus enteritis in Nigeria, focusing specifically on risk factors, vaccine efficacy, and clinical signs. Literature searches were performed using four major databases with a focus on Nigeria: Web of Science, SCOPUS, PubMed, and African Journals Online (AJOL). Search terms included combinations of "canine parvovirus," "CPV enteritis," "dogs," "Nigeria," "risk factors," "vaccine efficacy," and "clinical signs" which are the focus groups. Only peer-reviewed articles published between January 2009 and April 2025 were considered. The search was limited to English-language publications, and reference lists of relevant articles were manually screened to identify any additional studies. Eligibility criteria were established to ensure the inclusion of studies with direct relevance to the research objectives. Studies were included if they presented original data concerning canine parvovirus infections in Nigerian dog populations and addressed at least one of the three focus areas. Articles were excluded if they were reviews, case reports without epidemiological analysis, opinion pieces, or studies that focused exclusively on molecular characterization without clinical context. Duplicates were removed, and titles and abstracts were screened for relevance before full-text review. Data extraction was performed independently and included the following variables: study design, location, sample size, diagnostic methods employed, identified risk factors, and focus groups. Studies varied in methodology, including retrospective hospital-based surveys, cross-sectional field studies, and serological assessments. Due to variability in study designs and reported measures, a narrative synthesis was employed rather than a quantitative meta-analysis. Key findings were grouped and summarized under thematic headings to highlight common trends and discrepancies across different studies. The quality of included studies was critically appraised using adapted criteria for observational studies. Factors assessed included clarity of study objectives, appropriateness of sampling methods, validity of CPV diagnostic techniques, and robustness of statistical analysis. Studies with substantial methodological flaws or unclear reporting were excluded from synthesis. The final selection of 19 articles from an initial 1200 sources provided a comprehensive overview of the current understanding of canine parvovirus enteritis in Nigeria over the past 15 years, offering valuable insights into epidemiological patterns.

The retrospective cohort component of this study reviewed all laboratory-confirmed canine parvovirus cases presented to the Veterinary Teaching Hospital between January 2018 and December 2024. Electronic medical records and hospital logbooks were queried to identify dogs with positive fecal antigen ELISA or PCR confirmation of CPV infection. Records were screened according to predefined inclusion criteria for complete documentation of

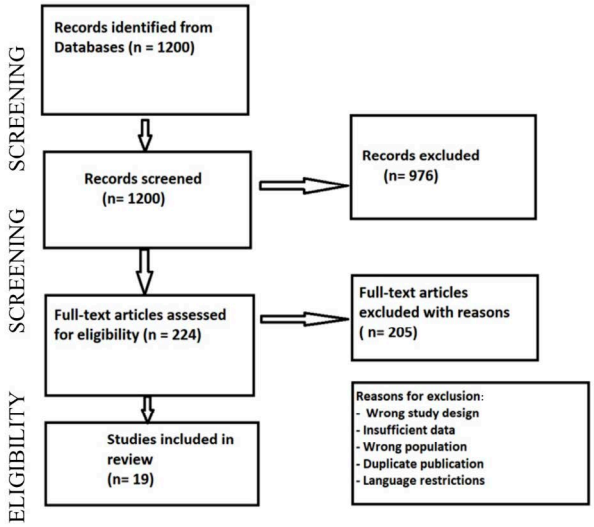

Fig. 1. PRISMA diagram showing the selection process for the articles used in the literature review and meta-analysis process in the study

signalment, history, and treatment outcome, and ineligible cases were excluded. A total of 415 cases met criteria and were entered into a standardized data collection spreadsheet. Extracted variables included demographic information (breed, sex, exact age), vaccination history (categorized as complete, incomplete, or not done), the number and type of clinical signs (e.g., hemorrhagic diarrhea, vomiting, lethargy, dehydration), and final outcome (survived, died, or lost to follow-up). All data were imported into SPSS v.25 for statistical analysis. Descriptive statistics summarized frequencies and percentages for categorical variables, which are the focus areas, and means with standard deviations for continuous variables (age). Sex and breed distributions were tabulated, and mean age comparisons by sex within each breed were assessed using independent-samples t-tests, with significance set at $p < 0.05$. Vaccination status was cross-tabulated against treatment outcome for the entire cohort and within subgroups stratified by clinical severity (those presenting with two or fewer clinical signs versus more than two signs) using Pearson's chi-square tests. Chi-square statistics and corresponding p-values were reported to evaluate the association between vaccination and survival across breeds and presentation severities.

Ethical Approval

The design and protocol for this retrospective cohort study and associated meta-analysis were reviewed and approved by the Director of the Veterinary Teaching Hospital and the Head of the Department of Veterinary Pathology, University of Ibadan. Written authorization was obtained for access and use of the anonymized clinical records from the hospital's database. For the component of the study involving telephone follow-ups to ascertain patient outcomes, verbal informed consent was procured from all dog owners after the nature and purpose of the contact were fully explained. All procedures adhered to established ethical principles for clinical research, ensuring client and patient confidentiality was maintained throughout the study.

RESULTS

Meta-analysis

Epidemiology of Canine Parvovirus in Nigeria

Canine parvovirus (CPV) has long been recognized as an endemic pathogen within Nigerian canine populations, with numerous serological surveys and antigen-detection studies documenting its widespread occurrence. In one notable longitudinal analysis conducted in Delta State, hospitalized dogs were screened over a fourteen-year interval, revealing an overall CPV enteritis prevalence of 13.4% and demonstrating cyclical peaks approximately every four to five years [3]. In a separate cross-sectional survey carried out in the southeastern region of the country, 37.3% of dogs presenting with clinical suspicion of parvoviral disease tested positive for CPV-2 via antigen detection in Yola State [4]. Collectively, these and other investigations underscore the persistent, year-round presence of CPV across diverse ecological zones in Nigeria, with young dogs, particularly those less than six months of age, bearing the brunt of infection [3].

Risk Factors Associated with CPV Enteritis

A variety of host- and environment-related factors have been implicated in heightened susceptibility to CPV infection in Nigerian dogs. Age remains the most consistently reported determinant; puppies under six months of age exhibit markedly higher infection rates than older animals. For instance, Shima documented that dogs aged zero to five months experienced significantly greater CPV incidence compared to their mature counterparts [3], while Ukwueze observed CPV prevalence of 42.9% in pups aged 0–6 months versus only 17.0% in dogs older than twelve

months [4]. These figures reflect both the immunologically naive young animals and the incomplete vaccination schedules typical in many Nigerian breeding operations. Breed-related vulnerability also emerges from Nigerian field studies [1, 3, 4]. In the Delta State survey, purebred German Shepherds (Alsatians), indigenous Mongrels, Rottweilers, and other locally kept breeds demonstrated higher rates of CPV enteritis than did mixed-breed dogs [3]. The southeastern study further highlighted that large breeds such as Rottweilers (63.4% prevalence), American Pit Bulls (50.0%), Great Danes (40.0%), Bull Mastiffs (39.4%), and Alsatians (36.0%) were disproportionately affected, while local mongrels exhibited a much lower infection rate of 11.1% [4]. These patterns may derive from genetic predispositions, breed-specific management practices such as kennel housing or breeding density, or differential levels of owner investment in preventive care. Sex as a risk factor has produced mixed findings. Shima reported a higher incidence of CPV infection in male dogs, whereas other investigations have failed to demonstrate a significant sex-based difference, suggesting that any apparent male bias may be confounded by ownership patterns or sampling variability [3, 4, 5]. Consequently, sex alone does not appear to be a robust predictor of CPV risk. Vaccination status is uniformly recognized as a critical determinant of outcome. In Delta State, unvaccinated dogs were significantly more likely to develop clinical parvoviral enteritis than their vaccinated counterparts [3]. Contrastingly, some southeastern studies have found no statistically significant correlation between self-reported vaccination status and CPV infection [4], a discrepancy attributable to factors such as incomplete immunization regimens, poor vaccine storage and handling, and unreliable record-keeping. In reality, many dogs classified as "vaccinated" receive only a single initial dose or are vaccinated at ages when maternally derived antibodies still block effective seroconversion. Environmental and management conditions further modulate CPV risk. Overcrowded kennels, suboptimal hygiene, and close contact with free-roaming or stray dogs amplify exposure to virulent CPV particles shed in feces [5]. Ogbu et al. (2021) emphasized that kennel management practices and breeder compliance with vaccination protocols are pivotal in determining CPV transmission dynamics [6]. Concurrent stressors such as parasitic infestations, early weaning, and nutritional deficiencies may depress immune function and predispose exposed dogs to fulminant disease [7]. Geographic and seasonal variables also play roles: Shima observed bimodal peaks in CPV hospital admissions during the dry season (January) and the rainy season (July), while Ogbu reported inter-provincial differences in prevalence, likely reflecting localized breeding and husbandry practices [3, 6]. However, recent sophisticated time-series analysis conducted in Ibadan revealed a statistically significant negative correlation with monthly rainfall ($r = -0.55$), pinpointing the dry-season months, particularly January and February, as the period of highest prevalence [8]. This seasonality, with its low-incidence trough during the wet months of September and October, is a crucial element for predicting outbreaks and allocating preventive resources efficiently.

Diagnostic Methods and Challenges

The diagnostic landscape for CPV in Nigeria is characterized by a tiered approach influenced by resource availability. The most widely employed method in clinical practice is the point-of-care immunochromatographic (IC) antigen test, valued for rapid turnaround and clinic-side accessibility, but with sensitivity and specificity limitations dependent on viral load and sampling timing [9]. These rapid tests can also produce false positives in animals recently vaccinated with modified-live vaccines due to transient shedding of vaccine antigens [9]. Molecular diagnostics, particularly PCR and sequencing, are available but largely confined to university research settings and referral centers, limiting their routine clinical utility [10, 11]. These methods are invaluable for confirming diagnoses and for molecular epidemiology studies that track viral evolution. In many rural and resource-limited practices, clinicians rely on a combination of classic clinical signs and response to supportive care for diagnosis, with necropsy and histopathology providing definitive diagnosis in fatal cases [12]. The overarching challenge is limited access to affordable, reliable confirmatory testing nationwide, underscoring the need for improved diagnostic algorithms tailored to different practice tiers [12]. This was the basis for the work by Akanbi in 2025, who reported that clincal signs, in addition to other laboratory markers (haematology), statistically compete favorably in diagnosis in resource-limited setting when used with PCR-confirmed cases [13].

Vaccine Efficacy and Immune Profiles

Preventive vaccination represents the cornerstone of CPV control, yet in Nigerian practice its potential remains underrealized due to operational shortcomings. Specific mutations reported in Nigerian field isolates, such as S297A and Y324I, may influence antigenicity and receptor interactions, raising concerns about potential impacts on vaccine effectiveness [11, 10]. Phylogenetic analyses indicate relationships between Nigerian strains and variants from Asia and other African regions, suggesting multiple introductions and local evolution (Tion et al., 2021; [11]). These findings emphasize the need for ongoing molecular surveillance and consideration of vaccine strain updates to ensure antigenic matching with circulating variants [11, 10]. The commercial vaccines in use, typically live attenuated CPV-2 or CPV-2b strains, are antigenically capable of protecting against circulating CPV-2a, -2b, and -2c field variants [1, 5]. However, on-the-ground effectiveness is compromised by several factors. Cost constraints and limited access frequently lead to truncated immunization schedules, with many puppies receiving only a single initial dose rather than the full primary series and boosters (at least 3 shots of DHLPP) [5]. This DHLPP vaccine is a 5-in-1 conventional vaccine, which protects against distemper, hepatitis, leptospirosis, parainfluenza, and parvovirus infections. In some instances, vaccines are administered too early, during the window when maternal antibodies remain at titers high enough to neutralize the vaccine virus and block active immunization [5]. This maternal antibody interference is well documented as a major contributor to vaccine failure in high-viral-load environments like Nigeria. While their new CPV component is combined with the more conventional MLV CDV component [14] intended to protect puppies against CPV infection at a very young age (4 weeks) by breaking MDA interference more effectively than previous generation vaccines [15], these vaccines are not commonly available in Nigeria as of the time of this publication. Serological studies provide insight into the population's immune status. In Abeokuta, Babalola found that 79.2% of dogs, regardless of reported vaccination status, exhibited high anti-CPV IgG titers, implying widespread exposure or repeated vaccination [16]. Similarly reported by Nwosu, seropositivity rates were 93.5% in vaccinated dogs and 87.9% in those unvaccinated, suggesting that natural infection contributes substantially to herd immunity [17]. Nonetheless, despite high seroprevalence, clinical cases of CPV continue to occur at alarming frequencies, indicating that serological markers alone may not equate to protective immunity in the field. Indeed, Babalola demonstrated significant correlations between antibody levels and variables such as age, breed, and interval since last vaccination, highlighting that immunity wanes over time and varies across subpopulations [16]. Genetic characterization of Nigerian CPV isolates confirms the co-circulation of CPV-2a, -2b, and -2c variants, with 2c increasingly predominant [1, 3]. Fortunately, existing vaccines appear to match field strains antigenically, and there is no compelling evidence to date of immune escape by emergent variants. Thus, vaccine failure in Nigeria stems less from antigenic mismatch than from logistical issues such as improper dosing intervals, cold-chain breakdowns, and maternal antibody interference.

Clinical Presentation of Parvoviral Enteritis

The key feature of CPV infection in dogs is acute, severe gastroenteritis characterized by profuse vomiting and hemorrhagic diarrhea [3, 4, 18, 19]. Owners frequently report rapid onset of these signs, accompanied by lethargy, anorexia, and high fever. Diarrhea is often described as foul-smelling and copious, containing frank blood and mucosal casts. In a cohort of post-mortem cases, two separate authors described gross pathological changes of extensive fibrinous and hemorrhagic inflammation of the small intestinal mucosa, with necrosis of crypt epithelial cells and profound lymphoid depletion in Peyer's patches and the bone marrow microscopically [18, 19]. These lesions correlate clinically with severe fluid losses and hypovolemic shock. Hematological abnormalities are a consistent laboratory feature. Leukopenia, particularly neutropenia and lymphopenia, reflects the virus's tropism for rapidly dividing cells, including those in the bone marrow. Olaifa reported that over one-third of CPV-infected dogs developed severe non-regenerative anemia, predominantly of the normocytic hypochromic and microcytic hypochromic types [20]. Thrombocytopenia was profound in 73.3% of cases, further compounding hemorrhagic tendencies. Biochemical profiles often reveal elevated liver enzymes (AST, ALT), signifying hepatic involvement, and in a subset of dogs, elevated creatinine indicates renal compromise [6, 20]. Though the classical presentation involves gastrointestinal and hematological derangements, very young puppies may occasionally manifest myocarditis, leading to

acute heart failure in the absence of overt GI signs. Clinically, such cases present with sudden collapse, respiratory distress, and arrhythmias. However, this cardiac form is relatively uncommon in Nigerian reports.

Retrospective cohort

Demographics & Sex Distribution

A total of 415 laboratory-confirmed CPV cases across 22 breeds were analyzed and presented over a six-year period at the Veterinary Teaching Hospital, University of Ibadan characterised by a steady rise in cases between 2018 and 2024, with the exception of a dip in cases during the 2020 pandemic (Figure 2). The breed distribution and demographic characteristics of the 415 confirmed CPV cases are summarized in Table 1 and Figure 3. Overall, 216 dogs (52.05%) were female and 199 (47.95%) were male. Boerboels (119/415; 28.67%) and German Shepherds (120/415; 28.92%) together accounted for 57.59% of cases, each with an approximately equal female-to-male ratio (Boerboels: 63 F/56 M; GSDs: 63 F/57 M). Rottweilers comprised 14.46% of the cohort (24 F/36 M), Eskimos 5.54% (13 F/10 M), and Caucasians 6.99% (13 F/16 M). Lhasa Apsos (19/415) and Pitbulls (11/415) showed marked female predominance, 14 F/5 M (73.68%) and 8 F/3 M (72.73%), respectively, whereas Neapolitan Mastiffs (2/415), Cane Corsos (1/415), Great Danes (1/415), and Pugs (1/415) were each represented solely by males. Several breeds appeared only once (Bull Mastiff, Golden Retriever, Pomeranian, Saint Bernard) or twice (Dobermann, Samoyed, Terrier), limiting sex-specific analysis in those groups.

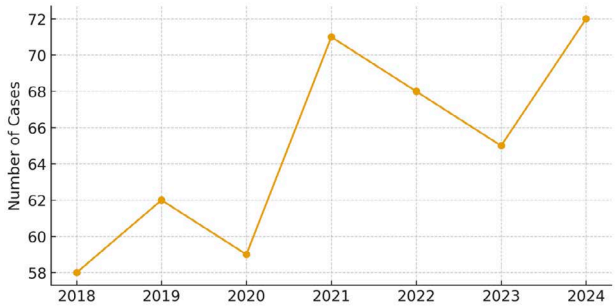

Fig. 2. Line graph showing temporal trends and annual distribution of CPV cases (2018–2024) presented to the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Ibadan, showing a gradual increase in cases during the time frame with a noticeable drop in 2020 during the COVID pandemic. The consistent caseload across years demonstrates the endemic nature of CPV in Nigeria, with notable variations potentially reflecting seasonal outbreaks or increased surveillance efforts.

Table 1: Key breed distribution of dog breeds and sex in the retrospective cohort (2018–2014) (n = 415)

Breed	Female	Male	Total	% of Cohort
German Shepherd	63	57	120	28.9%
Boerboel	63	56	119	28.7%
Rottweiler	24	36	60	14.5%
Other Breeds*	66	50	116	28.0%
Total	216	199	415	100%

Age Distribution by Breed & Sex

Age analysis (Figure 3) revealed that CPV predominantly affected young dogs, with a mean age at presentation of 21.44 ± 22.77 weeks (approximately 5 months). Breed-specific age variations were observed, though statistical significance was limited to Lhasa Apsos, where females presented at a significantly older age (24.36 ± 19.88 weeks) compared to males (12.00 ± 4.00 weeks; t = 2.20, p = 0.04). Mixed-breed dogs showed the widest age distribution, with females presenting at 40.67 ± 30.61 weeks compared to males at 13.75 ± 4.46 weeks, though this difference did not reach statistical significance (t = 2.14, p = 0.09), potentially due to small sample sizes. The consistent pattern of young age at diagnosis across breeds underscores the particular vulnerability of juvenile dogs to CPV infection in the Nigerian context.

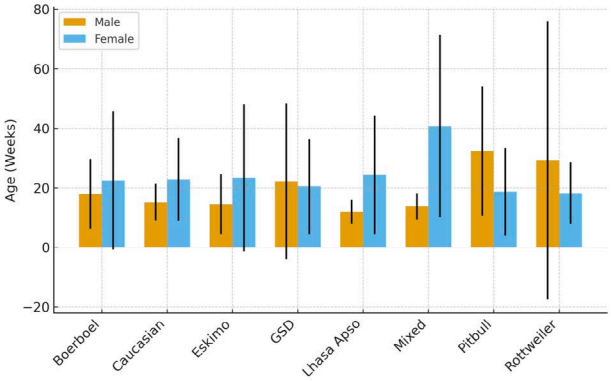

Fig. 3. Box plot showing age variation across major breeds, stratified by sex at presentation (mean ± SD) during the 6-year study at the Faculty of Veterinary Medicine (VTH), University of Ibadan. Lhasa Apsos show significant sex-based age differences (p = 0.04), with females presenting at older ages. Overall, the concentration of cases in young animals (<24 weeks) is evident across all breeds.

Vaccination Status vs. Survival

Complete vaccination and outcome data were available for 338 dogs (81.4% of the cohort). The analysis revealed a profound survival advantage for vaccinated dogs, with 76.6% (49/64) surviving compared to 49.6% (68/137) of

unvaccinated dogs ($\chi^2 = 33.95$, $p < 0.001$), quantitatively demonstrated in Table 2 and visually represented in Figures 4 and 5. This represents a 54% relative increase in survival probability for vaccinated animals. Dogs with unknown vaccination status (n = 137) demonstrated the poorest outcomes, with only 32.9% (45/137) surviving, highlighting the critical importance of documented vaccination history. Breed-specific survival among vaccinated vs. unvaccinated dogs included Boerboels: 14/15 (93.33%) vs. 18/39 (46.15%) ($\chi^2 = 12.81$, $p = 0.002$); German Shepherds: 15/20 (75.00%) vs. 17/29 (58.62%) ($\chi^2 = 15.70$, $p < 0.001$). The differential vaccine responsiveness across breeds is graphically represented in Figure 7, which quantifies the survival advantage conferred by vaccination for each major breed group. Other breeds showed variable χ^2 values ranging from non-significant to approaching significance, with vaccinated (both complete and incomplete) dogs generally exhibiting higher survival percentages. The survival benefit of vaccination was further substantiated by Kaplan-Meier analysis (Figure 5), which demonstrated significantly superior survival probability over time for vaccinated dogs compared to their unvaccinated counterparts (log-rank test: $p < 0.001$). The survival curves diverged early in the clinical course and maintained separation throughout the observation period, indicating that vaccination not only improves initial survival but also provides sustained protection against mortality throughout the disease progression. When examining outcome distribution by vaccination status (Figure 4), vaccinated dogs showed a dramatically different profile, with survival comprising the overwhelming majority of outcomes (76.6%), while unvaccinated dogs were nearly evenly split between survival (49.6%) and mortality (50.4%), reinforcing the substantial protective effect of vaccination against CPV-associated mortality.

Table 2: Vaccination status vs. survival outcomes seen in the retrospective cohort between 2018–2024

Vaccination Status	Survived	Died	Total	Survival Rate
Vaccinated	49	15	64	76.6%
Unvaccinated	68	69	137	49.6%
Unknown Status	45	92	137	32.9%
Total Known	117	84	201	58.2%

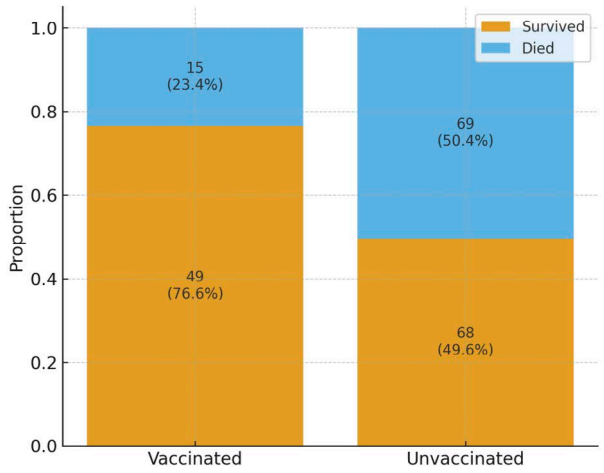

Fig. 4. Stacked bar chart illustrating the distribution of survival outcomes. Vaccinated dogs show a dramatically different outcome profile, with survival comprising 76.6% of cases compared to 49.6% in unvaccinated dogs. The visual emphasizes the substantial protective effect of vaccination against CPV-associated mortality.

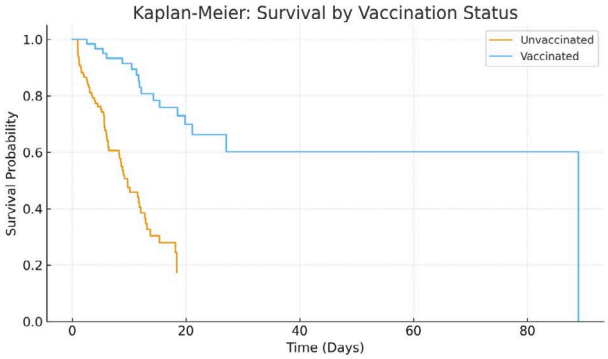

Fig. 5. Kaplan-Meier survival analysis by vaccination status. The survival curve demonstrates significantly superior survival probability over time for vaccinated dogs (blue line) compared to unvaccinated dogs (red line) (log-rank test: p<0.001).

Clinical Presentation Patterns

The clinical presentation of CPV enteritis in this Nigerian cohort aligned with classical descriptions of the disease. Hemorrhagic diarrhea was the most consistently reported clinical sign, present in 89.2% of cases, followed by vomiting (83.1%), lethargy (77.6%), and dehydration (71.3%) (Figure 6). Less common manifestations included hypothermia (18.3%) and abdominal pain (22.7%). The number of clinical signs strongly correlated with overall survival, with dogs exhibiting ≤2 signs showing 64.7% survival compared to 44.1% in those with >2 signs among unvaccinated animals. Hematological abnormalities consistent with CPV pathophysiology were commonly observed, including leukopenia (68.9%), neutropenia

(72.4%), and lymphopenia (65.8%). Thrombocytopenia was noted in 73.3% of cases, potentially contributing to the hemorrhagic manifestations. Biochemical alterations included elevated liver enzymes (AST and ALT) in 42.7% of cases and elevated creatinine in 18.9%, indicating potential hepatic and renal involvement in severe cases.

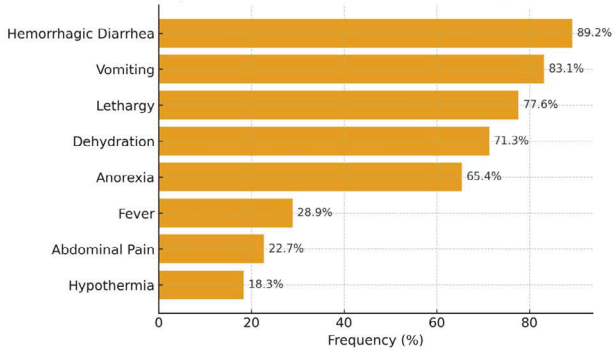

Fig. 6. Bar chart ranking the most common clinical manifestations. Hemorrhagic diarrhea (89.2%) and vomiting (83.1%) were the predominant signs, followed by lethargy and dehydration. This pattern aligns with the classical CPV enteritis presentation and informs early clinical recognition during the cohort study (2018-2024).

Breed-Specific Vaccination Benefits

The protective effect of vaccination was consistent across all major breed groups, though the magnitude of benefit varied (Figure 7 & 8). Boerboels demonstrated the most dramatic vaccination benefit, with 93.3% (14/15) of vaccinated individuals surviving compared to only 46.2% (18/39) of unvaccinated Boerboels ($\chi^2 = 12.81$, $p = 0.002$). This represents a doubling of survival probability with vaccination in this breed. German Shepherds also showed significant vaccination benefits, with 75.0% (15/20) of vaccinated dogs surviving versus 58.6% (17/29) of unvaccinated counterparts ($\chi^2 = 15.70$, $p < 0.001$). Among Pitbulls, though sample sizes were limited, a clear survival advantage emerged for vaccinated individuals, with 100% (1/1) survival in vaccinated dogs compared to variable outcomes in unvaccinated animals ($\chi^2 = 7.77$, $p = 0.021$). Other breeds, including Rottweilers, Caucasians, and Lhasa Apsos, consistently demonstrated higher survival percentages among vaccinated individuals, though statistical significance was limited by smaller subgroup sizes. The consistent direction of effect across all breed groups provides compelling evidence for the universal benefit of vaccination, irrespective of genetic background.

Vaccination Efficacy Across Clinical Severity Strata

To evaluate whether vaccination benefits persisted across varying disease severity, cases were stratified by the number of clinical signs at presentation (Figure 7). Remarkably, vaccination conferred survival advantages in both mild and severe clinical presentations (Figure 7). Among dogs presenting with two or fewer clinical signs (n = 98), representing earlier or milder disease, vaccinated dogs achieved 78.6% (11/14) survival compared to 64.7% (22/34) in unvaccinated dogs ($\chi^2 = 26.75$, $p < 0.001$). This demonstrates that even in less severe cases, vaccination provides a significant additional survival advantage of approximately 14 percentage points. Most notably, in dogs presenting with more than two clinical signs (n = 231), indicating more advanced or severe disease, the vaccination benefit was even more pronounced. Vaccinated dogs in this severe presentation group maintained a 76.0% (38/50) survival rate, dramatically higher than the 44.1% (45/102) survival observed in unvaccinated severely affected dogs ($\chi^2 = 16.39$, $p = 0.003$). This represents a 72% relative increase in survival probability for vaccinated dogs even when presenting with advanced clinical signs (Table 3, Figure 3). The stratified analysis revealed that vaccination provided approximately 32 percentage points of additional survival benefit in severe cases, compared to 14 percentage points in mild cases. This counterintuitive finding suggests that vaccination may provide particularly crucial protection in the most critical cases, potentially by modulating immune responses or reducing viral load during the peak of clinical disease.

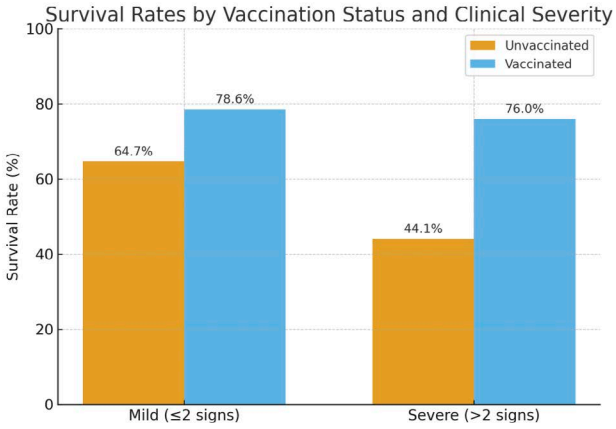

Fig. 7. Survival rates by vaccination status and clinical severity. The grouped bar chart demonstrating vaccination benefits across disease severity strata. Vaccinated dogs maintained high survival rates in both mild (≤2 clinical signs) and severe (>2 clinical signs) presentations. Notably, the survival advantage was most pronounced in severe cases, where vaccination provided a 32 percentage point improvement in survival probability.

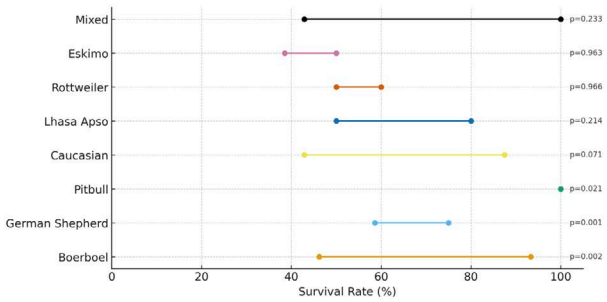

Fig. 8. Forest plot illustrating breed-specific vaccination benefits in the study between 2016–2025. Boerboels show the most dramatic vaccine response (93.3% vs 46.2% survival), while all major breeds demonstrate significant survival advantages with vaccination. Error bars represent 95% confidence intervals.

DISCUSSION

This integrated analysis, employing both a systematic review of the Nigerian literature and a substantial six-year retrospective cohort, provides a robust and nuanced understanding of Canine Parvovirus (CPV) enteritis in Nigeria. The dual-method approach allows for the triangulation of high-level, long-term trends with granular, real-world clinical data, offering insights that are both generalizable and directly actionable for veterinary practice in the region. The findings consistently underscore the endemic nature of CPV, firmly establish key demographic and management-related risk factors, and, most significantly, provide quantitative, stratified evidence of the profound survival benefit conferred by vaccination, even in the face of advanced clinical disease. The epidemiological picture painted by our cohort study is one of persistent, year-round challenge, consistent with the endemic status of CPV described in meta-analytical surveys from Delta and Yola States [3,4]. The steady rise in cases from 2018 to 2024, with a notable dip only during the 2020 pandemic restrictions (Figure 2), demonstrates the virus's relentless circulation and endemicity. The demographic profile of affected dogs further reinforces well-documented risk factors. The mean age at presentation of 21.44 weeks, with a clear concentration of cases in puppies under six months, highlights the persistent vulnerability of juvenile dogs. This window of susceptibility is a perfect storm of waning maternally derived antibodies (MDA) and frequently incomplete or improperly timed primary vaccination series [5]. Our data suggest that current public health messaging and owner compliance are insufficient to bridge this critical immunity gap. Breed predisposition emerged as a dominant theme, with Boerboels and German Shepherds collectively accounting for over 57% of the cases in our cohort (Table 1). This aligns with previous Nigerian studies that identified large purebreds like Rottweilers and Alsatians as disproportionately affected [4]. The reasons are likely multifactorial, involving a complex interplay of genetics, popular kenneling practices that facilitate viral spread, and potentially higher owner investment in seeking advanced care at a teaching hospital, creating a detection bias. However, the near-balanced sex ratio (52.1% female) in our cohort challenges some previous reports of a male bias [3]. This discrepancy suggests that any apparent sex-based difference in infection risk is more likely an artifact of local ownership patterns, sampling methods, or reporting biases rather than an intrinsic biological susceptibility, a conclusion that allows for a refined focus on management over sex-linked genetics.

The cornerstone finding of this study is the unequivocal, quantitative demonstration of vaccination's life-saving efficacy. The overall survival advantage for vaccinated dogs was stark: 76.6% versus 49.6% for unvaccinated dogs ($\chi^2 = 33.95$, $p < 0.001$). This represents a 54% relative increase in the probability of survival and is powerfully visualized in the Kaplan-Meier curve (Figure 5), which shows the survival probabilities diverging early and maintaining a significant gap throughout the clinical course. This data provides the much-needed numerical validation for the qualitative observations and clinical recommendations that have existed in the Nigerian veterinary community for decades. It transforms the assertion that "vaccination is important" into the evidence-based conclusion that vaccination is the single most significant modifiable factor affecting CPV outcome in this setting. Our meta-analysis provides the context for why this efficacy is not universally realized in the field. While commercial live-attenuated vaccines (CPV-2 or CPV-2b) are antigenically capable of cross-protecting against all circulating variants, including the now-predominant CPV-2c [1], their potential is undermined by a cascade of logistical failures. These include, as cited in the literature, cost-related truncated schedules, administration in the face of high MDA titers, and breakdowns in the cold chain [5, 14]. The poor outcomes for the "unknown vaccination status" group in our cohort (32.9% survival) serve as a sobering proxy for this systemic failure; where records are poor, protection is likely absent or inadequate. A novel and critically important insight from

our stratified analysis is that the protective effect of vaccination is not merely a function of preventing severe disease but also of dramatically improving outcomes when severe disease occurs. When we stratified cases by the number of presenting clinical signs, a clear marker of disease severity, a remarkable pattern emerged (Figure 7). In dogs with milder presentations (≤2 signs), vaccination provided a significant, though modest, 14-percentage-point survival advantage (78.6% vs. 64.7%). However, in dogs presenting with more than two signs indicating advanced, systemic illness, the benefit was nothing short of dramatic. Vaccinated dogs in this severe group maintained a 76.0% survival rate, compared to a mere 44.1% in their unvaccinated counterparts, a 32-percentage-point advantage. This counterintuitive finding has profound clinical implications. It suggests that a history of vaccination should not lead to therapeutic nihilism in critically ill patients. Instead, these animals possess a primed immune system that, even when overwhelmed to the point of severe clinical signs, confers a remarkable resilience, potentially by modulating the cytokine storm, facilitating a more rapid clearance of the virus, or mitigating the extent of bacterial translocation and endotoxemia. The breed-specific analysis, elegantly summarized in the forest plot (Figure 8), adds a further layer of nuance. While vaccination was beneficial across all major breeds, the magnitude of the effect was not uniform. The most striking response was observed in Boerboels, where vaccination was associated with a doubling of survival probability (93.3% vs. 46.2%). This indicates that the well-documented breed predisposition in Boerboels is not a fixed fate but a highly modifiable risk. The significant benefits also observed in German Shepherds and Pitbulls (despite low sample representation) reinforce the universal value of vaccination, irrespective of genetic background. This breed-specific efficacy data is invaluable for targeted client education, allowing veterinarians to present compelling, breed-relevant evidence to owners of high-risk dogs.

The clinical presentation in our cohort was classic for CPV enteritis, with hemorrhagic diarrhea (89.2%) and vomiting (83.1%) as the hallmarks (Figure 6). The high prevalence of hematological abnormalities, leukopenia, neutropenia, and a profound thrombocytopenia (73.3%), aligns with the known pathophysiology of the virus and recent reports [20, 21]. These findings underscore the necessity of supportive care, including broad-spectrum antibiotics to counter secondary infections from a compromised gut barrier and neutropenia, and potential plasma transfusions in cases of severe thrombocytopenia. The integration of these clinical and laboratory findings with the vaccination data presents a comprehensive picture of CPV enteritis in Nigeria, from etiology and risk to outcome and mitigation.

Limitations

Several limitations temper our conclusions. The meta-analysis pooled studies with heterogeneous designs, diagnostic criteria (serology versus antigen detection), and temporal spans, introducing potential publication and selection biases. Case definitions varied, and the cyclical nature of CPV outbreaks [3] may have skewed prevalence estimates. The retrospective cohort, while providing detailed clinical correlations, was constrained by missing data points, potential misclassification of vaccination status (especially following owner history), and a single-centre design that may not capture regional epidemiological diversity. The cohort's observational setup means the study can't nail down cause and effect perfectly. Things like exact vaccine timing against MDA, treatment intensity, or owner adherence could sway results without us measuring them. Being from one hospital gives rich details but might not capture Nigeria's full variety. Furthermore, smaller subgroups in breeds or severity levels limited deeper stats.

CONCLUSION

This integrated analysis, combining a systematic review with a six-year retrospective cohort, yields unequivocal evidence of the profound survival benefit conferred by vaccination in Nigerian dogs afflicted with canine parvovirus (CPV). The data demonstrate a stark disparity in outcomes, with vaccinated dogs exhibiting a 76.6% survival rate compared to only 49.6% in unvaccinated animals. This protective effect holds critical clinical relevance as it persists across the entire spectrum of disease severity; even among dogs presenting with advanced illness marked by more than two clinical signs, vaccination was associated with a robust 76.0% survival rate, vastly superior to the 44.1% survival seen in their unvaccinated counterparts. The epidemiological landscape of the infection was characterized by a significant burden among large purebreds such as Boerboels and German Shepherds, with the

former showing a particularly dramatic vaccine response, effectively doubling their survival probability. The disease predominantly affected puppies under six months of age, who consistently presented with the classic clinical signs of hemorrhagic diarrhea, vomiting, lethargy, and dehydration, underscoring their heightened vulnerability. While these findings point to a powerful and clinically meaningful association between vaccination and reduced mortality, the observational nature of this study necessitates a measured interpretation. The outcomes observed are likely influenced by a constellation of factors beyond mere vaccination status, including the precise timing and completeness of the vaccine regimen, interference from maternal antibodies, the quality of supportive care provided, and inherent variations in host immunity. The notably poor outcomes for dogs with unknown vaccination history further highlight how gaps in documentation can mirror the risks of being unvaccinated. Therefore, we assert that vaccination is strongly associated with improved survival, acknowledging that unmeasured confounders prevent a definitive declaration of causation. To translate these findings into actionable public health strategies and reduce the endemic burden of CPV in Nigeria, a concerted, multi-pronged approach is essential. This must include the standardization and digitization of vaccination records to ensure completion of primary and booster series, coupled with optimized vaccine delivery protocols designed to circumvent maternal antibody interference and maintain cold-chain integrity. Concurrently, strengthening veterinary capacity through early-detection training and emergency fluid-therapy protocols, alongside targeted owner education on hygiene and timely vaccination, is crucial. Future efforts should prioritize high-risk groups, including young puppies and susceptible breeds, and explore the broader adoption of modern vaccines engineered to overcome immunity barriers. To solidify this foundation, subsequent research must involve prospective, multi-centre surveillance, ongoing molecular monitoring of circulating variants, and field trials evaluating novel vaccination schedules and pilot intervention programs to build a more resilient defense against this pervasive pathogen.

Conflict of Interest Statement

The authors declare no conflict of interests.

Data Availability Statement

The raw data of this article will be made available by the authors.

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All procedures adhered to established ethical principles for clinical research, ensuring client and patient confidentiality was maintained throughout the study.

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Authors' Contributions

Olanrewaju S. Olaifa conducted the literature review, performed data analysis, drafted the manuscript, extracted and curated meta-analysis data, and contributed to the interpretation of findings. Olatunde B. Akanbi conceived and designed the study, conducted the literature review, extracted and curated meta-analysis data, and contributed to the interpretation of findings. He also reviewed the manuscript. Theophilus A. Jarikre oversaw statistical analyses and critically revised the methods and results sections and reviewed the manuscript. Benjamin O. Emikpe provided mentorship, reviewed and approved the final manuscript. Joseph Busari assisted in data collection.

All authors read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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ORIGINAL ARTICLE

AMELIORATIVE EFFECTS OF PIROXICAM ON ANTHRACENE-INDUCED LUNG TOXICITY IN FEMALE *RATTUS NORVEGICUS*

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

Anthracene present in cigarette smoke may cause tumors of the lungs and other organs. Hence, the therapeutic potential of piroxicam against anthracene-induced lung damage was investigated. Thirty-six female rats, aged seven weeks, were randomly divided into six groups of six rats per group and administered 100 mg/kg of oral anthracene, except the control group that received 2.5 mg/kg of water only, for a period of three consecutive weeks. After 24 hours, the rats were treated daily with different doses of piroxicam, carboplatin, and a combination of both for the next three consecutive weeks. Body weights and blood samples (2 ml) were obtained weekly. After 24 hours, the rats were euthanized with 100 mg/kg intraperitoneal sodium pentobarbitone. The lungs, kidneys, liver, and heart excised and weighed for allometric scaling significantly decreased ($p < 0.05$), except those treated with carboplatin and/or piroxicam. Serum carcinoembryonic antigen increased significantly in the piroxicam-treated group, whereas total oxygen consumption, heart rate, ventilation rate, lung volume, and tidal volume were significantly decreased ($p < 0.05$) in the anthracene-administered groups. Lung width was significantly decreased ($p < 0.05$) in experimental groups. Potential doubling time, growth factor, and cell loss factor were significantly increased ($p < 0.05$). Carboplatin remitted micronodular pulmonary lesions, and piroxicam reduced the lesions.

Keywords: anthracene; amelioration; carboplatin; lung damage; piroxicam; rat

INTRODUCTION

Lung cancer is currently one of the most common and deadliest forms of cancer worldwide. The average life expectancy of patients after diagnosis is typically between three and seven months [1]. Approximately 2.48 million new cases and 1.8 million deaths from lung cancer were reported in 2022, and this burden is projected to increase to 4.62 million new cases and 3.55 million deaths by 2050 [2]. An estimated 18 million cancer cases were recorded worldwide in 2018, including 9.5 million (52.8%) in men and 8.5 million (47.2%) in women, indicating a higher incidence and lower life expectancy among men [3]. In 2008, cancers accounted for an estimated 169.3 million disability-adjusted life years (DALYs) lost globally [4]. African countries recorded approximately 541,800 cancer-related deaths, representing about 0.3% of global cases [5]. In Nigeria, an estimated 100,000 new cancer cases occur annually, with a high case-fatality rate [6]. The country contributed about 15% (102,150 cases) of the 681,000 new cancer cases reported across Africa in 2008 [7]. The incidence of lung cancer in Nigeria is approximately 1.4%, ranking 14th among 35 types of cancers reported in the country [8]. Cigarette smoking, which exposes individuals to polycyclic aromatic hydrocarbons (PAHs) such as anthracene, remains a major risk factor for lung cancer. More than 80% of lung cancer cases are diagnosed after the age of 50 years [9].

Piroxicam is a nonsteroidal anti-inflammatory drug (NSAID) used in both humans and animals to relieve pain and fever and to treat inflammatory and rheumatic disorders, including mastitis [10]. It is highly bound to plasma proteins, has a biological half-life of approximately 50 hours in humans, and is excreted mainly in urine and feces [11]. In addition, piroxicam has been reported to offer protection against cerebral ischemia [12] and to exert hypotensive and sedative effects [13]. The central nervous system (CNS) effects of piroxicam have been attributed to metabolites formed through keto–enol tautomerism [14]. The median lethal dose (LD_{50}) of piroxicam has been reported to be less than 500 mg/kg in mice and rats and less than 1000 mg/kg in guinea pigs, chickens, monkeys, turkeys, and dogs [13]. Piroxicam has also demonstrated anticancer activity against transitional cell carcinoma [15]. Piroxicam suppresses tumor growth and malignant transformation [16] through the inhibition of cyclooxygenase, phosphodiesterase, and protein kinases, which are central to cancer initiation and promotion. It also interferes with transmembrane ion fluxes and cell-to-cell adhesion [17].

Anthracene is a solid polycyclic aromatic hydrocarbon (PAH) consisting of three fused benzene rings, commonly derived from coal tar. It has a molecular weight of 178.23 g/mol [16–18] and is generated during incomplete combustion processes. Human exposure occurs mainly through tobacco smoke and the ingestion of food contaminated with combustion by-products. Anthracene is a three-ring, semi-volatile compound [19], readily biodegraded in soil and susceptible to photodegradation in the presence of light [20]. However, data on its toxicity potential remain limited, although prolonged exposure has been associated with both topical and systemic adverse effects. Carcinogenicity bioassays involving anthracene in humans and experimental models have generally yielded positive results [21].

Cancer therapy over the past five decades has imposed a substantial global economic burden, estimated at over 900 billion USD, surpassing that of cardiovascular diseases [22, 23]. This underscores the need for the discovery of new drugs or the repurposing of existing NSAIDs for the treatment of anthracene-induced lung tumors. Despite the poor prognosis of lung cancer, metastasis and the lack of ideal therapeutic regimens remain major clinical challenges [24]. The present study was therefore designed to evaluate the potential of repurposing piroxicam (an NSAID) for the treatment of anthracene-induced lung tumors, given its long half-life and strong anti-inflammatory and immunomodulatory properties. Female rats were used in this study, as sex-related differences in susceptibility to chemically induced tumors have been reported in several experimental models [25].

MATERIALS AND METHODS

Drugs

Piroxicam (Shanxi Federal Pharmaceutical Co. Ltd., China; NAFDAC No. 04-6809) and carboplatin (German Pharmaceutical Company Ltd., Germany) were used for the experiment. Carcinoembryonic antigen (CEA) assay kits (AccuQink, India) were employed as a model tumor biomarker in this study.

Animals

Thirty-six female rats aged seven weeks and weighing 166.25 g ± 26.7 g were housed under a 12-hour light–dark cycle in the Animal House of the Department of Veterinary Physiology and Biochemistry, College of Veterinary Medicine, Joseph Sarwuan Tarka University, Makurdi, Benue State, Nigeria. Feed and water were provided ad libitum. The animals were cared for in accordance with the guidelines of the College Ethical Committee (Permit No. JOSTUM/CVM/ETHICS/2025/07).

Preparation of piroxicam solution

Approximately 20 g of piroxicam granules obtained from capsules were dissolved in 80 ml of distilled water to prepare a 20% (w/v) solution (equivalent to 200 mg/ml). The resulting preparation was a homogeneous and stable solution with a pH of approximately 6.

Selection of therapeutic doses

The therapeutic doses of piroxicam were selected based on a pilot study conducted in rats. Reported LD_{50} values from the literature were used as reference [13].

Selection of tumor-inducing dose of anthracene

Anthracene was used to induce tumor formation in rats. A dose of 100 mg/kg body weight was selected as the induction dose, corresponding to 2% of the reported oral LD_{50} (>5000 mg/kg body weight) [26]. The compound was administered orally once daily for 21 consecutive days.

Translation of human therapeutic dose to rat dose

The human equivalent dose (HED) formula was employed to extrapolate the therapeutic doses of carboplatin and piroxicam from humans to rats, following the method described in the literature [13].

$$HED\,(mg/kg) = \frac{Animal\ dose\,(mg/kg) \times Animal\ km}{Human\ km} \quad \text{.....(i)}$$

$$Km = \frac{Body\ weight}{Body\ surface\ area} \quad \text{..... (ii)}$$

Calculation of the dose of piroxicam was done according to reported methods [13, 27].

Weight of rats = 166.25 g

Weight of human = 60 kg

Hkm = 37.5

RKm = 6.7

$$HED\,(mg/kg) = \frac{RD\ x\ RKm}{HKm} \quad \text{.....(iii)}$$

RD = rat dose; RKM = rat metabolism constant; HKM = human metabolism constant

$$\frac{RD\,x\,6.7}{37.5}$$

$$\therefore RD\,x\,6.7 = 20\,x\,37.5$$

$$RD = \frac{20\,x\,37.5}{6.7} = 111.9\,mg/kg$$

$$2\%\ of\ therapeutic\ dose = \frac{2}{100}\,x\,111.9 = 2.238 \simeq 2.0\,mg/kg$$

But LD_{50} of piroxicam in rat = 259.4 ± 69.6 mg/kg which is 189.8 to 329.

$$2\%\ of\ LD_{50} = \frac{2}{100}\,x\,\frac{259.4}{1} = 5.188 \simeq 5.0\,mg/kg$$

Hence therapeutic dose of 2–5 mg/kg was chosen for piroxicam treatment.

The therapeutic doses of carboplatin were estimated based on previously reported toxicological data [27, 28]. Considering the reported oral LD_{50} value of 343 mg/kg in rats, 1–2% of this value (equivalent to 3.43–6.86 mg/kg) was selected as a safe and pharmacologically active dose range. Accordingly, two doses of 2.5 mg/kg and 5.0 mg/kg were chosen for comparative evaluation of efficacy and safety.

Experimental induction of lung tumorigenesis

A randomized repeated-measures experimental design was adopted. Thirty-six female rats were randomly assigned to six groups of six animals each. Groups 1, 3, 4, 5, and 6 received oral anthracene (100 mg/kg body weight) daily for three weeks to induce lung tumor formation, whereas Group 2 served as the negative control and received 2.5 mL of distilled water only. Baseline (pretreatment) blood samples (2 mL) were collected from each rat before anthracene or water administration, and additional samples were taken on days 7 and 21. Following the induction phase (day 0–21), the rats were treated orally for 21 consecutive days (days 22–42) as follows: Groups 1 and 2 received distilled water (vehicle control), Groups 3 and 4 received piroxicam (2.5 mg/kg and 5.0 mg/kg, respectively), Group 5 received carboplatin (2.5 mg/kg), and Group 6 received a combination of piroxicam (2.5 mg/kg) and carboplatin (2.5 mg/kg).

Hence 3.43–6.86 mg/kg was calculated. So, we decided to use 2.5 and 5.0 mg/kg for the two for comparative efficacy and safety.

Group	Drugs used
1	Tumor control group (anthracene, water)
2	Normal control group (no anthracene, water)
3	Piroxicam low-dose group (2.5 mg/kg) (anthracene, piroxicam)
4	Piroxicam high-dose group (5.0 mg/kg) (anthracene, piroxicam)
5	Carboplatin group (2.5 mg/kg) (anthracene, carboplatin)
6	Combination therapy group (piroxicam 2.5 mg/kg + carboplatin 2.5mg/kg) (anthracene, piroxicam, carboplatin)

Anthracene administration for tumor induction was discontinued after 21 days. All therapeutic treatments commenced on day 22 of the study and continued for 21 consecutive days. Throughout this period, the normal control group (Group 2) continued to receive distilled water only, without anthracene or drug treatment. On day 42, all animals were humanely euthanized by intraperitoneal injection of sodium pentobarbital (100 mg/kg body weight) for gross pathological and histopathological examination [29–31].

Analysis of tumor biomarker

Serum concentrations of carcinoembryonic antigen (CEA) were determined using an automated chemiluminescent immunoassay, as follows. Serum samples were transferred into individual cuvettes containing superparamagnetic microbeads coated with anti-CEA monoclonal antibodies. CEA in the samples was bound to the antibodies on the surface of the magnetic beads, and a magnetic field was applied to separate bound components from unbound components. Unbound substances were then washed away, and a second anti-CEA antibody labeled with alkaline phosphatase was added. The labeled antibody bound to a different epitope on the CEA molecule, forming a sandwich complex. The magnetic beads were again separated magnetically, and unbound labeled antibodies were removed by washing. Substrate reagents were then added to initiate a chemiluminescent reaction catalyzed by alkaline phosphatase, resulting in light emission. The light intensity was measured using a luminometer. The assay was performed using the AccuBind CEA chemiluminescent ELISA kit (Monobind Inc., USA). The analytical sensitivity (limit of detection) of the assay was 0.5 ng/mL, as specified by the manufacturer. Reference intervals for CEA were defined as follows: <3.0 ng/mL (normal, non-smokers), ≥5.0 ng/mL (abnormal), 5–10 ng/mL (slightly elevated), 10–20 ng/mL (significantly elevated), and >20 ng/mL (indicative of metastasis) [32]. Precision and performance characteristics, including repeatability, reproducibility, accuracy, linearity, analytical range, limit of detection, interference, specificity, carry-over, and reference interval verification, were validated according to the manufacturer's guidelines. All analyses were performed in triplicate.

Allometric scaling of pathophysiological and tumor parameters

The micronodular lesions, weight, length, width, and volume of the left and right lungs of the experimental rats were measured using a metric ruler and electronic balance. Allometric relationships between organ size and body weight were analyzed using the general allometric equation: $Y = aM^b$, where Y is the biological variable (e.g., organ weight or volume), M is the measure of body size (body weight), a is a proportionality constant, and b is the allometric scaling exponent. The weights of the kidneys, liver, spleen, and heart were also determined using an electronic analytical balance [33, 34].

Lung tumor kinetic parameters

Tumor growth parameters were calculated mathematically according to the method described by Saganuwan [34]. The parameters determined included tumor radius, volume, weight, doubling time, specific growth rate, cell proliferation rate (cells/day), labeling index, growth fraction, potential doubling time, and cell loss factor. These parameters were computed using the following equations [34].

$$Tumor\ volume\ (cm^3) = \frac{4}{3} x \pi x r^3 \quad \text{.....(iv)}$$

Where $\pi = 3.14159$ ' $r = \frac{d}{2}$, r = radius; d = diameter

$$Tumor\ volume\ index\ (V, mm^3) = \frac{a \times b^2}{2} \quad \text{.....(v)}$$

where a = tumor length (mm) and b = tumor width (mm). Tumor doubling time (per day) = 1g of tumor mass = 10^9 cells = 30 doubling time

$$Specific\ Growth\ Rate\ (SGR)\ (\%/day) = \frac{ln_2 x\ 100}{DT} \quad \text{.....(vi)}$$

DT = tumor doubling time; $ln_2 = 0.6931$

$$Cell\ cycle\ per\ day\ (day) = \frac{TW\ x\ 10^9}{1000} \quad \text{..... (vii)}$$

TW = tumor weight

$$Growth\ factor = \frac{P}{Q+P} \quad \text{(viii)}$$

$$Cell\ loss\ factor\ (\varphi, \%) = \frac{1 - DTpot}{DT} \quad \text{(ix)}$$

$$Potential\ doubling\ time\ (Tpot, days) = \frac{Tc}{GF} \quad \text{(x)}$$

where Tc = duration of the cell cycle (days), and GF = growth fraction (P/(P+Q)).

$$Tumor\ \log_{10}\ cell\ kill = \frac{T - C}{3.32\ XTd} \quad \text{(xi)}$$

Tc = duration of the cell cycle constant = 3.32; T–C = difference in time (days) for a treated tumor size compared to untreated (control); Td = tumor doubling time, P = proliferating cells; Q = quiescent cells.

Determination of metabolic and cardiorespiratory parameters

Oxygen consumption, lung ventilation rate, lung volume, tidal volume, blood volume, respiratory frequency, heart rate, and organ weights (lungs and heart) were estimated using allometric equations derived from body mass data obtained in this study [35].

Metabolic rate (Kcal/day): 3.52 $W^{0.75}$ (where W = body weight in kilograms) ..... (xii)

Total O_2 consumption, V_{02} (liter O_2 h^{-1}: 0.676 x $Mb^{0.75}$ (where Mb = body mass in kilogram) ..... (xiii)

Body mass is the total amount of matter in the body measured in kilograms.

O_2 consumption per kilogram (liter h^{-1} kg):

0.676 x $Mb^{-0.25}$ ..... (xiv)

Heart rate (min^{-1}): 241 x $Mb^{0.25}$ ..... (xv)

Lung ventilation rate (liter h^{-1}): 20.0 x $Mb^{0.75}$ ..... (xvi)

Lung volume (liter): 0.063 x $Mb^{1.02}$ ..... (xvii)

Tidal volume (liter): 0.0062 x $Mb^{1.01}$ ..... (xviii)

Respiratory frequency (min^{-1}): 53.5 x $Mb^{-0.26}$ ..... (xix)

The calculated parameters were verified to ensure consistency with the established allometric equations.

Statistical analyses

The modified Kaplan-Meier estimate

$$\equiv \frac{Number\ of\ surviving\ tumor\ cells - Number\ of\ dead\ tumor\ cells}{Number\ of\ surviving\ tumor\ cells}$$

was used, which relates the number of surviving and dead tumor cells [34]. Data were expressed as mean ± standard error of mean (SEM). Differences between groups and time points were analyzed using two-way repeated-measures analysis of variance (ANOVA), followed by the least significant difference (LSD) post hoc test to determine pairwise differences at a 5% significance level ($p < 0.05$). Statistical analyses were performed using standard formulas as described by Saganuwan [36].

RESULTS

Body and organ weights

The body weight of the female rats induced with anthracene and treated with high-dose piroxicam (Group 4) was significantly higher at day 42 in Group 4 ($p < 0.05$) on day 42 (162.97 ± 4.15 g) compared with Groups 1 (150.62 ± 2.39 g), 2 (138.89 ± 1.15 g), 3 (136.61 ± 2.57 g), 5 (148.30 ± 2.25 g), and 6 (141.53 ± 1.95 g) (Table 1). The mean weight of the lungs was significantly greater ($p < 0.05$) in Group 6 (0.87 ± 0.03 g) compared with Groups 1 (0.62±0.10 g), 2 (0.55 ± 0.06 g), 3 (0.74 ± 0.11 g), 4 (0.64 ± 0.10 g), and 5 (0.73 ± 0.06 g). Similarly, the mean weights of the kidneys (0.61 ± 0.04 g), liver (3.82 ± 0.81 g), spleen (1.16 ± 0.00 g), and heart (0.64 ± 0.00 g) in Group 6 were significantly higher ($p < 0.05$) compared with Group 2 (kidneys: 0.47 ± 0.03 g; liver: 2.93 ± 0.29 g; spleen: 1.13 ± 0.31 g; heart: 0.51 ± 0.03 g). Among the organs, the liver had the greatest mean weight, followed by the spleen, lungs, heart, and kidneys (Table 2).

Lung morphology and micronodular lesions

The effects of anthracene and piroxicam on lung dimensions and micronodular lesions in female rats are summarized in Table 3.

In all groups, the left lungs were generally longer than the right lungs ($p < 0.05$), with mean left lung lengths ranging from 2.10 ± 0.10 cm (Group 6) to 2.67 ± 0.33 cm (Group 1). Right lung lengths ranged from 1.75 ± 0.15 cm (Group 6) to 2.15 ± 0.35 cm (Group 3). Differences in lung width followed a similar pattern, with slightly larger values recorded for the left lung in most groups.

The dimensions of pulmonary micronodules were significantly greater ($p < 0.05$) in Groups 1 and 2 compared with all treated groups. In contrast, micronodule size was markedly reduced in Groups 3, 4, and 5 and completely

Table 1. Effects of anthracene and piroxicam on body mass (g) of female rats

Group number	Experimental Groups	Days of Treatments				
		0	7	21	35	42
1.	Induced not treated Water (2.5ml)	149.36±1.68^{bd}	150.62±1.66^{bd}	162.91±1.58^{ab}	148.60±3.66^{bd}	150.62±2.39^{bd}
2.	Neither induced nor treated Water(2.5ml)	145.83±1.81^{bd}	148.85±1.83^{ad}	146.32±1.98^{bd}	135.65±1.30^{bd}	138.89±1.15^{bd}
3.	Induced and treated Piroxicam (2.5mg/kg)	147.46±1.06^{bd}	150.12±0.96^{ad}	147.21±1.95^{bd}	135.98±2.50^{bd}	136.61±2.57^{bd}
4.	Induced and treated Carboplatin (2.5mg/kg) Piroxicam (2.5mg/kg)	147.97±1.01^{bd}	150.74±1.01^{bd}	134.02±2.81^{bd}	155.07±3.86^{bc}	162.97±4.15^{ac}
5.	Induced and treated Piroxicam (5mg/kg)	152.13±1.67^{bc}	154.25±1.65^{ac}	180.62±3.20^{ac}	148.45±2.17^{bd}	148.30±2.25^{bd}
6.	Induced and treated Carboplatin(2.5mg/kg)	151.75±0.92^{bd}	153.51±0.94^{ad}	142.63±1.43^{bd}	137.39±1.69^{bd}	141.53±1.95^{bd}

Keys: a = significantly higher along the row ($p < 0.05$); b = significantly lower along the row ($p < 0.05$); c = significantly higher along the column ($p < 0.05$); d = significantly lower along the column ($p < 0.05$); Mass = is the amount of matter in a material (e.g body)

Table 2. Weights (g) of some internal organs of the female rats treated with anthracene and piroxicam

Group number	Experimental Groups	Internal Organs (g)				
		Lung	Kidney	Liver	Spleen	Heart
1.	Induced not treated Water (2.5ml)	0.62±0.10^{bd}	0.54±0.04^{bd}	3.92±1.06^{ac}	0.64±0.08^{bd}	0.59±0.10^{bd}
2.	Neither induced nor treated Water(2.5ml)	0.55±0.06^{bd}	0.47±0.03^{bd}	2.93±0.29^{ad}	1.13±0.31^{bd}	0.51±0.03^{bd}
3.	Induced and treated Piroxicam (2.5mg/kg)	0.74±0.11^{bd}	0.54±0.03^{bd}	2.96±0.24^{ad}	0.97±0.21^{bd}	0.62±0.06^{bd}
4.	Induced and treated Carboplatin (2.5mg/kg) Piroxicam (2.5mg/kg)	0.64±0.10^{bd}	0.48±0.08^{bd}	2.97±0.37^{ad}	0.91±0.04^{bd}	0.65±0.14^{bc}
5.	Induced and treated Piroxicam (5mg/kg)	0.73±0.06^{bd}	0.49±0.04^{bd}	2.81±0.11^{ad}	1.07±0.21^{bd}	0.52±0.14^{bd}
6.	Induced and treated Carboplatin (2.5mg/kg)	0.87±0.03^{bc}	0.61±0.04^{bc}	3.82±0.81^{ad}	1.16±0.00^{bc}	0.64±0.00^{bd}

Keys: a = significantly higher along the row ($p < 0.05$); b = significantly lower along the row ($p < 0.05$); c = significantly higher along the column ($p < 0.05$); d = significantly lower along the column ($p < 0.05$)

Table 3. Dimensions of lung and micronodular lesions of rats administered anthracene and treated with piroxicam and carboplatin

Group number	Experimental Groups	Lung Parameters (cm)				
		Right lung	Left lung	Width of right lung	Width of left lung	Nodule
1.	Induced not treated Water (2.5ml)	2.00±0.19^{bd}	2.67±0.33^{ac}	1.23±0.23^{ad}	1.50±0.25^{bd}	0.28±0.09^{bc}
2.	Neither induced nor treated Water (2.5ml)	2.10±0.20^{bd}	2.30±0.30^{ad}	1.45±0.45^{bd}	1.45±0.35^{bd}	0.28±0.04^{bc}
3.	Induced and treated Piroxicam (2.5mg/kg)	2.15±0.35^{bc}	2.50±0.10^{ad}	1.45±0.05^{bd}	1.60±0.10^{bc}	0.20±0.04^{bd}
4.	Induced and treated Carboplatin (2.5mg/kg) Piroxicam (2.5mg/kg)	2.15±0.05^{bd}	2.25±0.05^{ad}	1.10±0.10^{bd}	1.45±0.05^{bd}	0.24±0.04^{bd}
5.	Induced and treated Piroxicam (5mg/kg)	2.05±0.05^{bd}	2.15±0.15^{ad}	1.05±0.05^{bd}	1.20±0.10^{bd}	0.20±0.06^{bd}
6.	Induced and treated Carboplatin (2.5mg/kg)	1.75±0.15^{bd}	2.10±0.10^{ad}	1.65±0.15^{bc}	1.50±0.00^{bd}	0.00±0.00^{bd}

Keys: a = significantly higher along the row ($p < 0.05$); b = significantly lower along the row ($p < 0.05$); c = significantly higher along the column ($p < 0.05$); d = significantly lower along the column ($p < 0.05$)

absent in Group 6, indicating a dose-dependent and synergistic effect of piroxicam and carboplatin on regression of lung lesions (Table 3).

Tumor kinetic parameters

The calculated tumor-kinetic parameters are summarized in Table 4. Rats in Group 1 (anthracene only, no treatment) exhibited the largest tumor dimensions, with a mean tumor diameter of 2.8 ± 0.9 mm, volume of 1149.1 ± 38.2 mm^3, and weight of 548.8. ± 18.2 mg. These values were significantly higher ($p < 0.05$) than those recorded in the treatment groups.

In comparison, rats treated with low-dose piroxicam (Group 3) showed markedly smaller tumors (radius 1.0 ± 0.2 mm; volume 418.8 ± 3.4 mm^3; weight 200.0 ± 1.6 mg), while those treated with high-dose piroxicam (Group 5) or 2.5 mg/kg carboplatin (Group 4) exhibited intermediate values. The combination treatment (Group 6) produced complete tumor regression, with no measurable tumor mass.

Tumor doubling time (TDT) was longest in Group 1 (164.6 ± 5.5 days) and shortest in the treated groups (60–104 days), whereas specific growth rate (SGR) and cell production per day followed the reverse trend.

Calculated potential doubling time was approximately 5.1 days for all groups except Group 6, which showed complete remission (0 days). Tumor cell kill was estimated at 0% for Groups 1 and 2, 14.3% for Group 4, 28.6% for Groups 3 and 5, and 100% for Group 6. The theoretical growth factor was 0.59 for all groups except Group 6 (0.00). The cell loss factor was estimated at 2.4% for Groups 1 and 2, 3.4% for Group 4, 6.8% for Groups 3 and 5, and 0% for Group 6.

Carcinoembryonic antigen (CEA)

Serum levels of carcinoembryonic antigen (CEA) increased significantly ($p < 0.05$) in all rats treated with anthracene by day 21 compared with the normal control group (Table 5). The mean CEA concentrations at day 21 and day 42 were as follows: Group 1 (2.48 ± 0.72 vs. 1.40 ± 0.18 µg/mL), Group 2 (3.51 ± 0.07 vs. 3.37 ± 0.06 µg/mL), Group 3 (4.08 ± 0.12 vs. 3.76 ± 0.11), Group 4 (3.59 ± 0.48 vs. 2.67 ± 0.08 µg/mL), Group 5 (2.59 ± 0.89 vs. 1.87 ± 0.46), and Group 6 (3.92 ± 0.07 vs. 2.78 ± 0.30 µg/mL. By day 42, CEA concentrations had generally returned to near pretreatment (baseline) levels, indicating partial or complete remission of anthracene-induced lesions following piroxicam and carboplatin treatment.

Cardiorespiratory parameters

Oxygen consumption was significantly higher ($p < 0.05$) on day 21 in group 5 (346.8 ± 6.14 $L/h^{-1}Kg^{-1}$) in comparison to group 1 (312.8 ± 30.4 mLh^{-1}), 2 (281.0 ± 38.1 mLh^{-1}), 3 (282.7 ± 37.4 mLh^{-1}), 4 (266.9 ± 53.8 mLh^{-1}), and 6 (273.9 ± 27.4 mLh^{-1}), respectively. Total oxygen consumption was significantly increased ($p < 0.05$) on day 42 in group 2 (30.00 ± 0.00 mL/kg/min) as compared to groups 1, 3, 4, 5 (10.00 ± 0.00 mL/kg/min), and 6 (20.00 ± 0.00 mL/kg/min), respectively. Heart rate was significantly increased ($p < 0.05$) on day 42 in group 4 (865.38 ± 5.48 beats/min) as compared to groups 1 (842.95 ± 4.56 beats/min), 2 (820.46 ± 3.57 beats/min), 3 (815.95 ± 4.67 beats/min), 5 (838.59 ± 4.47 beats/min), and 6 (825.64±4.26 beats/min), respectively. Ventilation rate was significantly higher ($p < 0.05$) on day 42 in group 4 (925.98 ± 235.51 l/h) in comparison to that of rats in group 1 (855.82 ± 136.2 l/h), 2 (789.15 ± 65.26 l/h), 3 (776.21 ± 145.90 l/h), 5 (842.62 ± 128.06 l/h), and 6 (804.17 ± 110.69 l/h), respectively. Lung volume was significantly increased ($p < 0.05$) on day 42 in group 4 (11.60 ± 1.80 ml) as compared to groups 1 (10.42 ± 0.86 ml), 2 (9.33 ± 0.31 ml), 3 (9.13 ± 0.94 ml), 5 (10.20 ± 0.78 ml), and 6 (9.57 ± 0.65 ml), respectively. Nevertheless, tidal volume was significantly increased ($p < 0.05$) in group 4 (1.08 ± 0.17 ml) in comparison with groups 1 (0.97 ± 0.08 ml), 2 (0.87 ± 0.03 ml), 3 (0.85 ± 0.17 ml), 5 (0.95 ± 0.07 ml), and 6 (0.89 ± 0.62 ml), respectively. Meanwhile, frequency of respiration increased significantly ($p < 0.05$) on day 42 from 1.56 ± 0.09 to 2.87 ± 0.09 in group 2 and decreased significantly in group 1 from 1.72 ± 0.09 to 1.07 ± 0.79 sec^{-1}, group 3 from 1.19 ± 0.09 to 0.98 ± 0.09 sec^{-1}, 4 (from 1.41 ± 0.09 to 0.52 ± 0.09 sec^{-1}), 5 (from 1.74 ± 0.09 to 1.17 ± 0.09 sec^{-1}, and 6 (from 1.86 ± 0.09 to 1.42 ± 0.10 sec^{-1}), respectively (Table 6).

DISCUSSION

Body and organ weights

The decreased body weight of the animals observed during the study shows that either anthracene or piroxicam can cause a decrease in body weight gain of female

Table 4. Kinetic parameters of anthracene-induced lung tumor in rats

Group Number	Experimental Groups	Diameter (mm)	Radius (mm)	TV (mm3)	TW (mg)	Kinetic parameter of lung tumor							
						TDT (day)	SGR (%/d)	CCD (day)	GF	TCK (%)	TQCs	TCP	DTpot (day)	CLF (%)
1.	Induced treated with water (2.5mg/kg)	2.8±0.9^{a}	1.4±0.5^{a}	1149.1±38.2^{a}	548.8±18.2^{a}	164.6±5.5^{a}	0.42^{a}	5.5x10^{8a}	0.59	0.0	1.96x10^{8}	2.8X10^{8}	5.1	2.4
2.	Non-induced treated with water (2.5mg/kg)	2.8±0.4	1.4±0.2	1149.1±3.4	548.8±1.6	164.6±0.5	0.42	5.5x10^{8b}	0.59	0.0	1.96x10^{8}	2.8X10^{8}	5.1	2.4
3.	Induced treated piroxicam (2.5mg/kg)	2.0±0.4^{b}	1.0±0.2^{b}	418.8±3.4^{b}	200.0±1.6^{b}	60.0±0.5^{b}	1.2^{b}	2 x10^{8b}	0.59	28.6	1.4x10^{8}	2.0X10^{8}	5.1	6.8
4.	Induced treated carboplatin (2.5mg/kg), piroxicam (2.5mg/kg)	2.4±0.4^{b}	1.2±0.2^{b}	723.6±3.4^{a}	345.6±1.6^{b}	103.7±0.5^{b}	0.7^{b}	3.5x10^{8b}	0.59	14.3	1.68x10^{8}	2.4X10^{8}	5.1	3.4
5.	Induced treated piroxicam (5mg/kg)	2.0±0.6^{b}	1.0±0.3^{b}	418.8±11^{b}	200.0±5.4^{b}	60.0±1.6^{b}	1.2^{b}	2 x10^{8b}	0.59	28.6	1.4x10^{8}	2.0X10^{8}	5.1	6.8
6.	Induced treated carboplatin (2.5mg/kg)	0.0±0.0^{b}	0.0±0.0^{b}	0.00±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}	0.0^{b}	0.0x10^{8b}	0.00	100	0.0	0.0	0.0	0.0

Keys: TV = tumor volume; TW = tumor weight; TDT = tumor doubling time; SGR = specific growth rate; CCD = cell cycle per day; TCK = tumor cell kill; GF = growth factor; CLF = cell loss factor; DTpot = doubling time potential; a= significantly higher along the column ($p>0.05$); b = significantly lower along the column ($p < 0.05$); 1g of tumor cells = 1 cm = 109 tumor cells; TDT for adenocarcinomas = 170 days; TCP = tumor cells population; period of induction = 21 days; In every 1000 lung tumor cells 600 to 800 are quiescent, hence 700 cells were chosen in the present study; TQCs = tumor quiescent cells; Tc = duration of cell cycle = 2–4 days, hence 3 days was chosen. Tumor cell kill was calculated with reference to tumor size and control group.

rats, which could be attributed to systemic toxicity, metabolic alteration, and reduced appetite. This agrees with the report indicating that piroxicam is antiproliferative [37]. However, the administration of anthracene/piroxicam increased weight gain, suggesting an antagonistic effect which is hormetic. Piroxicam affects the synthesis of DNA, which in turn affects protein synthesis [38]. The increased organ weight gain observed for the carboplatin as compared to the piroxicam-treated group shows that carboplatin may cause weight gain [39]. Decreased body mass caused by anthracene and carboplatin shows that the drugs could be used against obesity. The effects could be drug and dose-dependent. However, the increased body mass observed in the group administered carboplatin/piroxicam suggests that administration of the drugs could be used for management of weight loss [40].

The effects of anthracene, carboplatin, and piroxicam on morphological parameters of rat lung tumor

Presence of nodular lesions observed in all the groups except the group administered carboplatin only shows that group administered carboplatin could be used to treat lung micronodular lesions caused by anthracene. However, 5 mg/kg of piroxicam or 2.5 mg/kg of piroxicam in combination with carboplatin (2.5 mg/kg) reduced the micronodular lesion, which translates to decreased tidal volume and frequency of respiration observed in the present study [41]. This finding agrees with the report indicating that combination therapy that includes carboplatin (2.5 mg/kg) could improve survivability in lung cancer [42]. Calculation of carboplatin dose based on estimating glomerular filtration rate (eGFR) provides optimal therapeutic effect [43].

The effects of anthracene, piroxicam, and carboplatin on the kinetics of lung tumors

The tumor remission caused by piroxicam is corroborated by the report of Knapp et al., indicating that piroxicam has antitumor activity that may not be directly cytotoxic [44]. Also, the lung tumor remission caused by piroxicam/carboplatin in the present study agrees with the report indicating that the combination could induce tumor remission [45]. Hence, a combination of carboplatin (2.5 mg/kg) and piroxicam (2.5 mg/kg) could be highly beneficial in the management of lung cancer. Therefore, small cell carcinoma of the bladder and lung may have a convergent but different pathogenesis [46], suggesting pros-

Table 5. Effects of anthracene and piroxicam on carcinoembryonic antigen (CEA) of female rats

Group number	Experimental Groups	Carcinoembryonic antigen (µg/ml)		
		0	21	42
1.	Induced not treated Water (2.5ml)	1.40 ± 0.18^{bd}	2.48 ± 0.72^{ad}	1.40 ± 0.18^{bd}
2.	Neither induced nor treated Water (2.5ml)	3.37 ± 0.06^{bd}	3.51 ± 0.07^{ad}	3.37 ± 0.06^{bd}
3.	Induced and treated Piroxicam (2.5mg/kg)	3.76 ± 0.12^{bc}	4.08 ± 0.12^{ac}	3.76 ± 0.11^{bc}
4.	Induced and treated Carboplatin (2.5mg/kg) + Piroxicam (2.5mg/kg)	2.67 ± 0.08^{bd}	3.59 ± 0.48^{ad}	2.67 ± 0.08^{bd}
5.	Induced and treated Piroxicam (5mg/kg)	1.87 ± 0.47^{bd}	2.59 ± 0.89^{ad}	1.87 ± 0.46^{bd}
6.	Induced and treated Carboplatin (2.5mg/kg)	2.78 ± 0.30^{bd}	3.92 ± 0.07^{ad}	2.78 ± 0.30^{bd}

Keys: a = significantly higher along the row ($p < 0.05$); b=significantly lower along the row ($p < 0.05$); c = significantly higher along the column ($p < 0.05$); d = significantly lower along the column ($p < 0.05$)

taglandin-dependent and independent cancer suppression potential of piroxicam [47], thereby reducing edema [48]. Substitution on the carboxamide nitrogen with a heteroanyl group gives piroxicam sevenfold more anti-inflammatory activity than the anyl group substitution [49]. Piroxicam (3.5 mg/kg) provided a longer therapeutic effect with a high safety margin [50], suggesting that hepatoma and lung adenomas caused by anthracene in animals [51] could be ameliorated by piroxicam. The most common lung tumors are carcinomas with the diameter size of ≤ 1 cm [52] and doubling time of 170 days [53] as observed in the group of rats (164.6 ± 5.5 days). The ability of carboplatin to ameliorate lung toxicity caused by anthracene disagrees with the report indicating that resistance has been developed against carboplatin [54]; modifications of functional groups of carboplatin at position 3 of the cyclobutane ring improve anticancer activity [55]. Moreso, the mechanism of action of carboplatin via platinum-adduct formation and that of piroxicam via cyclooxygenase enzyme inhibition suggest that a high dose of carboplatin is required to attack DNA; hence, carboplatin is highly tolerated [56], causing allodynia and cold hyperalgesia [57]. Therefore, carboplatin may be relatively safe, effective, and deliverable in the growing animals [58]. Induced carboplatin binding in phospholipid increased its anticancer activity [59].

Carcinoembryonic antigen

The carcino-embryonic antigen CEA is a diagnostic and prognostic tumor biomarker used for monitoring cancer initiation and progression [60]. Therefore, CEA is used for detection and staging of cancer. It is a glycoprotein biomarker used for monitoring gastrointestinal, colorectal, lung, and mammary tumors [61]. High CEA levels have been observed in epithelial tumors and 40–48% of non-small cell lung cancer, with higher sensitivity in advanced cancer [62, 63], suggesting that increased CEA in the present study may suggest lung tumor progression, connoting that numerous biomarkers are required for detection of lung tumors at an early stage [64].

Effect of anthracene, carboplatin, and piroxicam on cardiorespiratory parameters of rats

The decreased total oxygen consumption per kilogram, lung ventilation rate, and tidal volume, which are suggestive of dyspnea and hypoxemia observed in this study, agrees with the report that recommended oxygen for lung cancer patients [65]. However, oxygen use is not recommended for patients with advanced lung carcinoma [66], but adjuvant chemotherapy could cause linear peak oxygen consumption, as observed in the group administered carboplatin/piroxicam [67]. Tidal volume is a predictor of pulmonary complication in lung surgery [68]. Increased and decreased heart rate observed in the study agrees with the report of Franklin et al., which indicated that heart rate is a predictor of survival in non-small cell lung cancer and as such represents a therapeutic target as observed in the piroxicam-treated group [69]. Findings have shown that anthracene has very high potential to cause lung damage

Table 6. Effects of anthracene and piroxicam on total oxygen consumption, oxygen consumption and heart rate of female rats

Group No.	Experimental Groups	Oxygen consumption (mLh^{-1})				
		0	7	21	35	42
1.	Induced not treated Water (2.5ml)	286.8±32.4^{bd}	289.3±31.8^{bd}	312.8±30.4^{ad}	285.4±47.6^{bd}	289.3±46.1^{bd}
2.	Neither induced nor treated Water (2.5ml)	280.7±34.8^{bd}	285.8±35.3^{ad}	281.0±38.1^{bd}	260.5±25.1^{bd}	266.7±22.1^{bd}
3.	Induced and treated Piroxicam (2.5mg/kg)	283.2±20.4^{bd}	288.3±18.4^{ad}	282.7±37.4^{bd}	261.2±48.1^{bd}	262.4±49.3^{bd}
4.	Induced and treated Carboplatin (2.5mg/kg) + Piroxicam (2.5mg/kg)	284.2±19.4^{bd}	289.5±19.4^{bd}	266.9±53.8^{bd}	297.8±74.1^{bc}	312.9±79.6^{ac}
5.	Induced and treated Piroxicam (5mg/kg)	292.2±32.1^{bc}	296.2±31.7^{bc}	346.8±61.4^{ac}	285.1±41.7^{bd}	284.8±43.3^{bd}
6.	Induced and treated Carboplatin (2.5mg/kg)	291.4±17.6^{bd}	294.8±18.1^{ad}	273.9±27.4^{bd}	263.8±32.5^{bd}	271.8±37.4^{bd}
		Total oxygen consumption (mL/Kg/min)				
1.	Induced not treated Water (2.5ml)	20.00±0.00^{ad}	20.00±0.00^{ad}	20.00±0.00^{ad}	10.00±0.00^{bd}	10.00±0.00^{bd}
2.	Neither induced nor treated Water (2.5ml)	10.00±0.00^{bd}	20.00±0.00^{bd}	20.00±0.00^{bd}	30.00±0.00^{bc}	30.00±0.00^{ac}
3.	Induced and treated Piroxicam (2.5mg/kg)	40.00±0.00^{ad}	40.00±0.00^{ad}	20.00±0.00^{bd}	10.00±0.00^{bd}	10.00±0.00^{bd}
4.	Induced and treated Carboplatin (2.5mg/kg) + Piroxicam (2.5mg/kg)	40.00±0.00^{ad}	40.00±0.00^{ad}	10.00±0.00^{bd}	10.00±0.00^{bd}	10.00±0.00^{bd}
5.	Induced and treated Piroxicam (5mg/kg)	20.00±0.00^{ad}	20.00±0.00^{ad}	10.00±0.00^{bd}	10.00±0.00^{bd}	10.00±0.00^{bd}
6.	Induced and treated Carboplatin (2.5mg/kg)	50.00±0.00^{ac}	50.00±0.00^{ac}	30.00±0.00^{bc}	20.00±0.00^{bd}	20.00±0.00^{bd}
		Heart rate (beats/min)				
1.	Induced not treated Water (2.5ml)	840.58±4.06^{bd}	842.95±4.04^{bd}	865.26±3.97^{ad}	839.16±4.61^{bd}	842.95±4.56^{bd}
2.	Neither induced nor treated Water (2.5ml)	834.60±4.16^{bd}	839.63±4.18^{ad}	834.85±4.28^{bd}	814.04±3.73^{bd}	820.46±3.57^{bd}
3.	Induced and treated Piroxicam (2.5mg/kg)	837.01±3.48^{bd}	842.00±3.36^{ad}	836.54±4.26^{bd}	814.71±4.63^{bd}	815.95±4.67^{bd}
4.	Induced and treated Carboplatin (2.5mg/kg) + Piroxicam (2.5mg/kg)	837.97±3.42^{bd}	843.17±3.42^{bd}	820.72±4.81^{bd}	851.17±5.35^{bc}	865.38±5.48^{ac}
5.	Induced and treated Piroxicam (5mg/kg)	845.75±4.04^{bc}	849.67±4.03^{bc}	895.54±5.02^{ac}	838.87±4.42^{bd}	838.59±4.47^{bd}
6.	Induced and treated Carboplatin (2.5mg/kg)	845.05±3.31^{bd}	848.28±3.34^{ad}	827.76±3.84^{bd}	817.50±4.06^{bd}	825.64±4.26^{bd}
		Ventilation rate (liter h^{-1})				
1.	Induced not treated Water (2.5ml)	848.65±95.94^{bd}	855.82±94.33^{bd}	925.60±90.01^{ad}	844.34±140.78^{bd}	855.82±136.21^{bd}
2.	Neither induced nor treated Water (2.5ml)	830.65±103.23^{bd}	845.76±104.53^{ad}	831.38±112.81^{bd}	770.75±74.26^{bd}	789.15±65.26^{bd}
3.	Induced and treated Piroxicam (2.5mg/kg)	837.76±60.24^{bd}	852.94±54.33^{ad}	836.44±110.69^{bd}	772.66±142.26^{bd}	776.21±145.91^{bd}
4.	Induced and treated Carboplatin (2.5mg/kg) + Piroxicam (2.5mg/kg)	840.76±57.41^{bd}	856.50±57.52^{bd}	789.89±159.39^{bd}	881.12±219.23^{bc}	925.98±235.51^{ac}
5.	Induced and treated Piroxicam (5mg/kg)	864.38±94.86^{bc}	876.45±93.79^{bd}	1026.22±181.79^{ad}	843.48±123.51^{bd}	842.62±128.06^{bd}
6.	Induced and treated Carboplatin (2.5mg/kg)	862.24±52.27^{bd}	872.19±53.57^{ad}	810.38±81.13^{bd}	780.63±96.20^{bd}	804.17±110.69^{bd}

				Lung volume (ml)		
1.	Induced not treated Water (2.5ml)	10.30±0.53^{bd}	10.42±0.52^{bd}	11.59±0.48^{ad}	10.23±0.89^{bd}	10.42±0.86^{bd}
2.	Neither induced nor treated Water (2.5ml)	10.01±0.58^{bd}	10.26±0.59^{ad}	10.02±0.66^{bd}	9.04±0.37^{bd}	9.33±0.31^{bd}
3.	Induced and treated Piroxicam (2.5mg/kg)	10.13±0.28^{bd}	10.37±0.24^{ad}	10.10±0.64^{bd}	9.07±0.91^{bd}	9.13±0.94^{bd}
4.	Induced and treated Carboplatin (2.5mg/kg) + Piroxicam (2.5mg/kg)	10.17±0.26^{bd}	10.43±0.26^{bd}	9.35±1.06^{bd}	10.84±1.64^{bc}	11.60±1.80^{ac}
5.	Induced and treated Piroxicam (5mg/kg)	10.56±0.52^{bc}	10.76±0.51^{bc}	13.34±1.27^{ac}	10.22±0.75^{bd}	10.20±0.78^{bd}
6.	Induced and treated Carboplatin (2.5mg/kg)	10.53±0.23^{bd}	10.69±0.24^{ad}	9.67±0.42^{bd}	9.57±0.53^{bd}	9.57±0.65^{bd}
				Tidal volume (mL)		
1.	Induced not treated Water (2.5ml)	0.96±0.05^{bd}	0.99±0.05^{bd}	1.08±0.05^{ad}	0.96±0.08^{bd}	0.97±0.08^{bd}
2.	Neither induced nor treated Water (2.5ml)	0.94±0.06^{bd}	0.96±0.06^{ad}	0.94±0.06^{bd}	0.85±0.04^{bd}	0.87±0.03^{bd}
3.	Induced and treated Piroxicam (2.5mg/kg)	0.95±0.03^{bd}	0.97±0.02^{bd}	0.95±0.06^{ad}	0.85±0.08^{bd}	0.85±0.17^{bd}
4.	Induced and treated Carboplatin (2.5mg/kg) + Piroxicam (2.5mg/kg)	0.95±0.03^{bd}	0.97±0.03^{bd}	0.87±0.10^{bd}	1.01±0.15^{bc}	1.08±0.17^{ac}
5.	Induced and treated Piroxicam (5mg/kg)	0.98±0.05^{bc}	1.01±0.05^{bc}	1.25±0.12^{ac}	0.96±0.07^{bd}	0.95±0.07^{bd}
6.	Induced and treated Carboplatin (2.5mg/kg)	0.98±0.02^{bd}	1.00±0.02^{ad}	0.91±0.04^{bd}	0.86±0.05^{bd}	0.89±0.62^{bd}
				Respiratory frequency (sec^{-1})		
1.	Induced not treated Water (2.5ml)	1.72±0.09^{bd}	1.76±0.09^{bd}	1.87±0.08^{ad}	1.03±0.09^{bd}	1.07±0.09^{bd}
2.	Neither induced nor treated Water (2.5ml)	1.56±0.09^{bd}	1.53±0.09^{bd}	1.38±0.09^{bd}	2.42±0.09^{bc}	2.87±0.09^{ac}
3.	Induced and treated Piroxicam (2.5mg/kg)	1.19±0.09^{bd}	1.67±0.09^{ad}	1.42±0.09^{bd}	1.02±0.09^{bd}	0.98±0.09^{bd}
4.	Induced and treated Carboplatin (2.5mg/kg) + Piroxicam (2.5mg/kg)	1.41±0.09^{ad}	1.40±0.09^{bd}	0.87±0.09^{bd}	0.57±0.09^{bd}	0.52±0.09^{bd}
5.	Induced and treated Piroxicam (5mg/kg)	1.74±0.09^{bd}	1.77±0.09^{ad}	0.73±0.09^{bd}	1.23±0.09^{bd}	1.17±0.09^{bd}
6.	Induced and treated Carboplatin (2.5mg/kg)	1.86±0.09^{ac}	1.74±0.09^{bc}	2.15±0.10^{bc}	1.71±0.11^{bd}	1.42±0.10^{bd}

Keys: a = significantly higher along the row ($p < 0.05$); b = significantly lower along the row ($p < 0.05$); c = significantly higher along the column ($p < 0.05$); d = significantly lower along the column ($p < 0.05$); total volume = 0.6–1.5ml; respiratory frequency= 1.17–2.5 breaths per second; lung volume = 6–12ml

despite the fact that the US Environmental Protection Agency has indicated that not enough information exists to classify anthracene as a cancer-causing substance [70].

CONCLUSION

Anthracene exposure in female rats resulted in reduced body and organ weights, altered lung morphology, and impaired cardiorespiratory function. Treatment with piroxicam and carboplatin ameliorated these effects in a dose-dependent manner. Carboplatin treatment completely resolved micronodular lung lesions, while piroxicam reduced their size, suggesting a potential synergistic benefit of combined therapy. These findings indicate that piroxicam, alone or in combination with carboplatin, may have therapeutic potential in managing anthracene-induced lung tumorigenesis.

Ethical Statement

All experimental procedures involving animals were conducted in accordance with the guidelines of the Animal Ethics Committee of the College of Veterinary Medicine, Joseph Sarwuan Tarka University, Makurdi, Nigeria (Permit No. JOSTUM/CVM/ETHICS/2025/07).

Conflict of Interest

The authors have no conflict of interest to disclose.

Data Availability Statement

The raw data of this article will be made available by the authors, without undue reservation.

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Authors' Contributions

SAS designed the study and analyzed the data. AMU carried out the study and collected the data. SAS, AMU, AMV and TA wrote, proofread and approved the manuscript.

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ORIGINAL ARTICLE

TAXA PROFILING AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF BACTERIAL PATHOGENS ASSOCIATED WITH CANINE SURGICAL WOUNDS AND OPERATING THEATRE SURFACES

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

Surgical site infections are a major cause of morbidity and mortality in small animal surgical patients. This study evaluated the microbial profile and antibiotic susceptibility pattern of bacteria isolated from canine surgical wounds and operating room surfaces. Sterile swab samples (n = 41) were obtained from surgical wounds, operating tables, surgical instruments, and the recovery room. Aerobic and anaerobic bacteria were isolated and characterized using standard phenotypic methods. Antibiotic susceptibility was determined using the Kirby-Bauer disk diffusion method and profiled for resistance pattern. Eleven genera of bacteria were isolated from surgical wounds, with *Staphylococcus aureus* being the most frequently isolated. Seven genera of bacteria were each isolated from surgical instruments, and kennels, respectively, while four genera of bacteria were isolated from surgical tables, with *Escherichia coli* being the most frequently isolated. The median number of isolated aerobic bacteria was significantly higher ($P \leq 0.05$) than anaerobic bacteria. Bacteria isolated showed resistance to amoxicillin + clavulanic acid, cefuroxime, ampicillin + cloxacillin, and ceftriaxone. *Staphylococcus aureus, Bacillus mycoides, Escherichia coli,* and *Flavobacterium* species showed lower than 10% antibiotic resistance rates. The results provide insight into possible nosocomial bacteria transmission with high-level multidrug resistance. Improvement in decontamination practices and regulation of antibiotic usage is recommended to prevent the emergence of theatre-related multidrug-resistant bacteria.

Keywords: antibiotics; bacteria; dogs; incision; surgery

INTRODUCTION

Surgical incision results in breakage of the integrity of the skin and underlying connective tissues. This process allows for entry, contamination, multiplication, and proliferation of infecting microorganisms within deeper tissue with an accompanied risk of surgical site infection [1]. Surgical site infections (SSI) are the most common nosocomial infections in human patient populations, accounting for 16% of all human infections and 38% of nosocomial infections among surgical patients [2].

In veterinary medicine, SSI is a complication of small animal surgical procedures with significant variations based on surgery type [2, 3]. Risk factors associated with SSI in dogs include age, breed, physical and nutritional status, type of surgical procedure, type and duration of anaesthesia, and skin antisepsis [2, 4, 5]. Bacterial contamination of wounds is a major cause of complications of surgical sites with resultant delay in the wound healing process. Despite the progress made in the field of veterinary surgery, surgical site infection remains a problem, accounting for significant morbidity and mortality. This may be associated with poor infection prevention and control programs in veterinary practices, inadequate guidelines on antibiotic stewardship, and the emergence of antibiotic-resistant bacteria [6].

In spite of the prevalence of surgical site infections and antimicrobial-resistance in small animals, clinical microbiological data on the associated bacteria are not available in most African countries [7]. With growing concerns for antimicrobial resistant bacteria, critical appraisal on the appropriate use of antibiotics in veterinary medicine is important. An understanding of the type of bacteria associated with surgical wounds and the antibiotics susceptibility pattern is also critical.

Currently, the prevalence rate of SSI and associated antimicrobial resistant bacterial strains in canine surgical patients in sub-Saharan Africa is unknown. Hence, this study evaluated the microbial profile and the antibiotic susceptibility patterns of bacteria associated with canine surgical wounds, surgical instruments, and various surfaces of the operating room.

MATERIALS AND METHODS

Informed consent was obtained from the Veterinary Teaching Hospital, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria, prior to commencement of the study. In addition, ethical approval (FUNAAB/COLVET/CREC/2024/03/05) was received from the COLVET Research and Ethics Committee (CREC), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Sampling

Sterile swabs were used to obtain samples from the operating tables (n = 6), sterile surgical instrument packs (n = 6), admission kennels (n = 5), and surgically created wounds from dogs immediately before surgical closure of the wounds (n = 12) and at 48 hours post-surgery (n = 12) and preserved in transport broth (Bactec, Becton Dickinson & Company, Laoghaire, Ireland). The biodata distribution of sampled dogs consisted of males (n = 3) and females (n = 9) (Fig. 1A), which comprised three breeds, namely Boerboel, Alsatian, and local dogs (Fig. 1B), with ages ranging between 6 months and four years (Fig. 1C). Only dogs undergoing clean routine surgical procedure in any part of the body, such as castration, ovariohysterectomy, and splenectomy, were included, while dogs undergoing a clean-contaminated or contaminated surgery such as gastrotomy, cystotomy, intestinal resection, and anastomosis, were excluded.

Routine medical records of each dog were retrieved from the hospital database prior to surgery, while physical examinations of the dogs were performed to adjudge the dogs to be apparently healthy. All the dogs were subjected to at least six hours of routine fasting prior to surgery while routine aseptic preparation of the surgical site was performed. Steam (autoclave, Astell, UK) at temperatures 121°C–134°C was used for instruments sterilization, while surgical site disinfection was done using either povidone-iodine (5%) or chlorhexidine solution (0.05%). Each dog was fitted with an Elizabethan collar post-surgery to prevent contamination of their surgical wounds.

Bacterial biotyping and abundance estimation

A total of forty-one swab samples were collected and inoculated on MacConkey agar without salt (Oxoid CM 516, UK) and 7% blood agar (Oxoid, UK). All the inoculated plates were incubated at 37 °C for 18 to 24 hours

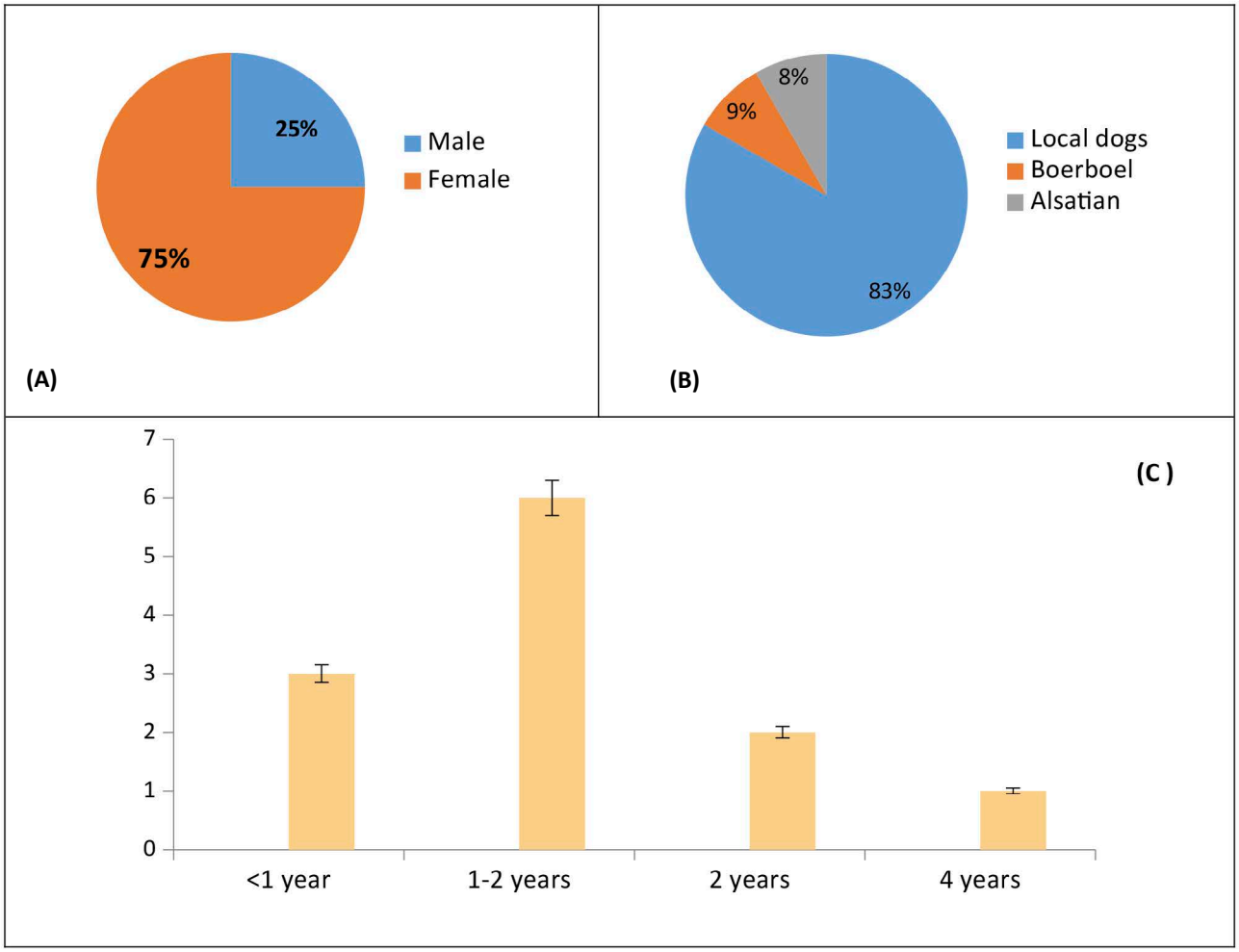

Fig. 1. Biodata distribution of selected dogs based on (A) gender, (B) dog breed and (C) age

and examined for cultural and cellular morphology by the Gram staining method [8]. Biochemical tests were performed using the Analytical Profile Index (API 20E and S36). The proportion of each strain of bacteria obtained from each source was estimated [9].

Antibiotic susceptibility testing

Susceptibility of each recovered bacteria isolate was analysed using the Kirby-Bauer disk diffusion antibiotic testing method [10]. Briefly, 3 to 5 pure colonies of the test organism were emulsified in 3–4 ml of sterile normal saline, and the suspension was adjusted to 0.5 McFarland (1.5×10^8 CFU/mL) turbidity. A sterile swab was dipped into the tube containing the bacteria suspension and spread uniformly on the Mueller Hinton agar surface. After a few minutes, antibiotic discs of cefuroxime (CXM), levofloxacin (LBC), gentamycin (GN), ofloxacin (OFX), iminepem (IMP), cefotaxime (CTX), ampicillin + cloxacillin (ACX), cefixime (ZEM), amoxicillin + clavulanic acid (AMC), and ceftriaxone (CRO) were gently placed on the inoculated agar surface and incubated at 37°C for 24 hours. After incubation, the zones of inhibition for each disc were measured and interpreted based on the criteria and breakpoints set by the Clinical and Laboratory Standards Institute [11].

Data analysis

Continuous variables, such as age and weight of the dogs, were assessed for normality. Data that were not normally distributed were presented as medians (ranges), while prevalence of SSI was presented as a percentage. Data on microbial distribution was presented using measures of central tendency. A p-value less or equal to 0.05 was considered significant. All statistical analysis was performed using STATA 15 [12].

RESULTS

High abundance rates of aerobic bacteria were isolated from operating tables, sterile surgical instruments, admission kennels, and surgical sites of the dogs (Fig. 2). High rates of *Escherichia coli, Staphylococcus saprophyticus, Staphylococcus aureus, ß-Streptococcus* species *Flavobacterium* species, *Proteus vulgaris,* and *Bacillus subtilis* were predominantly found in surgical wounds and surgical table samples (Tables 1 and 2). Samples from the surgical instruments and kennel floors yielded lower rate of *Bacillus subtilis, Staphylococcus saprophyticus, Proteus vulgaris,* and *Enterobacter* species (Tables 3 and 4). The bacteria isolated from the surgical site samples were mostly *Staphylococcus aureus, α-Streptococcus spp., ß-Streptococcus* spp., *Escherichia coli, Staphylococcus saprophyticus, Bacillus subtilis, Proteus vulgaris, Pseudomonas fluorescens, Klebsiella oxytoca, Flavobacterium* spp., and *Bacillus mycoides*.

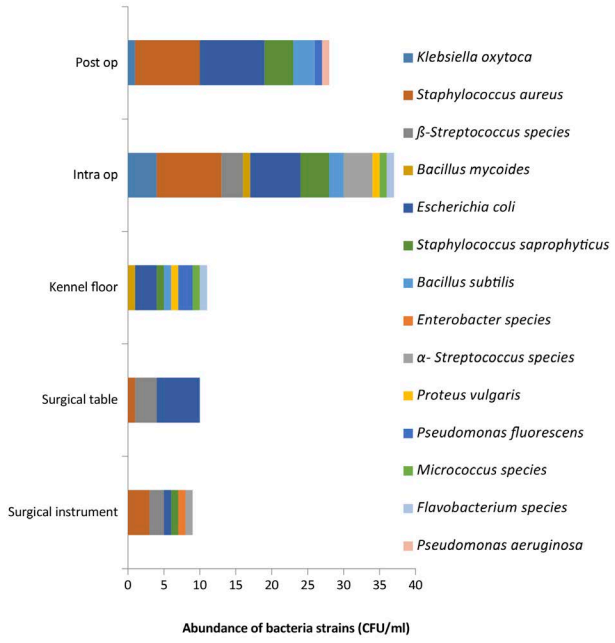

Fig 2. Bacterial populations from different operating room surfaces, surgical wounds, and kennel floors

Comparative analysis of microbial population from surgical incision prior to wound closure showed significant and higher abundance of *Staphylococcus aureus, α-Streptococcus* spp., *ß-Streptococcus* spp., *Escherichia coli, Staphylococcus saprophyticus, Bacillus subtilis, Proteus vulgaris, Klebsiella oxytoca, Flavobacterium* spp., *Bacillus mycoides* and *S. aureus* compared to the microbial population obtained from the surgical sites 48 hours post-surgery ($p = 0.002$; Table 5, Fig. 2).

Bacteria isolates recovered from surgical wound samples were resistant to augmentin, ceftriaxone, cefuroxime, ampicillin/cloxacillin, cefixime, levofloxacin, and gentamycin, while isolates from the kennel floor and surgical table showed resistance to ampicillin + cloxacillin, cefuroxime, and ceftriaxone, (Fig. 3A). Lower than 10% resistance rates were shown by *Klebsiella oxytoca*, *Staphylococcus aureus, Bacillus mycoides*, *Escherichia coli, Staphylococcus saprophyticus*, *Enterobacter* spp., *Streptococcus* spp., *Proteus vulgaris, Pseudomonas fluorescens*, *Micrococcus* spp., and *Flavobacterium* spp. (Fig. 3B).

DISCUSSION

The result of this study showed the presence of several bacteria genera from different operating room surfaces, as well as canine surgical wound prior to and 48 hours after wounds closure, suggesting nosocomial transmission of bacteria in canine surgical wounds. In addition, the antimicrobial susceptibility test performed on the isolated bacteria samples showed resistance to multiple antimicrobial agent indicating multidrug resistance.

The samples used for this study were obtained from dogs presented for clean surgeries such as orchidectomy, ovariohysterectomy, and splenectomy. This was done to prevent contamination of the surgical incision from bacteria within the gastrointestinal or urinary tract mucosa. In spite of sampling animals undergoing clean surgeries, the majority of the dogs still showed evidence of surgical site contamination. This supports the theory that there are several exogenous sources of wound contamination, such as the patient's skin, the operating room environment, surgical instruments, and the surgeons [13]. The small sample size of dogs in the study reflected the cultural practice of surgical procedures such as orchidectomy and ovariohysterectomy in sub- Saharan Africa where dog owners do not embrace neutering of their dogs because of financial gain [14].

Recycling of surgical instruments and linens may account for one of the sources of surgical site contamination in this study. Instruments are routinely cleaned after surgical procedures and then thermally disinfected using an au-

Table 1. Microbial population isolated from surgical wounds

Sample Identification	Bacteria Isolated	Number of Bacteria Genera
Intra Op 2	*Klebsiella oxytoca, S. aureus, ß-Streptococcus* spp., *E. coli, Bacillus mycoides*	5
Intra Op 3	*S. aureus., E. coli, S. saprophyticus, Flavobacterium* spp.	4
Intra Op 4	*Klebsiella oxytoca, S. aureus, E. coli*	3
Intra Op 5	*Klebsiella oxytoca, S. saprophyticus*	2
Intra Op 6	*Klebsiella oxytoca, S. aureus*	2
Intra Op 7	*S. aureus, α-Streptococcus* spp., *E. coli, S. saprophyticus*	4
Intra Op 8	*S. aureus, α-Streptococcus* spp., *E. coli, Proteus vulgaris*	4
Intra Op 9	*S. aureus, α-Streptococcus* spp., *E. coli, Bacillus subtilis*	4
Intra Op 10	*S. aureus, ß-Streptococcus* spp., *E. coli, S. saprophyticus, Bacillus subtilis*	5
Intra Op 11	*S. aureus, α-Streptococcus* spp., *ß-Streptococcus* spp.	3

Median number of bacteria genera isolated = 4

Table 2. Microbial population isolated from surgical tables

Sample Identification	Bacteria Isolated	Number of Bacteria Genera
T2	*E. coli*	1
T3	*E. coli, S. aureus, ß-Streptococcus* spp.	3
T4	*E. coli*	1
T5	*E. coli, ß-Streptococcus* spp.	2
T6	*Bacillus subtilis, E. coli, ß-Streptococcus* spp.	3
T7	*S. aureus, E. coli*	2

Median number of bacteria = 2

Table 3. Microbial population isolated from surgical instruments

Sample Identification	Bacteria Isolated	Number of Bacteria Genera
S2	*E. coli*	1
S3	No growth	0
S4	*S. saprophyticus, Enterobacter* spp.	2
S5	*S. aureus, ß-Streptococcus* spp., *Monococcus* spp.	3
S6	*S. aureus, ß-Streptococcus* spp., *Monococcus* spp.	3
S7	*S. aureus, ß-Streptococcus* spp.	2

Median number of bacteria = 3

Table 4. Microbial population isolated from kennel floors

Sample Identification	Bacteria Isolated	Number of Bacteria Genera
K2	*Bacillus subtilis, Proteus vulgaris*	2
K3	*E. coli, Pseudomonas fluorescens*	2
K4	*Pseudomonas fluorescens*	1
K5	*E. coli, Micrococcus* spp.	2
K6	*E. coli, Staphylococcus saprophyticus, Micrococcus* spp., *Flavobacterium* spp.	4

Median number of bacteria = 2

Table 5. Microbial population isolated from surgical site 48 hours after wound closure

Sample Identification	Bacteria Isolated	Number of Bacteria General
Post Op 2	*S. aureus, E. coli, S. saprophyticus*	3
Post Op 3	*S. aureus, E. coli, Pseudomonas fluorescens*	3
Post Op 4	*S. aureus, E. coli, Bacillus subtilis*	3
Post Op 5	*Klebsiella oxytoca, E. coli*	2
Post Op 6	*S. aureus, E. coli*	2
Post Op 7	*S. aureus, E. coli, S. saprophyticus, Bacillus subtilis*	4
Post Op 8	*S. aureus, E. coli, Micrococcus* spp., *Pseudomonas aeruginosa*	4
Post Op 9	*S. aureus, E. coli, S. saprophyticus*	3
Post Op 10	*S. aureus, E. coli, Bacillus subtilis*	3
Post Op 11	*S. aureus, E. coli, S. saprophyticus*	3

Median number of bacteria = 3

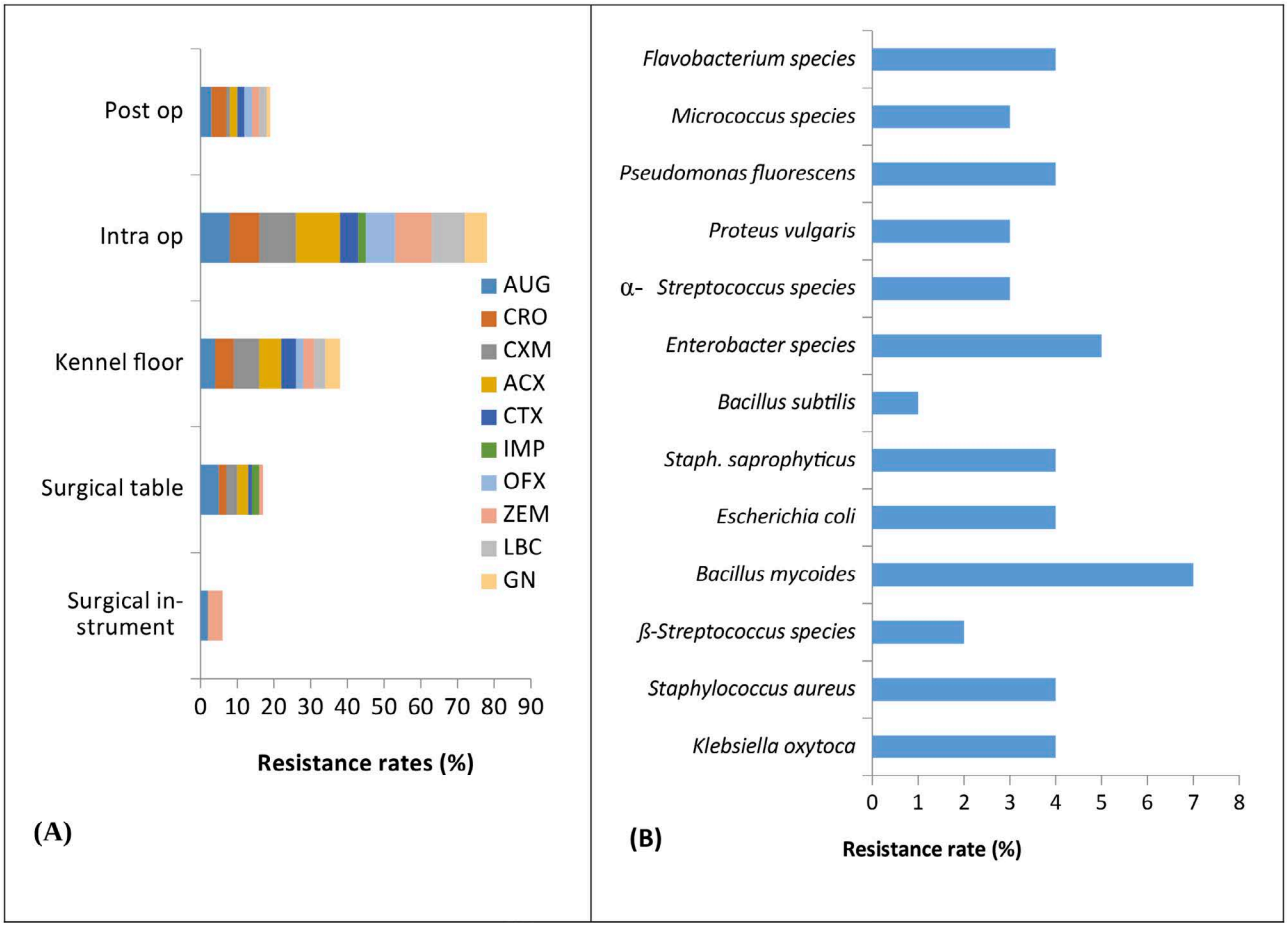

Fig. 3A. Antibiotic resistance pattern of bacteria isolated from surgical wounds and different theatre surfaces
Fig. 3B. Antibiotic resistance rates of different recovered bacteria isolates
Keys: CXM: cefuroxime, LBC: levofloxacin, GN: gentamycin, OFX: ofloxacin, IMP: iminepem, CTX: cefotaxime, ACX: ampicillin + cloxacillin, ZEM: cefixime, AUG: augmentin, CRO: ceftriaxone.

toclave, and stored in the theatre until the next operation. Contamination of the instruments can occur at any stage of the processing. For example, sterile surgical instruments could be contaminated in the protective area of the operating room when left open [15]. Autoclave malfunction due to failure of the sterilization cycle can also result in suboptimal steam sterilization. In this study, distribution pattern of the surgical linen (drapes) and instruments showed that surgical linen and instruments were reused in all the cases. This may account for bacteria being isolated from them despite the routine sterilization process. This finding is similar to that reported in the human operating room [16]. While the major bacteria isolated from surgical instruments and tables from this study was *E. coli,* coagulase-negative staphylococci were mostly isolated bacteria in a similar study in a human operating room [17]. It is thought that the air in the operating room might be the source of contamination of the surgical instruments, tables, and linens [18].

In this study, the bacteria isolated from the surgical instruments were *Escherichia coli, Staphylococcus saprophyticus, Enterobacter* spp., *Staphylococcus aureus, ß-Streptococcus* spp., and *Monococcus* spp., with *Staphylococcus aureus* being the most frequently isolated bacteria. This finding is similar to that reported in the surgery department of a resource-limited country [19]. This phenomenon may be associated with recycling of surgical instruments and sterilization failure. It is worrisome that despite the use of an autoclave for the sterilization of the instruments, multidrug-resistant bacteria were still isolated from the instruments. Sterilization failure has been reported to range between 12 and 17 percent in human dental facilities and has been associated with inadequate personnel training, poor equipment maintenance, and incorrect equipment maintenance [20]. These factors might be responsible for the presence of bacteria in sterilized instruments and linen despite sterilization with an autoclave.

Escherichia coli was the most frequently isolated bacteria on both the surgical table and the floor of the kennel. This is contrary to the report from a similar study in which *Rothia* spp., coagulase-negative *Staphylococcus* spp., *Staphylococcus aureus*, and *Enterococcus* spp. were the bacteria isolated from the operating room and admission kennels of a veterinary hospital [21]. In another study, *E. coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Enterococcus* spp. were isolated [22]. The organisms isolated from the surgical tables and kennel floor may be associated with faecal contamination of these surfaces [21]. The presence of bacterial contamination of the surgical table despite sterilization with disinfectants such as chlorhexidine may be due to several factors, such as a lower concentration of the disinfectant used, the disinfectant not reaching the minimum contact time of the product with the surface, or taking very long cleaning intervals [22]. This will therefore necessitate strict disinfection protocol of all surfaces of the operating theatre to prevent the presence of contaminating bacteria.

The bacteria isolated from the surgical sites included *S. aureus, α-Streptococcus* spp., *ß-Streptococcus* spp., *E. coli, S. saprophyticus, Bacillus subtilis, Proteus vulgaris, Pseudomonas fluorescens, Klebsiella oxytoca, Flavobacterium* spp., and *Bacillus mycoides.* This finding is similar to that reported in previous studies, with the isolated bacteria probably being those present in the skin of the dogs rather than contamination from other sources [2, 23]. This observation underscores the importance of strict skin preparation, such as scrubbing and proper disinfection, prior to surgery. The use of a scalpel for shaving should also be discouraged, as it can help in the spread of normal bacterial flora of the skin to the incision site. Thus the use of clippers for hair removal is advocated.

It is important to note that the majority of the bacteria isolated from different surfaces of the operating room, kennel floor, and the surgical incision sites exhibited multidrug resistance pattern except *Bacillus subtilis* and β-*Streptococcus* spp. This finding is similar to reports from previous studies where multidrug-resistant bacteria have been isolated [2, 21, and 22]. This may not be unconnected with indiscriminate and inappropriate use of antibiotics in patients undergoing routine and elective surgical procedures in sub-Saharan Africa. Judicious use of antibiotics based on empirical data is therefore recommended.

Finally, there are few limitations in this study that will warrant that the present result be interpreted with caution. The samples studied were obtained from one hospital where it is expected that routine operating room practices such as disinfection and asepsis are similar. This makes it difficult to determine if a change in the operating room hygiene procedures will affect the pattern of bacterial contamination at the surgical site. The small number of the animals sampled is another limitation. However, small sample size reflects the cultural perception of dog owners regarding neuter surgery. It is important to note that in spite of the small size, a large number of bacteria were isolated from different surfaces of the operating room and the surgical site. The high number of bacteria isolated from different surfaces may be associated with the poor resource nature of the hospitals that resulted in the recycling of surgical instruments and linens.

CONCLUSION

In conclusion, aerobic bacteria populations from different surfaces of the operating room provide insight into possible nosocomial transmission in spite of routine disinfection protocol. The emergence of new strains with high-level multidrug resistance from contaminated surgical instrument and surgical incision sites during intraoperative and postoperative periods could serve as aetiological agent for postoperative systemic infection. From the study, it is evident that most bacterial contamination of the surgical site occurs intraoperatively rather than postoperative contamination of the wound at the kennel. Multi-centre bacteria profiling for comparative operating room practices, incorporating a larger sample size from veterinary surgical centres is recommended. The evidence of a high rate of sterilization failures suggested the need for improved personnel training, proper equipment maintenance, and adherence to proper equipment operation.

Data Availability Statement

Data sets for this study are available upon request from the corresponding author.

Ethical Statement

Ethical approval (FUNAAB/COLVET/CREC/2024/03/05) was received from the COLVET Research and Eth-

ics Committee (CREC), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

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Generative AI statement

No AI or AI-assisted technologies were used in the preparation of this manuscript.

Authors' Contributions

Conceptualization: ARA and TRA; Data Acquisition: TRA, SOL, OFK; Data Analysis: OFK, SOL, PAA; Manuscript Preparation: PAA, ARA. All the authors review and accept the final manuscript.

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FOLIA VETERINARIA, 69, 4: 73—78, 2025

ORIGINAL ARTICLE

COMPARATIVE PHARMACOKINETICS OF INTRAVENOUS AND INTRAMUSCULAR MELOXICAM IN AWASSI SHEEP: IMPLICATIONS FOR EXTENDED THERAPEUTIC EFFICACY

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ABSTRACT

Meloxicam, a COX-2 preferential NSAID, is broadly used in veterinary medicine for analgesic and anti-inflammatory activity, but pharmacokinetic data in sheep remain restricted. This study compared plasma pharmacokinetic profiles of meloxicam administered intravenously (IV) and intramuscularly (IM) in Awassi sheep. A two-phase crossover design study with a 12-day washout was conducted in five healthy male Awassi sheep (26–34 kg). Meloxicam (0.5 mg/kg) was administered by IV or IM. Blood samples were collected at determined intervals (IV: 0.083–24 h; IM: 0.5–24 h). Plasma concentrations were quantified via spectrophotometry, and Pharmacokinetic (PK) parameters resulted from non-compartmental analysis. IV administration showed rapid distribution (C_{max}: 7.17 ± 0.17 µg/mL at 0.083 h) and prolonged $t_{1/2\beta}$ (13.02 h). IM administration showed delayed absorption (T_{max}: 4 h; C_{max}: 7.08 ±0.04 µg/mL) and comparable $t_{1/2\beta}$ (14.77 h). Both routes maintained plasma concentrations >1 µg/mL for >24 h. Key parameters include volume of distribution (V_d) (IV: 0.094 L/kg; IM: 0.078 L/kg), clearance (Cl) (IV: 0.0050 L/h/kg; IM: 0.0036 L/h/kg), and area under the plasma concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$) (IV: 98.95 µg×h/mL; IM: 135.36 µg×h/mL). Meloxicam revealed satisfactory PK properties with sustained therapeutic concentrations beyond 24 hours, supporting once-daily dosing for long-term pain management in sheep.

Key words: Awassi sheep; intramuscular; intravenous; meloxicam; pharmacokinetics

INTRODUCTION

Meloxicam, a selective cyclooxygenase-2 (COX) inhibitor, is extensively utilized in veterinary medicine for its anti-inflammatory and analgesic effects [1]. While its pharmacodynamics have been well-documented in humans [2], horses [3], calves [4], and cattle [5], data in sheep, particularly for intramuscular administration, remain sparse. In ruminants, meloxicam is employed to manage pain associated with surgical procedures, mastitis, and dystocia [6]. However, interspecies variability in drug metabolism, driven by differences in cytochrome p450 enzyme activity and protein binding, necessitates species-specific PK evaluation [7].

Existing studies in sheep report an elimination half-life ($t_{1/2\beta}$) of 10.85−14 hours flowing IV dosing [8, 9], but IM data are limited to a single study using a higher dose (1 mg/kg) [10]. This study addresses this gap by comparing IV and IM PK profiles of meloxicam (0.5mg/kg) in Awassi sheep, a breed prevalent in Middle Eastern livestock system. The findings aim to optimize dosing regimens and inform clinical use in ovine medicine.

MATERIALS AND METHODS

Animals & study design

Five healthy male Awassi sheep (11–12 months; 26–34 kg) were acclimatized in individual pens with *ad libitum* water and forage. A randomized crossover design with a 12-day washout period was employed. Sheep received meloxicam (0.5 mg/kg; Ashish Life Science, India) via IV (jugular vein) or IM routes. Blood samples were collected into EDTA tubes at specified intervals (IV: 0.083, 0.5, 1, 2, 4, 8, 16, 24 h; IM: 0.5, 1, 2, 4, 8, 16, and 24 h). Plasma was separated by centrifugation (1500 ×g, 10 min) and stored at −18°C.

Preparation of the standard solution

A standard solution of meloxicam (0.5 mg/mL) was prepared using 50% methanol, and secondary standard dilutions were prepared using methanol at concentrations of 0.5, 1, 1.75, 2.5, 5, 7.5, and 10 µg/mL.

Meloxicam quantification in the plasma protocol

The quantification of meloxicam in plasma was performed using a validated UV-visible spectrophotometric method, with sample extraction steps adapted from the HPLC protocol of Bae et al. (2007) [2]. Briefly , 500 microliter aliquots of plasma or calibration standards were transferred to glass test tubes and acidified with 100 microliter of 5 M hydrochloric acid (HCl), followed by brief homogenization by vortex mixing. Liquid-liquid extraction was initiated by adding 6mL of diethyl ether, after which samples were agitated for 30 minutes to ensure phase equilibration. Post- extraction, samples were vortexed briefly and centrifuged at 2500 rpm for 10 minutes to separate phases. The organic layer (diethyl ether) was quantitatively transferred to a fresh tube and evaporated to dryness under a controlled nitrogen stream. The residue was reconstituted in 1mL of methanol, and meloxicam concentration was determined spectrophotometrically by measuring absorbance at 355 nm using UV-vis spectrophotometer. A calibration curve (0.5–10 µg/mL; R^2 = 0.9325) was constructed from reference standards to calculate plasma concentrations of meloxicam by linear regression. This modified method was validated for linearity, precision, and reproducibility within the therapeutic concentration range relevant to sheep pharmacokinetic analysis.

Meloxicam quantification in sheep plasma analysis

The quantification of meloxicam concentrations in ovine plasma was achieved via a validated spectrophotometric method, with absorbance measured at 355 nm. A standard calibration curve was created through simple linear regression analysis (R^2 = 0.9325), resulting from laboratory-prepared reference dilutions of known meloxicam concentrations. The regression equation is expressed as y = 239.63x + −236.36, where y represents the absorbance value, x denotes the analyte concentration (µg/mL), m (slope) is 239.63, and b (y-intercept) is –236.36. Unknown plasma concentrations were calculated by readjusting the regression equation to x = (y + 236.36)/239.63, ensuring traceable calibration of experimental samples. Data processing and regression analysis were conducted using Microsoft Excel 2013, with methodological details demonstrated in Figure 1. This approach obeys to robust analytical principles for pharmacokinetic studies, leveraging linear regression's predictive accuracy within the validated dynamic range.

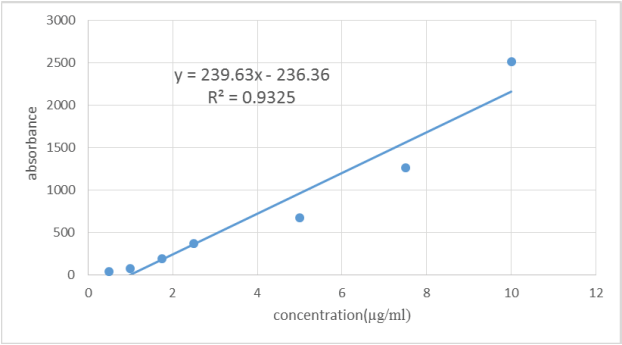

Fig. 1. Standard meloxicam calibration curve (0.5–10 µg/mL; R^2=0.932)

Pharmacokinetics of meloxicam in sheep

The objective of this study was to determine the pharmacokinetic profile of meloxicam following single-dose intravenous (IV) and intramuscular (IM) administration in sheep at a dosage of 0.5 mg/kg. Plasma concentrations of meloxicam were quantified through spectrophotometric analysis at determined time intervals. Pharmacokinetic parameters were derived via non-compartmental pharmacokinetic analysis using the pharmacokinetic modeling software PK Solver [11]. Key parameters measured included elimination half-life ($t_{1/2\beta}$), elimination rate constant (kel), volume of distribution (Vd), total systemic clearance (Cl), mean residence time (MRT), time to maximum concentration (T_{max}), peak plasma concentration (C_{max}), and the area under the plasma concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$). These parameters were evaluated to describe the drug's absorption, distribution, and elimination kinetics through both administration routes, verifying a comprehensive understanding of its *in vivo* behavior in the ovine model.

RESULTS

Plasma concentrations and pharmacokinetic parameters following intravenous administration

Meloxicam (0.5 mg/kg) was administered intravenously (IV) to sheep; plasma concentrations peaked at 7.17 ± 0.17 µg/mL within 0.083 hours (5 minutes) and declined progressively over 24 hours (Table 1). Pharmacokinetic analysis showed an elimination half-life ($t_{1/2\beta}$) of 13.02 hours, a volume of distribution (Vd) of 0.094 L/kg, and a total clearance (Cl) of 0.0050 L/h/kg. The area under the plasma concentration-time curve ($AUC_{0-\infty}$) was 98.952 µg×h/mL, and the mean residence time (MRT) was 18.27 hours (Table 2).

Table 1. Plasma concentrations of meloxicam after intravenous administration (0.5 mg/kg) in sheep

Time (h)	Concentration µg/mL
0.083	7.17±0.17
0.5	6.50±0.10
1	5.66±0.11
2	5.00±0.04
4	4.20±0.06
8	3.30±0.08
16	2.06±0.05
24	1.46±0.03

Table 2. Pharmacokinetic parameters of meloxicam after intravenous administration (0.5 mg/kg) in sheep

parameters	values	units
$t_{1/2\beta}$	13.02	h
T_{max}	0.08	h
C_{max}	7.17	µg
K_{el}	0.053	h^{-1}
MRT	18.27	h
V_d	0.094	L/kg
Cl	0.0050	L/h/kg
$AUC_{0-\infty}$	98.952	µg× h/mL

$t_{1/2\beta}$ = Terminal Elimination Half-Life, T_{max} = Time to Maximum Plasma Concentration, C_{max} = Maximum Plasma Concentration, K_{el} = Terminal Phase Elimination Rate Constant, MRT = Mean Residence Time, V_d = Volume of Distribution, Cl = Clearance, $AUC_{0-\infty}$ = Area Under the Curve from time 0 to infinity.

Plasma concentrations and pharmacokinetic parameters following intramuscular administration

Intramuscular (IM) administration of meloxicam (0.5 mg/kg) resulted in a delayed T_{max} of 4 hours, with a C_{max} of 7.08 ± 0.04 µg/mL (Table 3). The elimination half-life ($t_{1/2\beta}$) was prolonged to 14.77 hours, with an $AUC_{0-\infty}$ of 135.362 µg×h/mL. The volume of distribution (Vd) and total clearance (Cl) were 0.078 L/kg and 0.0036 L/h/kg, respectively, while the MRT extended to 20.21 hours (Table 4).

Table 3. Plasma concentrations of meloxicam after intramuscular administration (0.5 mg/kg) in sheep

Time (h)	Concentration µg/mL
0.5	5.98±0.17
1	6.31±0.13
2	6.67± 0.07
4	7.08±0.04
8	4.13±0.16
16	2.78±0.06
24	1.95±0.06

Table 4. Pharmacokinetic parameters of meloxicam after intramuscular administration (0.5 mg/kg) in sheep

parameters	values	units
$t_{1/2\beta}$	14.77	h
T_{max}	4	h
C_{max}	7.08	µg
K_{el}	0.046	h^{-1}
MRT	20.21	h
V_d	0.078	L/kg
Cl	0.0036	L/h/kg
$AUC_{0-\infty}$	135.362	µg× h/mL

$t_{1/2\beta}$ = Terminal Elimination Half-Life, T_{max} = Time to Maximum Plasma Concentration, C_{max} = Maximum Plasma Concentration, K_{el} = Terminal Phase Elimination Rate Constant, MRT = Mean Residence Time, V_d = Volume of Distribution, Cl = Clearance, $AUC_{0-\infty}$ = Area Under the Curve from time 0 to infinity.

Comparative pharmacokinetic profile

The $AUC_{0-\infty}$ and MRT were notably higher following IM administration compared to IV (135.362 vs. 98.952 µg×h/mL and 20.21 vs. 18.27 h, respectively). Both routes sustained plasma concentrations above the therapeutic threshold (1 µg/mL) for over 24 hours, with IM administration exhibiting a delayed absorption phase but comparable bioavailability (Figure 2).

DISCUSSION

The study employed a randomized two-phase crossover design with a 12-day washout period, verifying elimination of residual drug between IV and IM administration. This approach reduces inter-individual variability through using the same animals for both routes. However, the small sample size (n = 5) raises questions about statistical power and generalizability. While common in veterinary studies including large animals, bigger cohort would support conclusions. The use of Awassi sheep, a breed predominant in arid regions, additions ecological relevance but highlights the need for breed-specific pharmacokinetic (PK) data due to potential genetic and metabolic differences.

The present study explores the pharmacokinetic characteristics of meloxicam in Awassi sheep after intravenous (IV) and intramuscular (IM) administration at doses of 0.5 mg/kg. Both routes showed a sustained plasma concentrations above the therapeutic threshold (≥1 µg/mL) [12] for over 24 hours, highlighting the drug's prolonged therapeutic efficacy in this species. Remarkably, the elimination half-life ($t_{1/2\beta}$) of meloxicam in sheep (13.02 h IV; 14.77 h IM) in consist with prior reports in ruminants but diverges from values detected in humans [2], horses [3], and avian species [13], conforming interspecies variability in drug metabolism.

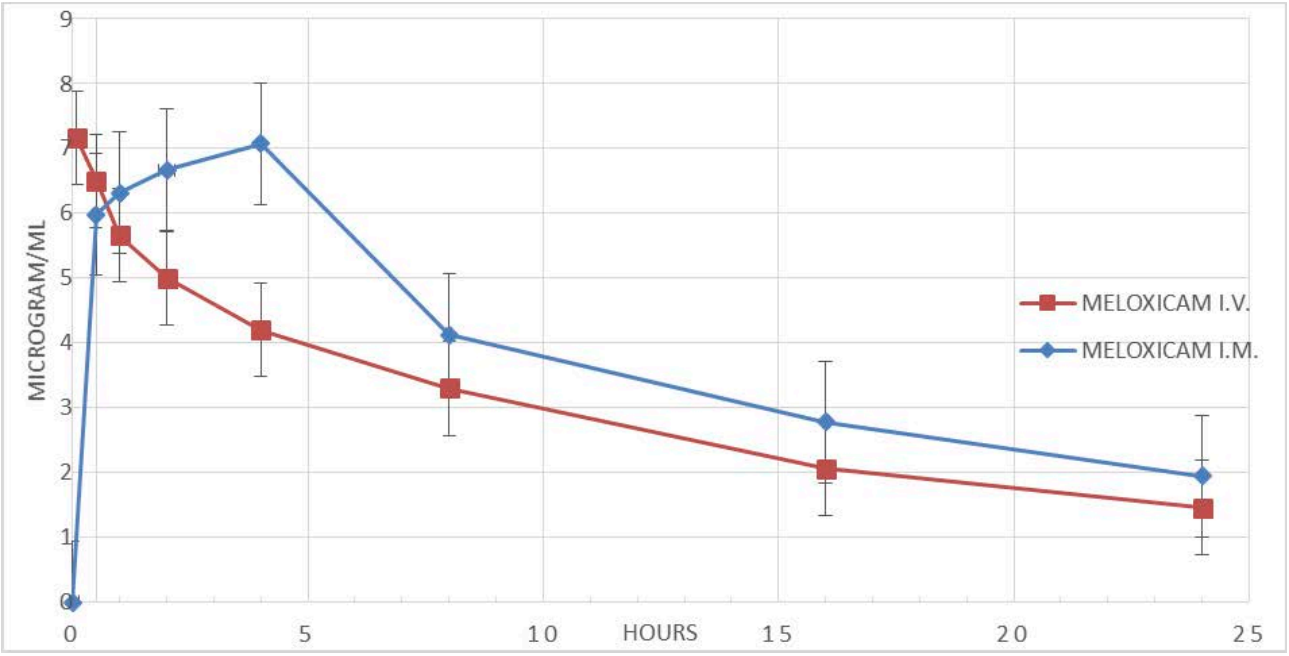

Fig. 2. Plasma concentration-time profile following IV and IM administration (Mean ± SE)

The prolonged $t_{1/2\beta}$ and mean residence time (MRT) observed in this study recommend meloxicam's potential for once-daily dosing in sheep, a critical advantage in clinical settings demanding prolonged analgesia. The significantly higher $AUC_{0-\infty}$ following IM administration for IV (135.362 vs. 98.952 µg×h/mL) indicates near-complete bioavailability by this route, consistent with efficient systemic absorption from the muscle compartment. However, the delayed T_{max} (4 h IM vs. 0.08 h IV) reflects slower absorption kinetics inherent to IM delivery, possibly influenced by formulation characteristics such as solvent composition or drug solubility.

Comparative analysis with present literature reveals notable inter-study variability in ovine pharmacokinetic parameters. For example, the IV $t_{1/2\beta}$ (13.02 h) aligns closely with values reported by Stock et al. (14 h) in Dorset cross sheep [9] but differs from Shukla et al.'s results (10.85 h) in crossbred females [8]. Such differences may arise from breed-specific dissimilarities in metabolic enzyme activity, principally cytochrome P450 isoforms responsible for meloxicam's hepatic metabolism. Similarly, the IM C_{max} (7.08 µg/mL) and $t_{1/2\beta}$ (14.77 h) in this study contrast with Woodland et al.'s data (9.77 µg/mL; 12.63 h) at a higher dose (1 mg/kg) [10], further highlighting the influence of genetic and methodological factors on pharmacokinetic outcomes.

The sustained therapeutic plasma levels (>1 µg/mL) observed beyond 24 hours post-administration confirm meloxicam's efficacy in managing chronic inflammatory conditions in sheep, such as mastitis or post-surgical pain. This prolonged activity, coupled with its satisfactory safety profile in ruminants, positions meloxicam as a valuable NSAID in veterinary practice. However, the lower volume of distribution (Vd) and clearance (Cl) following IM administration propose potential tissue-binding differences between routes, warranting further investigation into tissue-specific pharmacokinetics.

Therapeutic outcomes

•Sustained Therapeutic Levels: Both routes sustained plasma concentrations >1 µg/mL for >24 hours, supporting once-daily dosing in clinical settings. This is critical for managing chronic conditions (e.g., post-surgical pain, mastitis) in sheep.

•Practical Advantages of IM Route: The delayed T_{max} and prolonged $t_{1/2\beta}$ make IM administration ideal for field use, avoiding the need for IV catheterization.

Limitations of the study

•Small Sample Size: Limits statistical robustness and generalizability.

•Single-Dose Design: Precludes assessment of drug accumulation, safety with repeated dosing, or dose-response relationships.

•Lack of Clinical Endpoints: PK parameters alone do not approve therapeutic efficacy. Future studies should correlate plasma levels with pain relief or anti-inflammatory outcomes.

•Bioavailability Paradox: The higher AUC for IM despite identical dosing requires clarification, potentially through mass spectrometry validation or urinary excretion studies.

CONCLUSION

In conclusion, this study provides robust pharmacokinetic evidence supporting the clinical use of meloxicam in sheep, with both IV and IM routes offering sustained therapeutic coverage. The delayed absorption kinetics of IM administration and breed-related variability in drug metabolism underscore the need for tailored dosing regimens. Future research should explore the impact of formulation additives and genetic factors on meloxicam's disposition to optimize therapeutic outcomes in diverse ovine populations. This work underscores the necessity of species- and breed-specific PK studies to optimize veterinary drug regimens and improve animal welfare.

Data Availability Statement

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Statement

The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the College of Veterinary Medicine, University of Mosul, approval number (9/13/1526).

Conflict of Interest

No conflict of interest.

Funding

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Authors' Contributions

Yasser M. Albadrany: Conceptualization, Methodology, Writing and editing.

Nagham M. Abdelqader & Ahmed T. Mohammed: Sampling and methodology, Data collection, Data analysis.

Generative AI Statement

The authors declare that no generative AI was used in the creation of this manuscript.

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FOLIA VETERINARIA, 69, 4: 79—84, 2025

CASE REPORT

THERAPEUTIC APPROACH TO MAMMARY FISTULA IN A GOAT – SURGICAL INTERVENTION: A CASE STUDY

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OPEN ACCESS

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ABSTRACT

In June 2023, a goat presenting with a mammary gland fistula was admitted to our facility. Upon clinical examination of the patient, apart from the fistula located on the left half of the mammary gland, no other pathological findings were detected. Following diagnosis, the owner consented to surgical intervention involving excision of the lesion. An elliptical excision encompassing all tissue layers down to the mammary cistern was performed. The defect was subsequently closed using a three-layer suture with absorbable suture material. During the postoperative period, systemic antibiotic therapy was administered for ten days. Intramammary antibiotics and a non-steroidal anti-inflammatory drug were applied for three days, in combination with lavage of the mammary cistern using warm physiological saline and local wound care. At the time of removing the tension-relieving sutures, on the eighth day after surgery, the surgical site exhibited no signs of inflammation, and healing was proceeding adequately. The animal was alert, in good clinical condition, and showed interest in feed. On the tenth day, the patient was returned to the owner and reintegrated into the herd. The presented case describes the successful surgical treatment of a mammary fistula in a goat, resulting in complete healing without postoperative complications.

Key words: fistula; goat; udder

INTRODUCTION

Mammary gland and teat fistula is defined as an abnormal tract connecting the internal environment of the mammary gland or teat with the external environment, clinically manifesting in lactating animals by milk discharge [1]. From an etiological perspective, the fistula is described as either congenital or acquired. The acquired form of mammary gland fistula most commonly results from trauma or represents a complication following surgical treatment [2]. Traumatic injuries that do not involve the mammary cistern or teat canal may be managed by removal of debris

and necrotic tissue in combination with local therapy. If the trauma precludes machine milking, the placement of a sterile teat cannula is indicated. Hygiene management is crucial during patient care, especially when using a teat cannula, due to the high risk of mastitis development [3]. Mastitis is defined as inflammation of the mammary gland resulting from infection [4]. The inflammatory process induces a series of changes dependent on the extent of parenchymal damage and the causative pathogen. These changes include decreased milk production in both clinical and subclinical forms, reduced lactose content, altered casein proportion, and decreased lipoid concentration [5]. Surgical treatment is indicated for any laceration involving the mammary cistern or teat canal; otherwise, a permanent fistula may develop. Therapy is often challenging due to secondary bacterial infection [3]. The initial step of therapy involves thorough cleaning and debridement of the laceration to remove pathologically altered tissue, especially in chronic cases. Excision of wound edges is also recommended, with careful consideration of subsequent tissue adaptation. Precise debridement is essential not only for removing necrotic and non-viable tissue, but also for achieving proper wound closure during suturing [6].

CASE PRESENTATION

Case description

In June 2023, at the owner's request, a goat (crossbreed, 3 years old, 55 kg) was admitted to the Ruminant Clinic of the University Hospital at the University of Veterinary Medicine and Pharmacy in Košice. The owner reported an issue affecting the left half of the udder as the reason for presentation.

MANAGEMENT AND OUTCOMES

Clinical examination

General parameters were within reference ranges during the clinical examination. Examination of organ systems revealed no pathological findings other than the owner- reported issue involving the mammary gland. Parasitological examination of the patient was included as part of the general clinical examination. On clinical palpation, the udder exhibited a firm elastic consistency without enlargement, asymmetry, pain, or changes in temperature. On the left half of the udder, at the transition between the mammary cistern and the teat canal, a fistula was identified (Fig. 1).

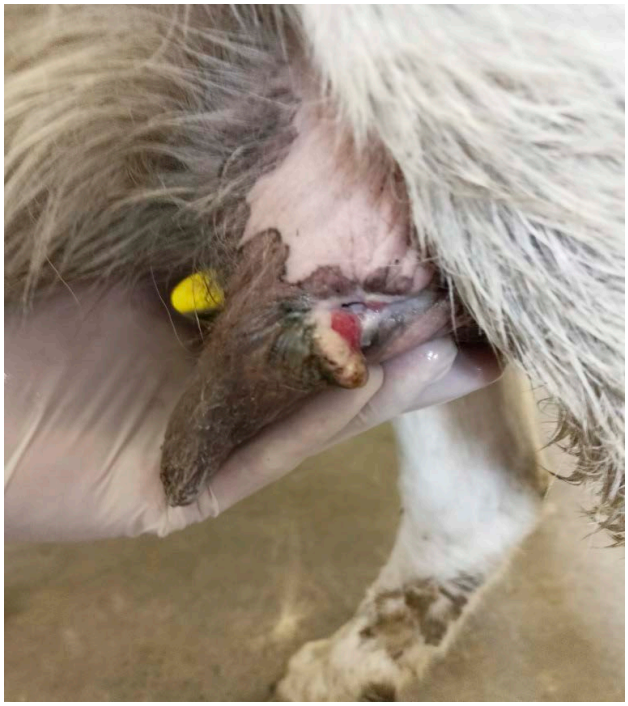

Fig. 1. Fistula of the mammary gland identified during clinical examination

It was characterized by skin adherent to the underlying parenchyma and a small amount of secretion. Scar tissue was evident in the surrounding area. The owner had attempted to dry off the udder, which was successful on the right side; however, the affected left half continued to produce a small amount of secretion, which discharged through the fistula. For hematological and biochemical evaluation, blood samples were collected from the jugular vein (*v. jugularis*). Biochemical analysis revealed no abnormalities, with all parameters within reference ranges. Hematological analysis, however, confirmed leukocytosis. Based on the clinical findings, a diagnosis of fistula of the left half of the mammary gland was established. The owner was informed of the diagnosis, clinical status, and therapeutic options and consented to surgical treatment.

Surgical treatment

As part of the surgical procedure, patient preparation included a 24-hour preoperative fasting period with free access to water. For general anesthesia, xylazine (0.2 mg/kg) was administered in combination with ketamine

(11 mg/kg). Anesthesia was subsequently maintained with the administration of half the initial dose. Prior to standard surgical field preparation, the mammary fistula was flushed with warm physiological saline. An elliptical incision was made around the fistula, approximately 1 cm from its margins, encompassing all tissue layers down to the mammary cistern (Fig. 2).

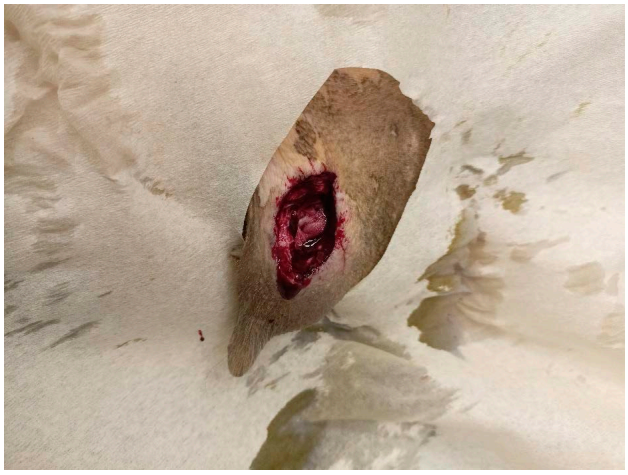

Fig. 2. Surgical excision of mammary fistula

Following excision of the fistula, the mammary cistern was flushed again, and an intramammary antibiotic was administered. Wound edge adaptation and three-layer closure of the surgical site were then performed. The first layer – the mucosa was closed using a mattress suture pattern with absorbable polydioxane suture material. The second layer, the submucosa, was closed with a simple continuous suture, also using absorbable material (PDX 3). The final layer, the skin, was closed intradermally with absorbable suture material (Fig. 3) and reinforced with three tension-relieving sutures. Finally, the surgical site was cleaned and treated with an antiseptic aluminum spray.

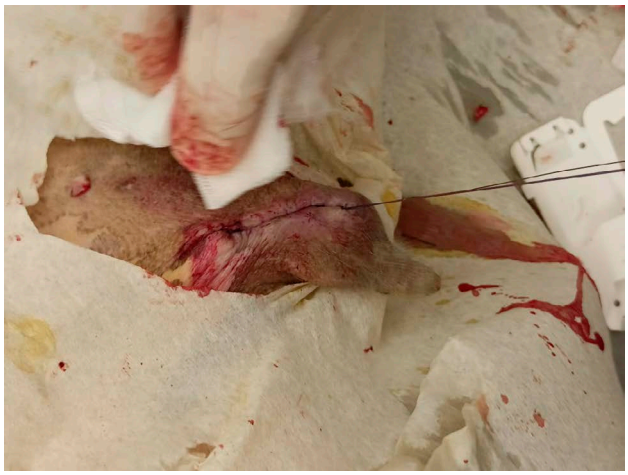

Fig. 3. Intradermal closure of the mammary gland after fistula excision

Postoperative care

The patient was housed in an individual pen during recovery from general anaesthesia and for the subsequent 10 days following surgery. Pharmacological treatment includes systemic antibiotic administration for 10 days (amoxicillin at a dose of 15 mg/kg), as well as intramammary antibiotics and non-steroidal anti-inflammatory drugs (flunixin meglumine at 2.2 mg/kg) for three days postoperatively. For the first three days following surgery, the mammary gland was irrigated with warm physiological saline, which was subsequently milked out to remove blood clots formed after the procedure. Each lavage was followed by intramammary antibiotic administration. By the fourth postoperative day, the mammary gland secretion was free of blood clots and fibrin flakes. On the eighth day, the three tension-relieving sutures were removed, and local wound care was performed. By the tenth day, the patient's condition was stable, the surgical site showed no pathological signs, and the patient was returned to the herd (Fig. 4).

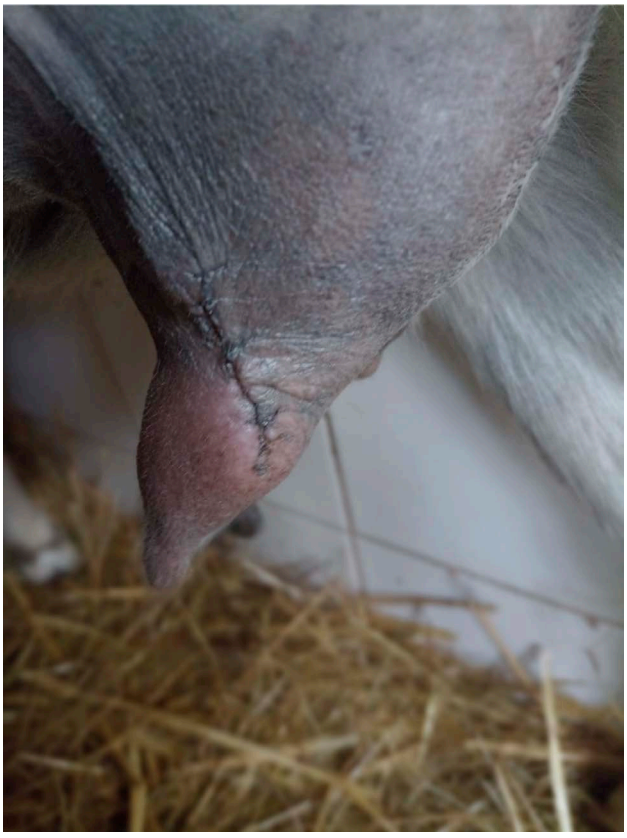

Fig. 4. Healing wound on the 10th day after surgery

DISCUSSION

The mammary gland is an exceptionally sensitive organ, highly susceptible to injury. Even minor trauma can lead to significant health complications and negatively affect milk production. Minimal dysfunction of the physiological barrier may result in chronic inflammation or infection of the gland, which in severe cases can lead to culling of the animal from the herd [7]. Postpartum enlargement of the mammary gland increases the risk of trauma. Injuries may occur due to other animals in the herd, foreign objects such as metal sheeting or wire, or even milking equipment. Both the mammary gland and the teats are at risk, as they are directly exposed to environmental factors. A fistula of the mammary gland or teat is considered an acute condition, especially in lactating animals, due to the increased risk of mastitis and the associated economic losses. Immediate intervention and closure of the fistula within 12 hours of injury (optimally within 3 hours) significantly improve prognosis [8]. After 12 hours, massive edema of the wound margins typically occurs. In such cases, it is advisable to delay repair for 2–3 days until the swelling subsides [9]. If the injury is left untreated, the wound heals secondarily, resulting in the formation of a fistula. Fistula formation is generally complete approximately four weeks post-injury [10].

In the present case, we performed surgical treatment of a mammary gland fistula most likely caused by trauma, as suggested by visible scar tissue. The chronic nature of the lesion was also indicated by the adherence of skin to the gland's mucosa and submucosa. The absence of clinical signs of inflammation in the mammary gland improved the prognosis. Surgically, an elliptical incision approximately 1 cm from the fistula margins was made, extending through all tissue layers down to the mammary cistern. Roberts et al. [11] emphasize that incisions in this region should be performed cautiously to avoid excessive tissue removal, which can complicate adaptation of wound edges. In our case, tissue was excised conservatively to ensure healthy tissue alignment. No issues with adaptation were observed due to the adequate amount of free tissue and the anatomical location of the defect, situated at the transitional zone between the mammary cistern and teat canal. Following tissue excision, the cistern was flushed with sterile saline, intramammary antibiotics were applied, and the wound was sutured. Although the most widely recommended suturing technique is a two-layer continuous suture, with first layer involving only the mucosa and the second the submucosa [12, 13] in this case, we performed a three-layer closure. The mucosal layer was closed with a continuous mattress suture, followed by a simple continuous suture of the submucosa. The skin was closed using an intradermal suture, reinforced with three tension-relieving sutures.

Experimental studies have shown that three-layer closure of mammary cistern and teat lacerations offers superior outcomes and minimizes the risk of postoperative fistula formation compared to two-layer techniques [14]. As for suture materials, synthetic absorbable options such as polyglactin, polyglycolic acid, or polydioxanone are recommended [12, 14]. These materials are particularly suitable for deep tissue repairs such as those involving the mammary cistern because they induce minimal inflammatory response and are degraded by hydrolysis [12, 15]. In contrast, chromic catgut is considered inappropriate for mammary surgery due to its intense inflammatory and phagocytic reaction and rapid loss of tensile strength, which adversely affects healing [16]. In this case, polydioxanone was used for suturing all three layers. No postoperative wound reactions suggestive of suture intolerance were observed. By day 4, postoperative edema had markedly subsided and wound edge adaptation had improved. Sutures should ideally be removed on day 9, depending on the healing progress. Prolonged presence of sutures may result in excessive fibrosis or localized infections [10]. On postoperative day 8, the surgical site showed no pathological signs, and wound edges were well adapted. Common complications associated with surgical repair of mammary gland or teat lacerations include suture dehiscence, fistula formation, abscess development, mammary cistern fibrosis, and mastitis [17].

Postoperative care should include systemic antibiotics, NSAIDs, and intramammary antibiotic therapy. Wound protection, particularly during summer months with increased fly activity, is also advised [18]. In our case, during the first three postoperative days, warm 0.9% saline lavage was performed to remove blood clots from the mammary cistern, followed by milking. By the fourth day, milk was clear and free of discoloration, clots, or fibrin flakes. Despite the owner's attempt to dry off the affected udder, complete involution occurred only on the right side. The left affected side continued to produce a small volume of

milk, which increased postoperatively. This residual milk production was beneficial for therapy, as it facilitated the removal of clots and fibrin from the cistern. California Mastitis Test (CMT) results on days 3 and 4 were positive; however, by day 8, the result was negative. Upon discharge, the owner was advised to continue milking the affected udder until postoperative day 15, followed by a gradual dry-off process.

CONCLUSION

This case report confirms that surgical management of a mammary gland fistula is an effective therapeutic approach, allowing for the restoration of both glandular morphology and milk production, thereby reducing economic losses in the herd. From a material and technical standpoint, the procedure is not demanding; however, postoperative care plays a crucial role and significantly influences the therapeutic outcome.

Data Availability Statement

The raw data of this article will be made available by the authors, without undue reservation.

Ethical Statement

All examinations and therapeutic procedures were performed in accordance with established standards and with due regard for the patient's welfare. The owner consented to the therapeutic process under the given conditions.

Conflict of Interest

The authors have no pertinent financial or non-financial conflicts of interest to declare.

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Generative AI Statement

The authors declare that no Gen AI was used in the creation of this manuscript.

Authors' Contributions

Marián Kadaši – article processing, clinical examination and diagnostics, surgery, article revision, Mekková Simona – clinical examination and diagnostics, surgery, article revision, Hisira Vladimír – clinical examination, postoperative therapy, article revision, Glembová Veronika – clinical examination of patient, anesthesiology, article revision, Gomulec Pavel – surgery, article revision Dolník Michal – clinical examination, medical care, article revision.

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ORIGINAL ARTICLE

VERIFICATION OF THE PRESENCE OF ANTIBIOTIC RESIDUE IN SHEEP MEAT

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

Antibiotics are substances with a direct action on bacteria that are used for treatment or prevention of infectious diseases. Their usage in food-producing animals may result in deposition of their residues in animal products. The sheep sector is considered an overall lower user of antibiotics, but there is still a need to monitor residues in sheep meat also. The aim of this study was to verify whether antibiotic residues are present in samples of sheep meat on the market from various local suppliers in Ireland, and if so, to tentatively identify them. Our study was carried out using two microbial inhibition tests, the tube Explorer 2.0 test with photometrical reading of the results and the multi-plate Screening Test for Antibiotic Residue (STAR), for the antibiotic group identification in the samples tested positive. Out of the 15 random samples tested, 2 samples yielded a positive result using the Explorer 2.0 test. Using the STAR, both positive samples were considered suspicious for the presence of beta-lactam antibiotics. Another fresh sample from each supplier that tested positive already had a negative result. Reliable monitoring of meat from food-producing animals in accordance with European Union law contributes to the protection of public health and strengthens consumer confidence.

Key words: antibiotic residues; screening; sheep meat

INTRODUCTION

"Antibiotic" means any substance with a direct action on bacteria that is used for treatment or prevention of infections or infectious diseases [1]. Antibiotics bring both benefits and adverse effects, but they need to be used in a responsible manner to prolong their efficacy. Benefits of using antibiotics in food-producing animals include health and welfare purposes of the animal, carcass quality, growth and production efficiency, economics, protection of the environment, decreased morbidity and mortality, and human health [2]. Adverse effects of residues on human health include development of drug resistance, carcinogenicity, mutagenicity, hypersensitivity reaction, bone marrow de-

pression and toxicity, disruption of normal intestinal flora, immunopathological effects, autoimmunity, and teratogenic effects [3, 4].

Strict EU legislations exist around the levels of residues of pharmacologically active substances, including antibiotics, known as maximum residue limits (MRLs), which must be officially controlled in food-producing animals and foods of animal origin. MRL means the maximum concentration of a residue of a pharmacologically active substance, which may be permitted in food of animal origin [5]. MRLs for pharmacologically active substances authorised as veterinary medicinal products are set out in the Commission Regulation (EU) 37/2010 [6]. Regulation (EU) 2017/625 [7] and its delegated and implementing acts lay down specific rules for the performance of official controls in relation to substances whose use may result in residues in food and feed. Commission Delegated Regulation (EU) 2022/1644 [8] lays down rules for the performance of official controls in regard to the range of samples and the stage of production, processing and distribution, in which the samples are to be taken regarding the use of pharmacologically active substances authorised as veterinary medicinal products or as feed additives and of prohibited or unauthorised pharmacologically active substances and residues thereof. The uniform practical arrangements for the performance of official controls as regards the use of pharmacologically active substances authorised as veterinary medicinal products or as feed additives and of prohibited or unauthorised pharmacologically active substances and residues thereof, on specific content of multi-annual national control plans and specific arrangements for their preparation, are set in Commission Implementing Regulation (EU) 2022/1646 [9]. The rules on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results, as well as on the methods to be used for sampling, are laid down in the Commission Implementing Regulation (EU) 2021/808 [10].

Analytical methods can be classified into five different cases based on the type of analysis (screening, confirmation) and the mode of analyte determination (qualitative, semi-quantitative, quantitative) [11]. “Screening method” means a method that is used for screening of a substance or class of substances at the level of interest (MRL). “Confirmatory method” means a method that provides full or complementary information enabling the substance to be unequivocally identified and, if necessary, quantified at or below the maximum residue level for authorised substances [10].

The current residue control strategy is based on two sequential steps: screening and confirmation. Screening techniques can generate qualitative (positive or negative), semiquantitative (high, medium/low), or quantitative results [12]. They are based on different detection principles (e.g., microbiological, immunological, physico-chemical) and must be able to screen for potential non-compliant results [13, 14]. A sample categorized as potentially non-compliant (“Screen Positive”) is subsequently analysed with a confirmatory test [15].

There are no restrictions for screening methods regarding the suitability of specific approaches or detection principles [15]. Microbial inhibition tests (MIT) are commonly used as screening tests for antibiotic residues, as they are relatively cheap, easy to perform, and have a broad spectrum of sensitivity. These screening tests are based on the principle of bacterial growth inhibition produced by concentrations of antimicrobial residues above the limit of detection of the test. There are two main groups of broad-spectrum MITs: tube and multi-plate tests [16, 17, 12, 18, 19]. Multi-plate tests provide more information than tube tests but in turn are more laborious and therefore more suited to official laboratories, whereas a slaughterhouse required to analyse a large number may find tube tests more suitable [20].

Tube tests have the added advantage of being ready to use, and results are available comparatively faster than multi-plate tests. Generally, tube tests consist of a medium (agar base and nutrients) seeded with the susceptible bacteria and a growth indicator (pH or redox based). The interpretation of the result is based on a colour change of the medium. Generally, a colour change indicates antibiotics are not present, and a lack thereof would imply a non-compliant sample [19, 21]. Plate or multi-plate test systems are based on a combination of different test organisms that are sensitive to a group or groups of antibiotics. The interpretation of the result is based on the production of an inhibition zone. The diameter of the inhibition zone theoretically correlates with the concentration of the antimicrobial compound in the sample [18].

Many MITs have been developed or commercialized for the screening of antibiotic residues in the meat

of food-producing animals. Among the most widely used ready-to-use tube tests for the screening of antibiotic residues in meat and offal of food-producing animals are the Premi®Test produced by R-Biopharm in Germany and the Explorer 2.0 test developed by Zeulab in Spain. Both tests rely on agar diffusion of the antibiotic residues, which is expressed by the absence of colour change from purple to yellow. The advantage of the Explorer 2.0 test over the Premi®Test is the interpretation of results by measuring colour change with the e-Reader. The e-Reader uses internal software to integrate time and colour parameters to determine the endpoint of the assay, stopping automatically and interpreting qualitative results. Performance of the Explorer 2.0 combined with an automated e-Reader device for the detection of antimicrobial residues in muscle was validated in line with MRL according to European Commission Decision 2002/657/EC [22] by Mata et al. (2014) [20]. Premi®Test was validated for the detection of antimicrobial residues in animal muscle tissues according to AFNOR rules (French Association for Normalisation) by Gaudin et al. (2008) [23].

The plate methods developed so far are using one to seven plates, different pH and media combinations, and one or different bacterial strains to improve the capability detecting different antibiotic groups [24]. Samples are considered positive if they gave the inhibition zone around the sample or the punch hole in the diameter set by the method. The most widely used plate method is the five-plate test known as the Screening Test for Antibiotic Residues (STAR), which was developed by the AFSSA Community Reference Laboratory in Fougeres for screening antimicrobial residues in muscle [25].

As antibiotic residues continue to be a public health concern, the aim of our study was to verify the presence of antibiotic residues in sheep meat originating from supermarkets and butchers in Ireland using the Explorer 2.0 test in conjunction with the e-Reader, followed by preliminary identification using the STAR method.

MATERIALS AND METHODS

Sheep meat samples

A total of 15 sheep meat samples for this study were collected from butchers and supermarkets in Ireland over several months. All fifteen samples were from a shoulder cut of the carcass. Samples were stored in a freezer at -18 °C before the analysis.

Explorer 2.0 test coupled to e-Reader

The first laboratory test that was carried out was a qualitative test, Explorer 2.0, developed by Zeulab S.L. (Zaragoza, Spain) with e-Reader (the equipment that incubates, stops the assay, and interprets the results). The principle of the Explorer 2.0 test is based on the inhibition of microbial growth. The kit is supplied in a single-tube format. Each tube contains agar medium spread with *Geobacillus thermophile* spores and a pH indicator. When the test is incubated at 65 °C, spores germinate, and cells grow, producing acid and changing the agar pH. Variations of pH will produce changes of the agar colour from purple to yellow. When samples contain inhibitors at higher concentrations than the limit of detection, the bacteria will not grow, and no colour change will be observed.

Sample preparation and assay procedure for the Explorer 2.0 test

3 g ± 0.5 g of lean meat muscle without adipose or connective tissue was weighed in a screw top tube. The tube was closed slightly but not fully sealed. The tube was put in a beaker of water. The beaker was then put in a microwave on the defrost setting for 3-4 minutes, until the meat was fully cooked. The meat was then removed, and the meat juice was used for analysis. A lyophilized negative control sample provided by Zeulab S.L. (ZE/EX/CN5) was used to determine the optimal incubation of the assay in each run.

100 µl of the negative control sample and 100 µl of each sample were pipetted onto the surface of the agar medium in the test tube. The tubes were then incubated at room temperature for 20 minutes. After 20 minutes, the tubes were turned upside down to remove the remaining sample. The tubes were washed with distilled water. The water was removed by placing the tube upside down and dried on absorbent paper. The tubes were sealed with adhesive foil and placed into the e-Reader for incubation at the temperature of 65 ºC. The procedure was terminated automatically, and results were read and displayed on the screen. The device detects a colour change of the negative control by photometric reading and automatically terminates the incubation time of the samples, with a positive sample expressed as a value ≥56 and a negative sample as a value <56.

STAR method

The method used for sample analysis is adopted from the STAR protocol developed by the Community Reference Laboratory in Fougeres, described by Gaudin et al. (2010) [25].

Test organisms and agar media used in the STAR protocol

Five culture media were used: 1) *Bacillus subtilis* BGA 5 x 10^4 spores/mL (Merck 10649, Darmstadt, Germany), Antibiotic medium 11 (Difco 259310, Detroit, USA), pH 8.0; 2) *Kocuria rhizophila* ATCC 9341 5 x 10^4 germs/mL (Czech Collection of Microorganisms, Brno, Czech Republic), Merck 10664 medium (Darmstadt, Germany), pH 8.0; 3) *Bacillus cereus* ATCC 11778 3 x 10^4 germs/mL (Czech Collection of Microorganisms, Brno, Czech Republic), Merck 10663 medium (Darmstadt, Germany), pH 6.0; 4) *Escherichia coli* ATCC 11303 10^5 germs/mL (Czech Collection of Microorganisms Brno, Czech Republic); Merck 10664 medium (Darmstadt, Germany), pH 8.0; 5) *Bacillus stearothermophilus* var. *calidolactis* ATCC 10149 5 x 10^5 spores/mL (Merck 1.11499, Darmstadt, Germany); Diagnostic Sensitive Test agar (Oxoid CM 261, Basingstoke, United Kingdom), pH 7.4 supplemented with trimethoprim (Fluka 92131, Buchs, Switzerland) 0.005 µg/mL. Culture media were prepared according to the manufacturer's instruction.

Sample preparation and assay procedure for the STAR method

5 ml of inoculated medium was added to each Petri dish. A cylindrical plug of 8 mm in diameter and 2 cm in length was cut in frozen muscle using a cork borer. Both ends of the cylindrical core were discarded, and 2 mm thick slices of muscle were cut by a sterile lancet. Two tissue discs were placed on one plate directly across from each other. The plates are then incubated as follows: *Bacillus subtilis* BGA for 18 hours at 30 °C, *Escherichia coli* ATCC 11303 for 18 hours at 30 °C, *Kocuria rhizophila* ATCC 9341 for 24 hours at 37 °C, *Bacillus cereus* ATCC 11778 for 18 hours at 37 °C, and *Bacillus stearothermophilus* var. *calidolactis* ATCC 10149 for 15–16 hours at 55 °C. A sample is positive when the inhibition zone around the sample is equal to or more than 2 mm on *Bacillus subtilis* BGA, *Escherichia coli* ATCC 11303, *Kocuria rhizophila* ATCC 9341, and *Bacillus cereus* ATCC 11778 test plates, and equal to or more than 4 mm on *Bacillus stearothermophilus* var. *calidolactis* ATCC 10149 test plates. The diameters of the inhibition zones were measured by an electronic caliper (Mitutoyo, Kawasaki, Japan). The diameter of the inhibition zones was expressed as the mean ± standard deviation (SD) of six measures.

Two paper discs (9 mm, Whatman No. 1, Maidstone, United Kingdom) soaked with 30 µg/L of control standard solutions of reference antibiotics were placed on the surface of the agar medium to verify the sensitivity of the plates. The following control solutions were used: sulfamethazine (S 5637, Sigma, St. Louis, MO, USA) at 1000 µg/L on *Bacillus stearothermophilus* var. *calidolactis* ATCC 10149 plate, streptomycin (S 6501, Sigma, St. Louis, MO, USA) at 2000 µg/L on *Bacillus subtilis* BGA plate, tylosin (T 6134, Sigma St. Louis, MO, USA) at 1000 µg/L on *Kocuria rhizophila* ATCC 9341 plate, chlortetracycline (C4881, Sigma, St. Louis, MO, USA) at 200 µg/L on *Bacillus cereus* ATCC 11778 plate, and ciprofloxacin (17850, Fluka Chemie GmbH, Buchs, Switzerland) at 100 µg/L on *Escherichia coli* ATCC 11303 plate. The diameters of the inhibition zones around the positive control paper discs should present the inhibition zones as follows: streptomycin 4.5 ± 1.5 mm, tylosin 5.5 ± 1.5 mm, chlortetracycline 6.0 ± 1.5 mm, ciprofloxacin 5.5 ± 1.5 mm, and sulphamethazine 5.0 ± 1.5 mm.

RESULTS

The results of the Explorer 2.0 test were obtained from the e-Reader when the assay reached the endpoint that was electronically determined by the e-Reader itself. The results were displayed on the screen, and the colour indicated the sample as positive (red) or negative (green). The test results of the Explorer 2.0 test are shown in Table 1, with sample 7 and sample 11 being positive. As can be seen from Table 1, the e-Reader coupled to the Explorer 2.0 test determined the test endpoint at a cut-off of 56 and generated the results as negative (<56) or positive (>56). The cut-off of a screening test is the response or signal, which indicates that a sample contains an analyte at or above the target concentration, thereby ensuring discrimination between positive and negative samples [15]. The test results from the e-Reader are shown in Figures 1, 2, and 3.

Table 1. Explorer 2.0 test results

Sample No.	Source	Explorer 2.0	Cut-off value
1	Supermarket	Negative	<56
2	Supermarket	Negative	<56
3	Butcher	Negative	<56
4	Supermarket	Negative	<56
5	Supermarket	Negative	<56
6	Butcher	Negative	<56
7	Butcher	**Positive**	**57**
8	Supermarket	Negative	<56
9	Supermarket	Negative	<56
10	Supermarket	Negative	<56
11	Butcher	**Positive**	**95**
12	Butcher	Negative	<56
13	Butcher	Negative	<56
14	Supermarket	Negative	<56
15	Butcher	Negative	<56

Fig. 1. Results of Explorer 2.0 with e-Reader for samples 1-5

When the Explorer 2.0 test was repeated with new sheep meat samples from both positive suppliers, the results for both samples were negative, as shown in Table 2. The test results generated by the e-Reader as negative for both new samples (green, cut-off <56) are shown in Figure 4.

Fig. 2. Results of Explorer 2.0 coupled to e-Reader for samples 6-10

Fig. 3. Results of Explorer 2.0 coupled to e-Reader for samples 11-15

Table 2. Explorer 2.0 test results for additional samples from positive suppliers

Sample No.	Source	Explorer 2.0	Cut-off value
7a	Butcher	Negative	<56
11a	Butcher	Negative	<56

Fig. 4. Results of Explorer 2.0 coupled to e-Reader for additional samples from positive suppliers

As sample 7 and sample 11 had a positive result from the Explorer 2.0 test coupled to e-Reader, the STAR method was used to confirm the positive results and to preliminarily identify antibiotics in these samples. Both positive food samples showed the formation of inhibition zones on the plates seeded with test organism *Bacillus stearothermophilus* var. *calidolactis* ATCC 10149 and *Kocuria rhizophila* ATCC 9341. The mean diameter of the inhibition zone on the plate seeded with test organism *Bacillus stearothermophilus* var. *calidolactis* ATCC 10149 was 2.86 ± 0.29 mm for sample 7 and 4.89 ± 0.42 mm for sample 11. The mean diameter of the inhibition zone on the plate seeded with test organism *Kocuria rhizophila* ATCC 9341 was 2.76 ± 0.35 mm for sample 7 and 2.91 ± 0.16 mm for sample 11. According to the width of the inhibition zones set for the positive results on the plate seeded with test organism *Bacillus stearothermophilus* var. *calidolactis* ATCC 10149, sample 7 was considered negative and sample 11 positive for antibiotic residues. According to its sensitivity to a group of antibiotics, sample 11 is suspected of the presence of beta-lactams or sulphonamides. According to the width of the inhibition zones set for the positive results on the plate seeded with test organism *Kocuria rhizophila* ATCC 9341, both samples were considered positive for antibiotic residues. Based on its sensitivity to a group of antibiotics, the samples are suspected of the presence of macrolides and beta-lactams. No inhibition zones were observed on the plates seeded with other test organisms of the STAR method. The mean diameter of the inhibition zones around the samples detected positive by the Explorer 2.0 test coupled with the e-Reader is presented in Tables 3 and 4.

Table 3. Mean diameter of the inhibition zones around the samples detected on *Bacillus stearothermophilus* var. *calidolactis* ATCC 10149 test plates

Sample No.	Source	Diameter, mm ± SD	Result
7	Butcher	2.86 ± 0.29	Negative
11	Butcher	4.89 ± 0.42	Positive

SD – standard deviation. All data are the mean ± SD of six measurements.

Table 4. Mean diameter of the inhibition zones around the samples on *Kocuria rhizophila ATCC 9341* test plates

Sample No.	Source	Diameter, mm ± SD	Result
7	Butcher	2.76 ± 0.35	Positive
11	Butcher	2.91 ± 0.16	Positive

SD – standard deviation. All data are the mean ± SD of six measurements.

The susceptibility of test organisms to control antibiotics determined by the STAR method was confirmed before analysis. This was done by creating inhibition zones around positive control paper discs with diameters corresponding to the sizes of the inhibition zones set for control antibiotics by the STAR method.

DISCUSSION

The direct risk to human health posed by antibiotic residues in food has been well documented. Direct risks include hypersensitivity reactions, which may range from a cutaneous reaction to acute anaphylaxis, liver injury, and disruption of the human intestinal microbiome in cases of short-term exposure to antibiotic residues [16, 26]. Long-term exposure can result in carcinogenicity, teratogenicity, and mutagenicity. Coupled with these concerning direct health risks, the indirect threat from antibiotic residues in food is that of antimicrobial resistance. There is no doubt that the use of antibiotics in livestock leads to antibiotic resistance in human medicine. However, antibiotics are important in modern farming practices for maintaining animal health and animal welfare and food production standards [16].

Due to the many negative implications posed to public health by antibiotic residues in animal products, a screening programme exists to ensure they comply with set standards [27]. Strict legislation exists around residues of antibiotics in meat in food-producing animals within the EU. MRLs, the maximum concentration of a residue of a pharmacologically active substance that may be permitted in food of animal origin, are established according to Regulation (EC) 470/2009, with a view to public health and safety [5]. MRLs are listed in Commission Regulation (EU) No 37/2010 [6]. Monitoring of MRLs is the responsibility of individual countries within the EU, as is the establishment and implementation of a monitoring plan, according to the Commission Implementing Regulation (EU) 2022/1646 [6]. The requirements for the analytical methods that may be used in the testing of official samples and the criteria for the interpretation of analytical results are laid down in Commission Implementing Regulation (EU) 2021/808 [10]. The strategy for the control of residues is based on two steps: screening methods and confirmatory methods [17, 12].

There are many ways to test for antibiotic residues in food, and these can include microbiological assays, physical and chemical assays, immunoassays, aptasensors, and whole-cell biosensors [28]. Qualitative tests are used to determine whether a particular chemical is present in a sample. Quantitative tests are used to determine the absolute or relative abundance of a particular substance present in a sample. Qualitative tests are further divided into microbiological and immunological methods. Microbiological tests involve either test tubes or Petri dishes. Immunological tests involve ELISA, radioimmunoassay, and receptor-binding techniques [26].

In our study, initial screening for antibiotic residues in sheep meat sourced in Ireland was performed using the Explorer 2.0 tube microbial inhibition assay coupled with the e-Reader. Of the 15 randomly collected sheep meat samples, 2 samples were positive. When screening other samples from both positive suppliers, the sheep meat was already negative for antibiotic residues. Microbial inhibition tube tests are qualitative screening methods, which means that they give binary results (positive or negative). Even though these kinds of tests are ready to use and only require an incubator, they need to be halted at an adequate point in time to avoid over- or under-incubation and, thus, to obtain the best results in terms of sensitivity. Moreover, a visual reading of results can lead to misinterpretation, as well as discrepancies between lab results and those obtained by users [29]. The use of an e-Reader in conjunction with the Explorer 2.0 test proposed by Mata et al. (2014) [20] allows for the automation of the test. This device includes a thermostatic incubator adapted to the test tubes and an optical system to monitor colour change. Both are integrated with software that controls device functions and automatically detects the end-time of the assay to provide an objective result. Analysis can be performed at any location (on a farm or during lairage time at the slaughterhouse), even by untrained staff [21]. The manufacturer instructions specify that the ampoules cannot be interpreted based on the colour change following incubation in the e-Reader. With the cut-off value set to 56 units, the system reliably distinguishes positive samples from negative ones. The cut-off values of both positive samples were at or above the level of 56.

The two samples that tested positive using the Explorer 2.0 test were then tested using the STAR method. Inhibition zones greater than 2 mm on plates inoculated with Kocuria rhizophila ATCC 9341 and greater than 4 mm on plates inoculated with Bacillus stearothermophillus var. calidolactis ATCC 10149 confirmed the Explorer 2.0 test result and tentatively identified the antibiotic group in the examined samples. The test organism Kocuria rhizophila ATCC 9341 is set for the detection of macrolides and beta-lactams, and the test organism Bacillus stearothermophilus var. calidolactis ATCC 10149 is set for the detection of beta-lactams and sulphonamides. Two samples that tested positive with the Explorer 2.0 assay were subsequently tested with the STAR method. Zones of inhibition greater than 2 mm on plates inoculated with Kocuria rhizophila ATCC 9341 and greater than 4 mm on plates inoculated with Bacillus stearothermophillus var. calidolactis ATCC 10149 confirmed the Explorer 2.0 result and tentatively identified the antibiotic group in the samples studied. Based on the sensitivity of the test organisms to the respective antibiotic group(s), it can be predicted that of the two samples tested on plates with Bacillus stearothermophilus var. calidolactis ATCC 10149, only sample 11 was evaluated as positive for beta-lactams and sulphonamides, and on plates with the Kocuria rhizophila ATCC 9341, both samples were evaluated as positive for beta-lactams and macrolides. Because both test organisms are specific for ß-lactam antibiotics, we can assume a potential presence

of ß-lactam antibiotics in both sheep meat samples. For final confirmation of the result, whether the samples are positive for the presence of a beta-lactam antibiotic or a macrolide antibiotic or a sulphonamide, the screening result must be confirmed by subsequent confirmatory analysis.

Macrolides are effective against most aerobic and anaerobic gram-positive bacteria. They are used to treat systemic and local infections, such as upper respiratory tract infections, urinary tract infections, bronchopneumonia, and metritis. Sulphonamides are effective against both gram-positive, and gram-negative bacteria and some protozoa. They are used to treat systemic infections such as mastitis, metritis, respiratory infections, and toxoplasmosis. Beta-lactams are effective against gram-negative and gram-positive bacteria and are used to treat respiratory infections. Stricter measures surrounding veterinary prescriptions and record keeping came into effect on 28th January 2022 under Regulation (EU) 2019/6 [1], which strengthened food protection measures from a farming perspective.

The importance of the results that were found in this research shows that even though regulations and directives are in place on both a European and national level, there is still non-compliance in a small percentage of not only sheep but in other food-producing animals. Under the Article 19 of Regulation (EU) 2017/625 [7], each member state is required to implement a residue monitoring plan and to submit their programmes annually to the European Commission. The scope of testing under Ireland's National Residue Control Programme (NRCP) is comprehensive, covering 8 food production systems as well as milk, eggs, and honey and 19 distinct residue groups. These residue groups include prohibited substances having an anabolic effect and unauthorised substances (Group A) and authorised veterinary drugs and contaminants (Group B). In Group B, B1a relates specifically to aminoglycosides/antibiotics, B1b to insecticides/fungicides/anthelmintics/anti-parasitics, B1c to sedatives, B1d to non-steroidal anti-inflammatory drugs/corticorticoids/glucocorticoids, and B1e to other pharmacologically active substances.

The Department of Agriculture, Food and the Marine and local authorities in Ireland carry out extensive testing as part of the NRCP, which is then published in a report available on the DAFM website. The most recent report details residue monitoring for 2023, in which 2345 samples of meat from Irish food-producing animals were tested for Group B1a substances and 1591 samples for B1b substances. From 250 sheep/goat samples from Group B1a, no sample was positive; however, in Group B1b, 9 samples were positive. Of the 9 suspect samples that were confirmed to be non-compliant, closantel was detected in eight samples and levamisole in one sample. From other animal species, bovine meat was positive for eight antibiotics, one anthelmintic, four nitrofurans, and one thyrostat, and equine meat was positive for phenylbutazone and oxyphenbutazone [30].

As antimicrobial resistance is still a major global concern, it is vital that not only food producers but those working in the human health sector and the veterinary sector cooperate to ensure that antibiotics remain effective to treat illness for years to come. From this research it can be concluded that food needs to be safeguarded from residues of drugs, as a small percentage of food products can be overlooked in regard to testing random samples, as the consumer thinks that the food that they are buying is safe for consumption when it clearly is not.

CONCLUSIONS

The aim of our study was to determine whether there were antibiotic residues present in random sheep meat samples obtained from local sources in Ireland, using two microbial inhibition screening tests, the Explorer 2.0 test coupled to e-Reader and the STAR method. It is clear to see from the results gathered during the study that 2 of 15 samples tested positive for antibiotic residues using the Explorer 2.0 test coupled with an automatic system for reading and interpretation of positive and negative results. By further testing of both positive samples using the STAR method, it was found that based on the sensitivity of the test organisms of the STAR method, both samples were found to be positive for macrolides, beta-lactams, and sulphonamides. These antibiotics are commonly used to treat systemic infections in sheep. Although confirmatory analysis is required to declare a final result, microbial inhibition tests are and remain an effective tool in the screening of antibiotic residues in food of animal origin intended for human consumption.

Data Availability Statement

The raw data of this article will be made available by the authors, without undue reservation.

Ethical Statement

No ethical approval was necessary for this study.

Conflict of Interest

The authors declare no conflicts of interest.

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Conceptualization: I.K., D.H.; methodology: I.K.; sample collection: D.H.; investigation: S.H., D.H.; data collection: D.H.; data curation: I.K.; supervision: I.K.; writing – original draft preparation: D.H., I.K.; writing – review and editing: I.K. All authors have read and agreed to the published version of the manuscript.

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