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Zoological Research and its Role in Shaping Business Innovation

OPEN ACCESS

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Zoological research drives progress in developing new business ideas. Business managers and zoological researchers lead to new biological discoveries that benefit different industries because they develop new medical technology, such as agriculture practices, robotic systems, and energy technologies. Combining animal studies with business operations leads to different types of positive outcomes. Businesses gain better knowledge of complex biological systems to make decisions that improve resource use and product development and promote sustainable business operations.

Modern business leaders recognize the scientific breakthrough from zoological study as it produces robotic machines that help farming and produces energy breakthroughs. It has grown into a real business movement that leads companies in sustainability and innovation to make lasting strategic changes. Our society studies how nature handles problems when trying to solve human issues.

Biomimicry is the practice of learning nature's strategies to solve human challenges. Moreover, zoological research can play a critical role in driving sustainable innovation. As companies seek to reduce their environmental footprint and improve their social responsibility, nature provides a wealth of inspiration. Biomimicry can help develop more efficient resource use, reduce waste, and promote eco-friendly practices throughout the supply chain

Our approach to human problem-solving focuses on studying how nature performs these tasks. Biomimicry can help develop more efficient resource use, reduce waste, and promote eco-friendly practices throughout the supply chain.

Humans took inspiration for Velcro by observing how burrs cling to animal fur and used robotic factories that act based on animal locomotion patterns. All companies follow normal business procedures that include applying zoological sciences.

The technology that promotes vaccine creation is drawn from research on animal defense systems. The behavior of animals helps robotic engineers design drones and autonomous vehicle systems that assist with monitoring and cargo transport activities. Modern power technologies take flight inspiration from birds, and scientists use animal migration behavior to create their essential climate model for sustainability.

As we look to the future, Zoology remains fundamental to creating new business ideas that enhance commercial success. Through natural intelligence, companies can discover better ways to grow, protect our environment, and successfully reach their business targets.

In conclusion, the connection between zoological science and business development will help industries grow and create a sustainable future for every person. Our success depends on using nature's limitless power to reach breakthroughs in industry development.

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Review Article

Abortifacient Diseases in Bovine: A Comprehensive Review

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ABSTRACT

Livestock plays a vital role in the livelihoods of rural communities in developing countries including Pakistan, serving as a major source of income, nutrition, and social capital. The dependency on livestock has multifaceted implications for rural livelihoods and development. On one hand, it provides a source of income stability, particularly for smallholder farmers, landless laborers, and marginalized communities. Livestock also contributes to food security through the provision of milk, meat, and other animal products. There are several problems in the livestock industry one of them is abortifacient diseases that are responsible for pregnancy loss in bovine. Abortifacient diseases pose significant challenges to livestock production worldwide, resulting in substantial economic losses, decreased productivity, and threats to food security. This review provides an overview of the role of infectious diseases responsible for pregnancy loss in livestock specifically bovines, highlighting key pathogens, and modes of transmission. The impact of abortifacient diseases on cattle production extends beyond direct morbidity and mortality. These diseases disrupt reproductive performance, reduce fertility rates, decrease growth rates and impair feed conversion efficiency. In this review, common infectious diseases are described which are responsible for abortion in bovine, and their possible transmission, diagnosis and treatment are described.

INTRODUCTION

In Pakistan and other developing countries, livestock rearing and dairy production are the agricultural sector's principal economic activities. People living in rural areas are mostly dependent on agriculture and livestock. Improvements in reproduction can enhance the profitability of dairy producers. A high rate of pregnancy loss is one of the factors that decrease reproductive performance in some dairy herds [1]. One of the primary causes of decreased fertility is pregnancy loss (PL), which has a negative financial impact on dairy farmers [2, 3].

Dairy cattle abortions increase the expense of reproduction, medical care, feeding, culling, and replacement rates [4]. Pregnancy loss in bovine has both infectious and non-infectious causes [5, 6]. Perhaps the most commonly assumed cause of abortions in humans and domestic animals is infectious agents [7]. Because these pathogenic organisms have the potential to cause significant financial losses, safety precautions and disease prevention are necessary [8]. Early diagnosis can help prevent and treat infectious diseases like trichomoniasis

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and vibriosis. To manage and avoid reproductive issues in their herd, dairy farmers should work with their veterinarians to establish a disease treatment and prevention program [9]. It is difficult to make a confirmatory diagnosis in bovine abortion, primarily when there are insufficient diagnostic tests available in the field [10]. Abortion results impair the ability of dairy cattle to reproduce. Furthermore, a longer postpartum period in cows that experience pregnancy loss may result in a higher culling rate [11].

This study aims to focus on the common bacterial, viral, protozoal and fungal diseases that cause bovine to have reproductive problems. Clinical signs are detailed, along with the diagnostic methods necessary for laboratories to accurately confirm the cause of abortion.

Abortion in Dairy Cattle

Abortion occurs when a pregnancy is ended between 45 and 260 days of gestation, at a point when the ejected fetus is recognizable in size and not viable [12]. Another definition of abortion provided by [13] is the delivery of a fetus before it reaches the stage of viability at which it can be seen with the unaided eye. Certain zoonotic diseases, like brucellosis and leptospirosis, can also induce abortion in cattle [4, 14]. Important infectious agents that have been linked to calf abortions include viruses, bacteria, protozoa, and several fungal species [15]. Furthermore, abortion can result from any illness that raises a fever [16]. Common Abortifacient Diseases in Bovines [17, 18] are shown (Table 1).

Table 1: Common Abortifacient Diseases in Bovine

Diseases	Occurrence of Abortion (Trimester)
Brucellosis	Third Trimester (Usually Around 7th Month)
Leptospirosis	Third Trimesters
Listeriosis	2nd or 3rd Trimesters
Compylobacteriosis	Between 4-7 Months
Trichomoniasis	1st Month to 4th Month
Bovine Viral Diarrhea (BVD)	1st or 2nd Trimesters
Infectious Bovine Rhinotracheitis (Ibr)	4th Month to End of Pregnancy
Neosporosis	2nd or 3rd Trimesters

Brucellosis

Brucellosis is a highly contagious zoonotic infection that affects both humans and animals and is an economically significant disease. It is the most prevalent zoonotic disease in the world [19]. Brucellosis may cause great loss of production through Abortion, stillbirth, orchitis, low herd fertility and decreased milk production [20]. In bovine, *Brucella abortus* causes brucellosis, which results in abortion, weak babies, fetal membrane retention, and low milk production. Usually, an abortion takes place during the seventh month of pregnancy. *Brucella abortus* is the main bovine pathogen [21]. Transmission of brucellosis primarily through fomites or direct contact with infected animals. Numerous pathogens are present in the fluid and fetal membranes of infected fetuses as well as in the vaginal discharges of recently aborted cows, making them a significant source of infection for other animals. Infected wild animals can also spread the disease to domestic livestock [22]. In cattle, brucellosis can also be spread by licking fetal membranes, aborted fetuses, or carrier cattle that have calved normally, as well as by consuming contaminated pasture, feed, and water. However, the mucosa at the conjunctiva and respiratory system often become infected when the skin is injured [23]. It is possible to prevent brucellosis by receiving the RB51 vaccine, in pregnant animals but live vaccines should be discouraged. The optimal time to vaccinate heifers is between 4 and 12 months of age. In certain high-risk circumstances, adult cattle may receive vaccinations. 131 The vaccination typically protects 70–80% of vaccinated animals, but it is not 100% effective. It is possible to implement vaccination and test-and-slaughtering policies on any age of population because Rifampicin-resistant and rough, *Brucella abortus* strain RB51 lacks the lipopolysaccharide o-side chain and does not generate antibodies against it that can be detected by standard serological testing [24].

Campylobacteriosis

Campylobacteriosis in bovine is caused by *Campylobacter fetus*. It can result in infertility, abortion, and early embryonic death in infected animals. Two significant animal pathogens that primarily affect the gastrointestinal and reproductive systems belong to the genus *Campylobacter* [25]. Abortion, embryonic mortality and poor reproductive performance are linked to bovine venereal campylobacteriosis. Cattle reproductive tracts and the internal organs of aborted fetuses have been found to harbour *Campylobacter fetus* subsp. *venerealis (Cfv),* is the causative agent of this STD [25]. Bovine venereal campylobacteriosis is primarily spread by natural means, but it can also spread through artificial insemination (AI) when contaminated equipment or semen from infected bulls is used [26]. Direct transmission is unlikely between female cattle, the spread of infection but when animals are housed together during mounting behaviour, bull-to-bull can happen [27]. Endometritis and salpingitis are the results of the spread of bacteria to the womb and fallopian tubes in heifers as well as cows. After infection, pathology is most noticeable from 8 to 13 weeks later and usually goes away in 4 to 5 months. Though it usually results in a delayed return to oestrus and early embryonic death, infection does not affect conception. Although they can happen at any time, most abortions are discovered between four and six months of pregnancy. In female, the disease usually resolves on its own. The majority of cows recover and become pregnant in three to six months after infection, and

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immunity lasts for several years [28, 29]. Nevertheless, some cows may continue to harbour the infection for much longer [27]. In contrast, the infection in bulls does not cause lesions or the development of protective immunity. The preputial epithelium's crypts can become colonized by the bacteria, and the number and size of these crypts grow with bull age, allowing infection persistence [29, 28]. Investigating a possible case of bovine venereal campylobacteriosis begins with reviewing the reproductive history of the herd, performing a biosecurity audit, and determining whether or not there are any related clinical symptoms. Antigen detection in preputial washings is commonly accomplished by fluorescent antibody tests (FATs) [30]. After a Cfv abortion, secretory IgA (antigen-specific) antibodies can be found in the vaginal mucosa using enzyme-linked immunosorbent assay (ELISA). Although these antibodies have a long half-life, individual animals may experience false reactions due to antibody fluctuations [31]. Vaginal mucus agglutination tests (VMATs) are also frequently employed with a sensitivity of roughly 50%, to identify antibodies in vaginal mucus washings [26]. Bovine venereal campylobacteriosis can also be diagnosed using molecular techniques like sequence analysis and polymerase chain reaction (PCR) [32-34]. Evidence suggests that young bulls can recover spontaneously from bovine venereal campylobacteriosis. Bulls under three years old have reportedly responded well to both local and systemic antibiotic therapy; however, culling older bulls is typically advised. The most commonly used antibiotic is streptomycin, however, reports of C. fetus strains resistant to streptomycin have surfaced [35, 36]. It is not advised to treat infected cows and heifers because of the poor results and the majority of the females develop protective immunity that allows them to resist the disease [28, 29].

Leptospirosis

Leptospirosis is a major cause of meningitis, nephritis, septicemia, hepatitis, and abortion in cattle, especially in young animals. More than 260 antigenically different serovars from 25 serogroups, which are grouped into 9 pathogenic species, 5 intermediate, and 6 saprophytic species of *leptospira,* as well as a gram-negative bacterium from the Spirochaetales order, are the cause of leptospirosis [37]. The most common ways for transmission to happen are through contact with contaminated water, milk, post-abortion discharges, and the urine of infected animals. During pregnancy, cows may transfer bacterium to their womb transplacentally, and infected bulls may spread during coitus. Cattle with endemic reproductive issues due to *Leptospira serovars hardjo* and *pomona* experience abortions, premature births, fetal mummification, stillbirths, retained fetal membranes and the birth of weak calves. Furthermore, a more subdued syndrome marked by early embryonic death and subfertility has been linked to the disease [38]. Most abortions happen during the final trimester of gestation, but certain serovars can result in embryonic death, fetal mummification, or abortions in the second trimester. The abortion rate varies between 3 and 10% for L. hardjo and 50% for L. pomona. Histologically, certain cases show a slight inflammation of the fetal membranes, and certain silver stains may be able to detect the organism's presence. Renal tubular necrosis and interstitial nephritis occur in some fetuses [39]. The reverse transcription polymerase chain reaction (RT-PCR) is the best diagnostic technique to demonstrate leptospira DNA in the kidney of aborted fetuses. In vaccinated animals, serologic diagnosis of leptospirosis may prove difficult on a herd basis. Commonly used microscopic agglutination tests gauge. The quantity of antibodies in the mother's serum during the abortion and again two to three weeks later [40]. Laboratory techniques that are both direct and indirect are used to study animal leptospirosis. The detection is donr by methods that involve isolating the causative agent [41]. The diagnosis of leptospirae in urine or blood has been made directly by dark field microscopic inspection. However, leptospirae are frequently confused with artefacts, and the technique has poor sensitivity and specificity [42]. The identification of particular serum antibodies serves as the foundation for the indirect techniques of leptospirosis investigation. These techniques include the spot agglutination test, indirect immunofluorescence, and various ELISA tests that detect serum antibodies without serovar discrimination, or the microscopic agglutination test (MAT), which accurately detects the infecting serovars. The "gold standard" in serological testing is the microscopic agglutination test (MAT), despite its high labour costs and the need for maintaining multiple leptospiral serovars in the lab. Additionally, the test necessitates that the professional interpret the findings [43].

Bovine Viral Diarrhea

Bovine viral diarrhea (BVD) is a contagious disease caused by the Bovine Viral Diarrhea Virus (BVDV) that can lead to reproductive issues such as abortion, fetal malformations, and infertility in cattle. There is a significant economic threat from the BVDV to cattle worldwide. According to [44], it is a major factor in the reduced milk yield, reproductive issues, and diarrhea in affected herds. This virus is closely related to this *pestivirus,* which belongs to the *Flaviviridae family* [45]. The illness affects people and animals globally, with some animals experiencing subclinical infections [46]. Embryonic death may occur from fetal infection up to 45 days into pregnancy. Between 45 and 175 days of pregnancy, an abortion may happen after contracting cytopathic bovine viral diarrhea virus

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persistently infected (PI) if non-cytopathic bovine viral diarrhea virus (ncpBVDV) infection happens before the fetus develops immune competence, which is typically between days 45 and 145. Most new acute and fetal infections originate from These PI animals that shed the virus through a range of bodily fluids, including semen, and typically do not develop BVDV antibodies. Premature death is the primary cause of mortality for most PI animals, with mucosal diseases resulting from ncpBVDV mutating into cpBVDV and superinfection occurring frequently. Numerous techniques have been developed to identify cattle infected with BVDV [45]. Among these are the isolation of viruses in the kidney, lung, testis, and other tissues of cows. Because serological tests, like ELISA, are utilized to ascertain the serostatus of a large number of animals sampled in a population, they are frequently utilized in exploratory studies [45].

Infectious Bovine Rhinotracheitis (IBR)

Infectious bovine rhinotracheitis (IBR) is caused by Bovine Herpesvirus 1 (BHV-1). Infection with IBR virus can lead to abortion and infertility in cattle. This infection is the primary cause of abortion among viral diseases in cattle is which has a 5–60% abortion rate in unvaccinated herds. Cattle populations are endemic to BHV-1, which causes several clinical syndromes such as conjunctivitis, respiratory disorders, encephalomyelitis, abortion, vulvovaginitis, balanoposthitis and fatal systemic infections in newborns [46]. All strain of BHV-1 has the potential to develop into a latent infection. A latently infected animal's semen or respiratory and reproductive secretions may contain virus particles due to stressful events such as calving, transportation, corticosteroid treatment, and other stressful situations. Following infection, BHV-1 in pregnant cows may remain latent in the placenta and only become active against the fetus after a few weeks. After becoming infected, the fetus dies rapidly and experiences autolysis before being expelled after remaining in the womb for several days. There is reddish-tinged edema in the subcutis. The pericardium, as well as the abdominal and thoracic cavities, contain large volumes of reddish-pink fluid [39]. The identification of BHV-1 can be achieved through several techniques such as real-time PCR, isolation of virus from fetal liver, lung and other tissues, EDTA-treated whole-blood samples, or adult animal semen [47].

Trichomoniasis

Trichomoniasis is caused by the protozoan *Tritrichomonas foetus.* It primarily affects the cattle's reproductive tract Trichomoniasis is a venereal disease that primarily affects pregnant animals. It can also occasionally cause abortion and pyometra, which can result in severe infertility. It is found worldwide and third most common cause of abortion in cattle, behind leptospirosis and brucellosis [48, 49]. When this organism is present, neither the bull nor the cow shows any symptoms of illness. Pyometra and early abortions (1-6 weeks) are also signs of the infection. Sometimes an infection prevents an abortion from occurring, and a healthy, full-term calf is born instead [50]. After an abortion, parasites typically vanish from vaginal discharges within seven days, and the aborted foetus is typically fresh [51]. For confirmatory diagnosis demonstration of *T. foetus* organisms from specimens taken from female cattle's genital tract, bull prepucial material or aborted foetal, and placental tissues confirms the diagnosis [52]. Additional diagnostic techniques comprise organism culture, PCR, and immunological testing [53, 54]. Trichomoniasis affects non-pregnant cows with involuted uteruses. Many cows experience an improvement in fertility and some level of immunity after three or five cycles of sexual rest. However, males who contract the infection become lifelong carriers of the pathogen; as a result, only clean bulls or semen should be used in breeding, and cows with deviant genital tracts should be put down [47]. "Carrier" bulls should be culled since they have the potential to infect susceptible, healed, and treated females again. As for the treatment of trichomoniasis in cattle, there isn't an approved chemotherapy drug [54].

Listeriosis

The infectious disease listeriosis is found all over the world and can affect both humans and animals. The three primary clinical signs are septicemia, meningoencephalitis, and abortion [55]. The majority of clinical cases have an infection with *Listeria monocytogenes. L. ivanovii* has only been linked to a small number of documented cases [16]. There are numerous species of Listeria in the environment. Silage that has not been properly preserved, fermented, or acidified enough to kill the bacteria can contain listeria. *Listeria monocytogenes* causes septicemia, encephalitis, and abortions in cattle. The late winter or early spring is when listeria infections and abortions typically occur. Abortions are most frequently detected in the final trimester of pregnancy [56]. Although ingestion is the primary route from where Listeria spp. enter the body, they can also get in through the respiratory system, or they can be injected into broken skin or eyes. *Listeria monocytogenes* nucleic acids and antigens have been detected in the placenta, fetus (such as the contents of the fetal stomach), or uterine discharges after an abortion; in the blood of animals suffering from sepsis; in samples taken from sites of localization, such as ocular swabs or cerebrospinal fluid (CSF); and in postmortem tissue samples, including the liver, kidneys, spleen, and brain. PCR can be used to detect nucleic acids in tissues and secretions. For *L. monocytogenes* and *L. ivanovii,* loop-

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been published. Antigens in tissues can be found using immunohistochemistry or immunofluorescence.

Neosporosis

Neosporosis is a protozoal disease caused by the *neospora caninum*.There are structural and genetic similarities between this parasitic protozoan and *Toxoplasma gondii.* The two species of *Neospora* that are currently known to exist are *Neospora caninum*. Cattle are among the intermediate hosts of *N. caninum,* with dogs serving as the definitive host [57, 58]. Oocysts excreted in dog feces can contaminate feed and water, or cattle can contract the infection congenitally [59, 60]. According to reports from populations of beef and dairy cattle worldwide, this parasite is the main cause of abortion and neonatal mortality [61, 62]. *Neospora caninum* can cause abortions in cattle as early as three months of gestation, although they happen most frequently between five and six months. It has been reported that abortion storms in cows or endemic abortions are connected to *neospora.* An important factor in the epidemiology of neosporosis is congenital/vertical transmission from seropositive dams to their offspring, and the incidence of abortion is frequently repeated in subsequent gestations [63, 64]. These include the histopathology of tissues from stillbirths and aborted fetuses, the extraction of parasites from sacrificed animals, the inoculation of mice, the use of molecular techniques like PCR, and the recovery of oocysts from dog feces. Nonetheless, because serology can be performed both antemortem and postmortem, it is the most widely used method for diagnosing neosporosis (ELISA and immune-fluorescent antibody test). Since serology is utilized to accurately test exposure and infection in populations of numerous animals, it is helpful in epidemiological studies [63].

DIAGNOSTIC METHODS OF BOVINE ABORTION

When diagnosing abortion in dairy animals, general guidelines include gathering all epidemiological information, including recent introductions into the farm, counting the impacted animals, closely inspecting the affected dam or dams, and gathering the ejected fetus and placenta for microbiological and pathological analysis. A diagnosis is then made by compiling and analyzing the data [16]. However, due to the wide variety of pathogens involved and the possibility of factors affecting the dam, fetus, and placenta, the diagnostic rate for bovine abortions is extremely low [61, 65]. Additionally, an abortion frequently happens after an initial infection that may have persisted for a few weeks or months; by the time the abortion takes place, the etiology is frequently undetectable. The issue is further exacerbated by the high expense of laboratory testing required to help diagnose bovine abortion [66]. An organized herd's records are frequently helpful when looking into abortion-related issues [67]. It is necessary to remember that there are many different reasons why cattle abort, making diagnosis difficult in many cases [67]. To improve the interpretation of laboratory results, the field of investigation could be

CONCLUSIONS

Abortion is one of the necessary reproductive health issues faced by dairy cows when it comes to the financial effects. In cattle, abortion can be caused by infectious or non-infectious agents. Hereditary and non-genetic disorders are examples of non-infectious variables. Stress-related to heat, production, changing seasons, and seasonal effects is the non-genetic causes of abortion, neosporosis, leptospirosis, listeriosis, brucellosis, and bovine viral diarrhea are among the common infectious causes of abortion in cattle. These infectious diseases not only result in direct economic losses due to abortion and reduced productivity but also pose public health risks. Control measures are necessary to prevent abortion because these causes can lead to significant economic losses. The main focus of attention of this review is on the infectious causes of abortion. The causes of abortion should be investigated, and controls should be put in place.

Authors Contribution

Conceptualization: SAL, MR, AS, FURS, FH, TM, IU, AR
Methodology: SAL
Formal analysis: QK, MMR
Writing review and editing: SAL, AS, FURS, FH, TM, IU, AR

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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Review Article

Global Antimicrobial Resistance: Strategies and Challenges

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ABSTRACT

Antimicrobial resistance (AMR) is a critical health challenge worldwide, that arises when bacteria, viruses, fungi, and parasites become resistant to antimicrobial medications, making diseases more challenging to treat. The enzymatic breakdown of antibiotics, modifications to target locations, elevated efflux pumps, and changes in cell membrane permeability are some of the processes behind AMR. A key factor behind the development and transmission of AMR has been the unregulated use of livestock feed for preventive purposes. Rapid global expansion of antibiotic-resistant bacteria is posing a hidden pandemic risk to public health and demanding immediate action. The misuse and unnecessary overreliance on antibiotics in human medicine is one of the many contributing factors of AMR, veterinary practices, and agriculture, as well as inadequate infection prevention strategies, lack of diagnostic tools, and inadequate sanitation. Preventive measures against AMR involve promoting the rational use of antibiotics through antimicrobial stewardship, improving infection control practices, advancing rapid diagnostic technologies, reducing antibiotic use in food production, and increasing public awareness. Efforts must also focus on global collaboration to monitor resistance trends, enhance regulatory frameworks, and invest in research to develop novel antimicrobial agents and alternative therapies. Addressing AMR requires an interdisciplinary and coordinated approach to safeguard the efficacy of current antimicrobial treatments and reduce the occurrence of resistance.

INTRODUCTION

Antimicrobial resistance (AMR) has serious economic ramifications and is acknowledged as a major worldwide health problem for both people and animals [1]. This can cause infections to remain in the body longer and increase the chance that they will spread to other people. Due to prolonged illness, antimicrobial resistance is also responsible for rising treatment costs and decreasing labor productivity [2]. Examples of antimicrobial chemicals that may be employed against microorganisms to limit their growth potential, stop their multiplication, or even kill them include antibiotics, disinfectants, and food preservatives [3]. Bacterial resistance is considered a major threat in healthcare institutions. With the widespread use of antibiotics, there is a greater chance that bacteria may become resistant to them in more sophisticated ways. Some recently changed strains seem to have reduced the chances that the patients would respond appropriately to the therapies, which has serious repercussions that might lead to clinical problems or morbidity and death [4, 5]. It is estimated that drug-

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resistant illnesses cause 700,000 fatalities globally and 25,000 within the European Union (EU). If nothing is done, this number is predicted to rise to 10 million deaths annually by 2050 [6]. Antimicrobial resistance also contributes to increased treatment costs and decreased worker productivity because of extended sickness. AMR is thought to cost the EU EUR 1.5 billion annually in lost productivity and medical expenses. Drug-resistant diseases are predicted to have a cumulatively detrimental effect on the global economy of USD 100 trillion by 2050 [2, 6]. However, bacteria developed antimicrobial resistance (AMR) as a result of the prolonged, widespread usage of antibiotics. Because of the contradictory degrees of spontaneous genetic development, the emergence of antimicrobial resistance (AMR) is a significant worldwide health problem in the twenty-first century. Therefore, early intervention is required [7]. Antibiotic resistance in bacteria reduces the effectiveness of antibiotic use in healthcare, and there is strong evidence that suggests that the improper use of antibiotics may ultimately encourage the growth and spread of antibiotic-resistant bacteria [8]. Numerous synthetic, semi-synthetic, and natural chemicals have distinct mechanisms that can profoundly impact physiological and metabolic functions. Instances of these substances include β-lactams and glycopeptides, which change how cell walls are made; macrolides and tetracyclines, which prevent proteins from being made; sulfonamides, which stop metabolic processes; and fluoroquinolones, which stop DNA replication and translation [5]. Global occurrence of antimicrobial resistance is investigated [9] (Figure 1).

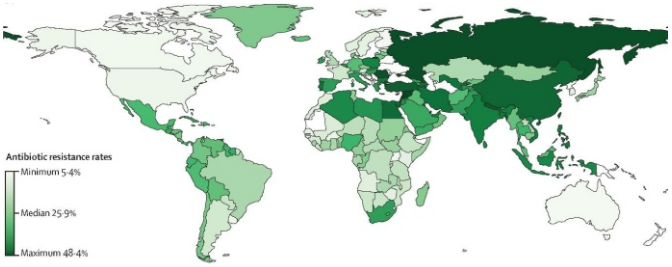

Figure 1: Global Occurrence of Antimicrobial Resistance

Mode of Action of Antibiotics

The vast majority of antimicrobial drugs used to treat bacterial diseases may be categorized according to their main mechanism of action. The four primary mechanisms of antibiotic action include blocking cell wall synthesis, inhibiting protein production, disrupting nucleic acid synthesis, and interfering with metabolic pathways [10]. Beta lactams, which include penicillins, cephalosporins, carbapenems, and monobactams, and glycopeptides, which include teicoplanin and vancomycin, are examples of antimicrobial medications that function by stopping the bacterial cell wall from being produced [10, 11]. By inhibiting the enzymes needed for the development of the peptidoglycan layer, beta-lactam drugs stop the bacterial cell wall from being produced [11]. By binding to the last D-alanine residues of the growing peptidoglycan chain, tecoplanin and vancomycin also prevent the development of cell walls.The cross-linking mechanisms required to form stable cell walls are halted as a result [3]. Macrolides, aminoglycosides, tetracyclines, chloramphenicol, streptogramins, and oxazolidinones are antibacterial agents because they inhibit protein synthesis [10, 11]. Ribosomes in bacterial and eukaryotic cells have different structures. Antibacterial medications target and prevent the growth of certain microbes by using these distinctions. The ribosome's 30S subunit is bound by tetracyclines, aminoglycosides, and macrolides, whereas the 50S subunit is bound by chloramphenicol [3]. Since fluoroquinolones interfere with DNA synthesis and induce deadly double-strand breaks during replication, they have antibacterial qualities [12]. Trimethoprim (TMP) and sulfonamides, however, block the route that produces folic acid, which halts the synthesis of DNA [13, 14]. In a common combination of antibacterial drugs, the sulfonamides sulfamethoxazole (SMX) and the folic acid analogue TMP block two steps in the bacterial folate synthesis enzymatic pathway. One possible fifth, less obvious mode of action is disruption of the structure of the bacterial membrane. Leakage of bacterial compounds out of membranes is assumed to be the mechanism by which polymyxins exert their inhibitory effects. The lipid tail of the cyclic lipopeptide daptomycin seems to pierce the bacterial cell membrane, leading to membrane depolarization and ultimately the bacterium's death [15] (Table 1).

Table 1: Class of Antibiotics Along with Their Mode of Action

Mode of Action	Antibiotics
Inhibitors of Cell Wall Synthesis	Penicillins, cephalosporins, carbapenems, and monobactams are examples of beta-lactam antibiotics
	Glycopeptides: teicoplanin and vancomycin
Inhibition of Protein Synthesis	Linezolid, quinupristin-dalfopristin, clindamycin, macrolides, and chloramphenicol attach to the 50S ribosomal subunit.
	Bind to the 30S ribosomal subunit: Tetracyclines, aminoglycosides
	Mupirocin attaches itself to the isoleucyl-tRNA synthetase in bacteria.
Disruption of Nucleic Acid Synthesis	Fluoroquinolones inhibit the production of DNA
	Rifampin inhibits the production of RNA
Blocking Metabolic Pathways	Sulfonamides and analogues of folic acid
Disordering of Bacterial Membrane Structure	Daptomycin and polymyxins

Mechanisms of Resistance

Antibiotics primarily target four key components in bacterial cells: protein synthesis, nucleic acid synthesis, the cell wall, and the cell membrane. The fundamental mechanisms of antimicrobial resistance include restricted

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drug uptake, modification of drug targets, drug inactivation, and increased active drug efflux. To develop acquired resistance, bacteria often employ strategies such as modifying drug targets, inactivating drugs, and expelling them through efflux mechanisms. In contrast, intrinsic resistance primarily arises from restricted drug uptake, drug inactivation, and active drug efflux [16]. Due to structural variations, gram-positive and gram-negative bacteria exhibit different mechanisms of drug resistance. Gram-positive bacteria, lacking a lipopolysaccharide (LPS) outer membrane and having a reduced capacity for efflux mechanisms against certain drug types, are less likely to rely on this approach to restrict drug absorption [17, 18]. However, studies have revealed that all four main drug resistance pathways are employed by gram-negative bacteria [18].

Inactivation of Drug

Certain bacterial species can inactivate antibiotics, resulting in drug resistance through two main mechanisms: either by breaking down the antibiotic or by attaching a chemical group to it. This can result in drug resistance. Members of the enterobacterales family generate hydrolyzing enzymes called beta-lactamases, which can render antibiotics that include beta-lactams inactive. Beta-lactamases target penicillin-binding proteins and disrupt their ability to interact with the Beta-lactam ring structure by breaking it at a specific site, ultimately inactivating them as penicillinases and cephalosporinases [16]. It is well known that several gram-positive bacteria, such as *Enterococcus faecalis*, *Staphylococcus aureus*, and *Enterococcus faecium* as well as other members of the Enterobacterales family, have beta-lactamase genes that are passed down by horizontal gene transfer. Additionally, the tet (X) gene in some bacteria produces an enzyme that hydrolyzes tetracycline [19]. The most often transferred chemical groups for pharmaceutical inactivation are adenyl, phosphoryl, and acetyl groups. The most often used technique to combat chloramphenicol is acetylation, fluoroquinolones, aminoglycosides, and streptogramins, whereas adenylation and phosphorylation are reported to be the most commonly used methods against aminoglycosides [18].

Drug Target Modification

The targets required for medication binding can be altered by bacteria, which results in either a poor or nonexistent drug attachment. This modification occurs due to spontaneous mutations in the gene or genes responsible for encoding the therapeutic target protein. In both gram-positive and gram-negative bacteria, fluoroquinolone resistance arises when mutations occur in the quinolone-resistance determining region (QRDR) of DNA gyrase (topoisomerase II and topoisomerase IV)[20]. Another target alteration method that is believed to be a highly successful means of establishing resistance is methylation. Methylation, as seen with erm methylases conferring resistance to lincosamides, macrolides, and streptogramin B antibiotics, occurs in both gram-positive and gram-negative bacteria. Additionally, the CFR gene has been associated with resistance in various bacterial species, including *E. coli, Proteus vulgaris, Staphylococcus, Enterococcus,* and *Bacillus* [21]. The mecA and mecC genes create a unique penicillin-binding protein that causes *Staphylococcus* species to show their affinity for beta-lactam medications has significantly decreased [22, 23].

Limiting Drug Uptake

In gram-negative bacteria, the outer membrane primarily includes lipopolysaccharide, a highly acylated glycolipid that prevents several substances, including antibiotics, from passing through. Moreover, changes in outer membrane protein permeability, particularly porin proteins, can contribute to acquired resistance to drugs. For hydrophilic antibiotics such as chloramphenicol, tetracyclines, fluoroquinolones, and beta-lactams, porins are the main point of entry. Bacterial susceptibility to antibiotics is influenced by the number and type of porin proteins, which also affect how these antibiotics enter the bacterial cell [24]. Furthermore, mutations that affect the function or expression of these porin proteins may lead to acquired resistance to antibiotics. There is an increase in resistance when mutations altering porin expression are coupled with additional mechanisms, such as efflux pumps or enzymatic antibiotic degradation [25]. Biofilm formation is another method in which some bacteria show antibiotic resistance. These include, among others, *Proteus mirabilis, Klebsiella pneumoniae, E. coli, Pseudomonas aeruginosa, Enterococcus faecalis, Streptococcus vitridans, Staphylococcus aureus,* and *Staphylococcus epidermidis.* A group of microbial cells immersed in their exopolysaccharide and attached to biotic or abiotic surfaces is called a biofilm. Among other ways, it is known to prevent antibiotics from penetrating, giving microorganisms resistance and tolerance to them. Additionally, it may stop antibiotics from developing at bactericidal concentrations across the whole biofilm [26, 27].

Efflux of Drug/ Decreasing Permeability

Efflux pumps are an active transport mechanism that helps bacteria resist aminoglycosides by removing the antibiotic from their cells. However, because of the aminoglycoside polycationic structures, there are only a few functional efflux pumps [28]. The cytoplasmic membrane contains an energy-dependent efflux pump that bacteria use to control the accumulation of antimicrobial agents, including antibiotics, within their cells. Efflux pumps help bacteria keep their internal environment stable by eliminating

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harmful compounds from within the cell, such as metabolites, antibiotics, and quorum-sensing signalling molecules. In the 1980s, scientists found that the first plasmid-encoded efflux pump in *Escherichia coli* was responsible for eliminating tetracycline from the bacterial cell. Since then, several resistant bacteria, both gram-positive and gram-negative, with various efflux pathways have been discovered. It is noteworthy that the majority of efflux systems rely on chromosomally encoded multidrug efflux mechanisms to guarantee intrinsic drug resistance in bacteria [29, 30]. Instead, genes on mobile genetic elements are more likely to be linked to efflux pumps that are unique to a given substrate, including those for tetracyclines, macrolides, and chloramphenicol [18]. The structure and energy source determines which of the six different drug efflux pumps are present. The ATP-binding cassette (ABC), resistance nodulation–division (RND), drug metabolite transporter (DMT), major facilitator (MFS), small multidrug resistance (SMR), and toxic compound extrusion (MATE) are among its super-families. Typically, a periplasmic protein, an outer membrane protein channel, and a cytoplasmic membrane pump comprise the RND superfamily, which includes the most clinically significant efflux systems in gram-negative bacteria [19]. Gram-positive bacteria, on the other hand, have most of their efflux pumps encoded by chromosomal genes or carried on plasmids, and they are members of the ABC and MFS families.

Causes of Antimicrobial Resistance

Various mechanisms contribute to antimicrobial resistance, including intrinsic traits of the microorganisms and a wide range of environmental variables affecting both prescribers and consumers. Antimicrobial resistance (AMR) factors can be broadly classified into subsections; environmental aspects (overcrowding, rapid transmission through mass travel, inadequate sanitation, ineffective infection control programs, and extensive agricultural use); drug-related issues (including counterfeit, substandard, and easily accessible over-the-counter medications); patient-related factors (such as non-adherence to treatment, poverty, lack of education, self-medication, and misconceptions); and physician-related contributors (which may involve any combination of these elements), medical and veterinary misuse (self-medication, overuse in agriculture) and economic and social factors (poverty, lack of education). Although there are many causes of antimicrobial resistance, the following are the most prominent ones.

Misuse or Unnecessary Use of Antibiotics

Antibiotic resistance occurs naturally, but its advancement has been significantly accelerated by the overuse of medications in both humans and animals. According to epidemiological studies, antibiotic use and the rise in bacterial resistance are causally related [31]. Despite repeated warnings from health groups, antibiotic misuse and abuse continue at a disproportionate rate worldwide, suggesting that the current situation is irreparable. Research has indicated that individuals worldwide, especially those from less educated backgrounds, hold misunderstandings and incorrect ideas regarding antibiotics. One such myth is that most viral diseases, such as the flu and the common cold, can be cured with medications. Additionally, antibiotics are commonly recommended as part of patient care; this is particularly true in many impoverished countries with limited diagnostic resources [32]. One such example of misuse is the administration of antibiotics without a clear indication. The accessibility of antibiotics as over-the-counter (OTC) drugs for both animals and humans contributes to the emergence and transmission of drug-resistant infections. Antibiotic abuse is also exacerbated by the absence of standard treatment guidelines and antibiotic policies, which are prevalent in developing countries, and by the over-prescription of antibiotics by veterinarians, pharmacy owners, and health professionals in many developing and underdeveloped countries [16]. The supply chain's use of subpar or low-quality medicines has made the antimicrobial resistance (AMR) problem worse in many developing nations. Antibacterial resistance may also result from giving antibiotics at the incorrect dosage or from providing long courses of treatment. Even though it is unethical, many doctors, particularly in developing nations, prescribe antibiotics without a prescription to meet patient expectations and occasionally receive financial incentives from pharmaceutical companies [33, 32] One more thing that contributes to resistance is drug self-medication [34, 35]. The illegal drug trade, particularly in nations like South and Central America, Asia, Europe, and Africa, lends support to this [7, 36]. Ineffective control measures would lead to drug addiction and easy access to less expensive medications [37].

Inappropriate Prescribing Patterns

Inappropriate administration of antibiotics has a major impact on the development of AMR [38]. Examples of "inappropriate antibiotic prescribing" include prescribing antibiotics when they are not needed, choosing the wrong medications, or administering antibiotics at the wrong dose and for the wrong amount of time [39]. A survey found that 50% of patients were prescribed antibiotics at least once while in the hospital without a valid reason. Approximately one-third of hospitalized patients were prescribed antibiotics without the required testing, and these prescriptions were kept on file for a longer period [40]. Antibiotic introduction should preferably be guided by previous bacterial isolation and antimicrobial sensitivity testing. In assisted living facilities, where around 75% of

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antibiotic prescriptions are written erroneously, with wrong dosages and time limits, the situation is even worse [41].

Use of Antibiotics in Animals

Because of several factors, including the recent growth in demand for animal protein, the usage of antibacterial medication in the livestock industry has expanded dramatically in the majority of developing countries. Antibiotic residues that can be found in animal-derived products, including muscle, liver, kidney, fat, eggs, and milk, are also contributing to the problem of antimicrobial resistance (AMR). Antibiotics can be used to prevent disease, prepare animal feed to promote growth, cure animal illnesses, and increase feed conversion efficiency, among other uses [42]. This strategy is more common in developing nations and has contributed significantly to the rise of AMR in humans due to a lack of government oversight and the need to increase revenue from food animal farms [43]. About 70% of the antibiotics that are sold in the United States for medicinal purposes are meant for use in animals [44]. There are significant issues when antibiotics given for human use are either closely related to or identical to those used in animal medicine in terms of their kinds, functions, and modes of action.

Easy Traveling Routes

There is compelling evidence that human migration has a major impact on the emergence and global dissemination of antibiotic-resistant bacteria. Convenient, contemporary transportation routes that are available to people, animals, and goods also have a big impact on AMR's global spread [45]. Travelers are likely to unintentionally bring antimicrobial-resistant organisms back to their home countries from their travel destinations. Research has shown that travelling to regions with high AMR prevalence can result in the presence of antibiotic-resistant bacteria in the human body can persist for up to 12 months, increasing the risk of spreading to vulnerable individuals [46].

Lack of Information

There is compelling evidence that the public and healthcare workers (HCWs) lack knowledge of the processes underlying antibiotic resistance and the proper use of antibiotics [47]. Surveillance is required to assess the extent of the antimicrobial resistance (AMR) issue and to create intervention plans such as antimicrobial stewardship. Regretfully, precise statistical information about the frequency of AMR and antibiotic use in the global agriculture and healthcare industries has not yet been obtained [47]. Surveillance data helps identify areas that should be the focus of strategic initiatives to achieve the greatest results and provides crucial information. Before implementing effective intervention strategies, several stakeholders must work together to close the existing knowledge gap (e.g., agriculture and animal production companies, consumers, international organizations, and the human and veterinary care sectors).

Strategies for Prevention of Antimicrobial Resistance (AMR)

To combat the growing threat of antimicrobial resistance (AMR), a multifaceted approach is essential. First, antibiotic stewardship programs must be implemented to promote the rational use of antibiotics, ensuring they are prescribed only when necessary and in the correct dosage and duration. Second, infection prevention and control (IPC) measures, such as improved hygiene, vaccination, and hospital sanitation, can reduce the spread of resistant pathogens. Third, public awareness campaigns are essential to educate communities about the dangers of misuse and overuse of antibiotics. Additionally, investing in research and development for new antibiotics, vaccines, and alternative therapies is vital to stay ahead of resistant strains. Public awareness campaigns and global collaboration among healthcare providers, veterinarians, policymakers, and farmers ensure a collective effort in combating AMR, preserving the effectiveness of life-saving drugs for future generations [47, 48]. Further details regarding AMR prevention is provided below.

Infection Control and Prevention

Preventing infectious illnesses is the best course of action since it stops the growth and spread of resistant bacteria and the need for medication. Monitoring prescription trends designing and enforcing effective infection prevention measures procedures, and teaching healthcare facilities like primary care clinics on how to use antibiotics appropriately are all ways to prevent antimicrobial-resistant infections. Antibiotic resistance can be prevented by tracking changes in resistance patterns, which requires identifying and tracking the main sources of antibiotic-resistant characteristics [48]. Monitoring and measuring food-borne illnesses, looking into outbreaks, teaching people how to handle food safely, identifying high-risk individuals, and encouraging hand-washing practices can all help to drastically slow the spread of infections. An efficient way to monitor patients and contacts who are at risk is through contact tracing. This guarantees that those who are vulnerable are appropriately recognized and given the required treatments.

Preventing Misuse of Antibiotics

The use of efficient evidence-based disease diagnosis and treatment techniques should be one component of improving antibiotic prescribing practices. Healthcare providers might be held responsible for improving patient safety by implementing such tactics. In addition, it is imperative to enact evidence-based policies to discourage the unnecessary prescription of antibiotics and guarantee the efficient execution of suggested policies. Physicians

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should be observed and their prescription practices should be regularly reviewed. To ensure optimal prescription optimization, patients and clinicians should instead receive educational support. The Get Smart initiative is one such successful tactic of center for Disease Control and Prevention. This program takes action to educate legislators, patients, and healthcare professionals about the severity and consequences of antibiotic misuse. It also supports state-based initiatives in this regard [47-49].

Antimicrobial Stewardship Program (ASP)

Antimicrobial stewardship involves a systematic and collaborative approach that efforts to stop the spread of microbial resistance by educating and convincing prescribers to use antimicrobial agents according to the right choice, dosage, and duration for better patient outcomes. Ensuring that healthcare professionals prescribe the best antibiotic at the right dose and duration for each patient is the primary objective of antimicrobial stewardship. The second objective is to stop antibiotics from being overused, abused, and misused. Limiting the development of resistance is the third objective. The two main overlapping strategies for accomplishing antimicrobial stewardship's main objectives are: Antibiotics can be used in two ways: (1) to enhance healthcare results; and (2) to provide long-term access for everyone who needs them. The Centers for Disease Control and Prevention (CDC) released the "Core Elements" of antimicrobial stewardship in 2014 to achieve these goals. All hospitals, regardless of size, can follow these principles, which include specific advice to support small and critical-access hospitals in their execution [50, 51].

Promotion of Education and Innovation

Stakeholder awareness can be raised by implementing creative educational programs that enhance and implement successful public health strategies. The mechanism, causes, effects on health, and hazards of antibiotic resistance should also be emphasized in educational programs. However, fact sheets, posters, or videos should be used to promote effective communication among the stakeholders. To prevent resistance, the importance of early recognition must be emphasized [47]. Therefore, information modules in this regard need to be provided to clinicians, health organizations, and health providers. Furthermore, since early intervention depends on the identification of novel resistant strains of microbes, it is imperative to support the development of cutting-edge technologies, laboratories, research projects, and instruments. Public-private partnerships can be introduced to facilitate the development of novel antimicrobial drugs. The legal obstacles that pharmaceutical companies face when conducting research and developing new medications may be addressed by introducing changes to the enforcement. It may be possible to support pharmaceutical companies by taking steps like streamlining regulatory approval or allocating additional funds for research [52, 53].

Establishing Checkpoints

To stop the unauthorized sale of antibiotics and the practice of self-medication, certain checkpoints should be established. The reason for this is that medications are sold in pharmacies without a prescription from a doctor. It is necessary to introduce legislation to restrict this unauthorized sale [54, 55]. Furthermore, a variety of factors, including the patient's wishes and presentation, affect the decision to prescribe antibiotics. Prescriptions should therefore be based on information rather than the preferences of the patient. It is effective for doctors to prescribe antibiotics at first consultations, but to postpone the patient's drug intake until the development of clinical symptoms. Information submitted to this network by surveillance organizations and institutions enables the surveillance and targeting of microorganisms that cause diseases linked to healthcare. Thus, it is critical to ensure the efficient execution of this network in healthcare environments such as nursing homes and hospitals. Clinicians must acknowledge the significance of conducting essential tests to identify resistant bacterial strains and resistance patterns that may have a substantial impact on patient outcomes [48].

CONCLUSIONS

Antimicrobial resistance (AMR), a significant global health issue that makes treating infections more challenging, poses a danger to the efficacy of medications. Bacterial genetic alterations or the Resistance genes acquired from other bacteria, typically via horizontal gene transfer, are the causes of AMR. These techniques allow bacteria to alter drug targets, neutralize antibiotics, or increase drug efflux, rendering treatment ineffective. AMR has several contributing components. Among the main causes are the overuse and abuse of antibiotics in veterinary and human medicine, insufficient infection control, inadequate personal cleanliness, and the use of antibiotics in agriculture to promote growth. Additionally, the problem has been made worse by the absence of new antibiotic research. To prevent AMR, coordinated preventative measures are required. Antibiotic usage in food production should be decreased, infection prevention and control (IPC) regulations should be strengthened, antimicrobial stewardship programs should be implemented to encourage the responsible use of antibiotics, and sanitation and hygiene should be improved.

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All authors have read and agreed to the published version of the manuscript.

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Review Article

Mealworms *(Tenebrio molitor L.)* as a Substituent of Protein Source for Fisheries and Aquaculture: A Mini Review

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ABSTRACT

Development of aquaculture and fisheries depend on the availability of sustainable feed sources. Using insects is one new option that is gaining popularity. The high protein content, rapid growth and little environmental impact of yellow mealworms have made them an appealing solution. Mealworms contain a substantial protein content (47-64%), essential amino acids, energy-dense lipids, and vital micronutrients, making them a viable substitute for traditional protein sources in fisheries and aquaculture. Historically consumed in various cultures, Mealworms have gained attention for their economic and nutritional value. In aquaculture, they enhance fish growth, feed efficiency, and overall health. Studies have shown that incorporating mealworm meal into fish diets improves immune functions, growth performance, and liver health, although excessive inclusion may have adverse effects. The nutritional content of mealworms can be optimized by adjusting rearing conditions, diet, and developmental stage. The European Union's approval of mealworms for human consumption further validates their safety and potential as a sustainable protein source. This review emphasizes the nutritional benefits of mealworms as a substitute for fishmeal, addressing protein shortages and environmental concerns associated with traditional feed production. By integrating mealworms into aquaculture, we can significantly improve sustainability, economic efficiency, and global food security.

INTRODUCTION

Insects from phylum Arthropoda are the most abundant animals in the world.They can reproduce rapidly and have large populations, serving as the most valuable source for human benefits.Because of their high protein, fat, and mineral content, are seen as a potential source of food and ingredients that can improve the quality of a wide range of food products. Dicke M in 2018 and Sabri NS *et al.*, in 2023 highlighted insects can also produce other useful products such as wax, dyes, silk, and also honey, royal jelly, and propolis that have various nutritional and functional values [1, 2]. Liceaga AM *et al.*, in 2022 and van der Fels-Klerx HJ *et al.*, in 2018 highlighted the concept of insect eating, or entomophagy is gaining interest as sustainable and nutritious alternative to traditional food sources.People have been eating insects for thousands of years before they had the means to hunt or cultivate, particularly in warmer regions of the planet where they could always find a variety of insects [3,4].Van der Fels-Klerx HJ *et al.*, in 2018 emphasized in Asia, Africa, Latin America (including Mexico), and Australia, there are 2000 and above insect species are thought to be consumed.In many areas, insects constitute a common component of the human diet, with significant cultural and gastronomic significance [4]. However, in Europe and other industrialized countries, entomophagy is often viewed as nasty [4].Baiano A 2020 and Liceaga AM *et al.*, 2022 pointed out China, Mexico, India, and Thailand are the countries in which highest numbers of edible insects have been reported [5, 6].

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Baiano A *et al.*, in 2020 underlined the consumption of insects is rare or even taboo in Western nations, despite compelling evidence of entomophagy in both ancient and modern times [5]. Hartmann C *et al.*, in 2017 and Garofalo C *et al.*, in 2019 outlined a number of research studies and evaluations focused on the European perspective on edible insects highlighting the overlooked potential of eating insects and need to reduce meat consumption and replace it with other protein sources [6, 7].The insects are used due to shortage of proteins, food security, cultural traditions, and environmental factors and for medicinal importance because they contain high protein content compounds and rich in nutritional value for feed and food.Furthermore, the nutritional content of insect is much better than that of plants both in qualitative and quantitative manner.The practice of consuming insects (entomophagy) as a source of nutrition has a rich history with archaeological and ethnographic records indicating historical use of insects. The ancient civilizations such as Aztecs, Mayans, Chinese, Greeks and Romans and Cambodian incorporated crickets, mealworms, locusts, silkworms and grasshoppers into their diets. Olivadese M *et al.*, in 2023 and Costa-Neto EM *et al.*,in 2016 highlighted in Africa, termite mounds are harvested for food.In Asia, silkworm pupae and bee brood were consumed. In Latin America, ants and their eggs were eaten; and among Native Americans, grasshoppers and crickets were used as food source [8, 9].Insects are utilized for various reasons, including protein deficiency, food security concerns, cultural traditions, and environmental considerations.Furthermore, insects possess medicinal importance due to their high protein content and other valuable nutritional properties.The concept of insect eating is perceived differently in different religions and cultures.Eating bugs is approved or even promoted in some religions because they are a nutrient-rich food source that aligns with a respect for nature.Sabri NS *et al.*, in 2023 pointed out however other religious beliefs have dietary guidelines that limit or restrict the consumption of certain animals, including insects, based on concepts of cleanliness, purity or symbolic significance [2]. Chung AY *et al.*, in 2000 and Sabri NS *et al.*, in 2023 highlighted the concept of insect consumption in non-Muslim countries of Asia is mainly reported in Japan, China, the Lao People's Democratic Republic, Vietnam and Thailand. However, in Muslim majority nations like Indonesia and Malaysia, insect consumption is less common due to a lack of historical dietary practices and concerns about its halal status [10, 2]. Tenebrio molitor (TM), commonly known as Mealworm, is a member of the family Tenebrionidae, class insecta and phylum Arthropoda.TM's exoskeleton is composed of calcium carbonate and chitin. TM grows mostly through molting of exoskeleton. Although TM is found all over the world, it is primarily found in the Mediterranean Sea. Since yellow TM has four tarsal segments on their legs while other beetles have five, TM may be easily distinguished from other beetles. It has four life stages as egg, larvae, pupa and adult.In Eberle S *et al.*, in 2022 study decaying fruits, vegetables, bird nests, chicken coop litter, and other decomposing organic materials.It grows best in a gloomy environment [11]. Heidari-Parsa S *et al.*, in 2018 showcased adult TM are dark brown, and yellow mealworm larvae are honey yellow as shown in Figure 1 [12].

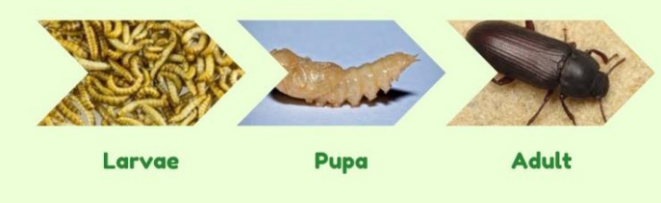

Figure 1: Physical Appearance of TM Life Stages

Melis R *et al.*, in 2018 explored their larvae are utilized as pet food for high nutritional qualities since they can readily develop on low nutrient trash [13]. Siemianowska E *et al.*, in 2013 discussed compared to huge animals, insects are better at converting plants into biomass. Insects have high nutritional value, take up less room, and eat less [14]. Growing Tenebrio larvae at home in a sterile container is simple. Its primary food sources at 28 degrees are carrots, lettuce, and other vegetables.It has the same protein content as soy and requires less land to produce (Cloudsley-Thompson, N.D.).The International Feed Industry Federation (IFIF) predicts that the world's population will surpass 10 billion by the year 2050.The food resources like meat from cows, poultry, pigs, etc. will never be sufficient for such large population. So, there would be other resources which will compensate the protein resources. Oonincx DG *et al.*, in 2012 demonstrated in recent years' entomophagy has been used as an alternate protein source because of its high nutritional values [15]. Veldkamp T *et al.*, in 2015 noted insects have all the essential amino acids, protein lipids and fatty acids which are useful for normal growth [16].Hong *et al.*,in 2020 indicated the larvae of TM are used in a powder form by freezing, chilling and drying. These procedures are done for maintain the nutrient composition [17]. Siemianowska E *et al.*, in 2013 presented many poor countries also use insects as a source of food due to their high nutritional content.The consumption level of insects as a food is less about 20% in Europe than other; such as in America it's 39% and in Africa is 30% [14].Grau T *et al.*, in 2017 identified the consumption of plant protein can be problematic for carnivorous species, so the protein from TM larvae is very effective for fishes.TM can also be supplemented to poultry and domestic birds feed [18].

TM as a Source of Protein for Fisheries

Due to increasing the population of the world it is estimated that consuming the animal protein is expending doubled

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than last some years.The consumption of protein is higher than its production because it is requirement in the feed of all the animals and human.In aquaculture fish is main source for human and other animals as a food and protein source. Dernekbaşi S highlighted the higher demand of fish oil and fish meal in aquaculture because these are basic food of aquaculture and having higher protein source and amino acids [19]. Aragão C *et al.*, in 2022 noted due to increasing demand of fish meal the number of fish decreasing worldwide.From some year's stakeholder is trying to find the proper source of amino acid and protein which can be used as a source of food for fish. In this way insects are promising to fulfill the requirements of proteins because they are almost free of cost, largely reproduce and give large amount of nutritional value [20].In recent years the production and expedition of farming the insects stand out as a basic protein source for mass production.Insects considered as large diversity of the world.In this way TM use as a protein source for all animals and humans.It is also estimated that dried yellow TM are safer and use as food for human.The European Union (EU) allowed yellow mealworm as food for human and these are the first insects that are used as a food in EU.Among these TM use as food source for both animals and human and are sustainable for environment.Now due to high demand of protein TM replaces the fish feed.These are also use as food for human. Nogales-Mérida S *et al.*, in 2019 presented according to this information insect are valuable as a protein source [21].The requirement of protein for omnivorous and carnivorous fish is higher than the herbivores and detritivores species.The requirement of protein for fish depends upon amino acid which is based on the requirement of corporal amino acid profile for each species.Many valuable proteins are abstracted from fish meat and fish oil.Due to this reason the requirement of fish is increasing which is reducing the population of fishes. Some plants and plant products are also used in place of fish meals but they are deficient in containing some amino acids as compared to other fish meal.The fresh leaves of Drumstick tree (Moringa oleifera) are used as diet for plant-eating fish such as tilapia, barbs, fancy carps, etc.The availability of amino acid for fish in insect's meal is alternative source of food. In aquaculture the TM use as a food because almost all the fishes eat them and obtain protein vitamins and fatty acids these are valuable for the growth of fishes.The amino acids which are essential for fish meal are arginine (Arg), histidine (His), isoleucine (Ile), leucine (Leu),methionine (Met), phenylalanine (Phe), threonine (Thr) and valine (Val).

Life Cycle of TM

Female TM produces eggs in dark shelter place and it has the ability to lay more than 300 eggs at a time and these eggs are hatched into larvae (mealworms) these larvae develop at different rates at different temperature.

Temperature plays an important role in the growth of larvae.Its larvae can be grown at different temperatures from 20 degrees to 30 degrees but above this temperature its growth does not increase further.Its survival rate is maximum between 26 to 27 degrees. Larvae converted into pupa and then pupa is then transformed into an adult beetle as shown in Figure 2.

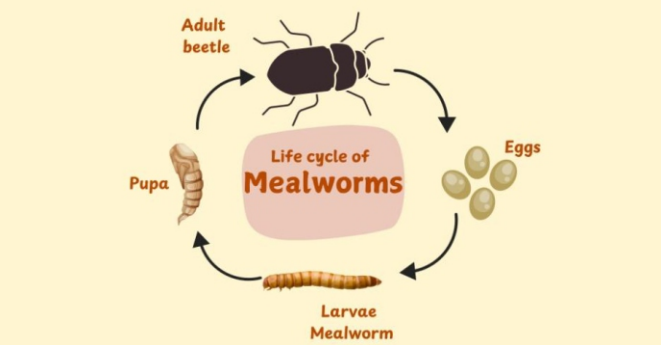

Figure 1: Life Cycle of TM

Nutritional Composition of TM

Ravzanaadii N *et al.*, in 2012 highlighted TM as a viable source of food in aquaculture and agriculture because a paradigmatic shift in the global food systems, yielding a great economic benefit and reducing environmental pressure associated with traditional feed sources. There is a great value of nutrition of TM especially as a protein supplier for domestic animals, fishes, plants and for human beings [22]. Bogusz R *et al.*, in 2024 noted TM act as a valuable resource for animal nutrition as it contains exceptional balance of bioactive compounds including micronutrients, macronutrients, vitamins and indispensable amino acids [23].

Table 1: Nutritional Composition of TM on Dry Matter basis [22]

TM Nutritional Composition	Dry Matter (%)
Proteins	55-60%
Fats	25-30%
Carbohydrates	10-15%
Vitamins	1.5-2.5%
Ash	3-5%
Fiber	5-10%
Essential Amino Acids	40-50%
Non-Essential Amino Acids	30-40%
Minerals	3-5%

The nutritional paradigm of yellow mealworms comprises a diverse array of nutrients, the concentrations of which can fluctuate in response to various factors such as ontogenetic stage, dietary regimen and somatic dimensions. Proteins (contain amino acids including valine, lysine, tryptophan, methionine, isoleucine, leucine, threonine, histidine, phenylalanine), Fatty acids (including oleic acid, stearic acid, palmitic acid, linoleic acid), minerals (calcium, phosphorus, iron, zinc, potassium,

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sodium, magnesium) and vitamins (including vitamin K, D3, A, E, B1, B12, B6, B1, B2) reveals its nutritional importance for food source.Ravzanaadii N *et al.*, in 2012 highlighted TM larvae constitute approximately 46%, adult 63%, exuvium 32% and excreta 18% protein content [22]. Noyens I *et al.*, in 2024 noted the excreta of TM even used in food recycling process as an additional supplement [24].The nutritional contents of the insects are found to be influenced by a number of factors such as diet, developmental stage and rearing environment.The temperature at which the insects were reared had a considerable impact on the larvae's fat content.Ghosh S *et al.*, in 2017 noted that the desired product from the insects by increasing or decreasing the factors that influence their nutritional content they contain [25].

Effect of TM Meal On Fish Growth

TM can be served as sustainable and effective alternative protein source for fisheries and aquaculture. Research has showed that replacing traditional fishmeal (FM) with TM meal is offering potential benefits for fish growth, health, and feed efficiency.A study conducted by Chen H *et al.*, in 2023 showed the effect of TM meal on juvenile largemouth bass growth performance, digestibility and hepatic health [26].The fish were fed with varying amounts of TM meal. The meal containing 24%TM had significantly higher weight gain rate, final body weight and specific growth rate. Appropriate replacement levels of fishmeal (FM) by TM meal can enhance the immune functions and antioxidant capacity in largemouth bass.However,high levels of FM substitution with TM meal can inhibit growth and can cause liver damage.The study suggests that TM is a feasible feed protein source for largemouth bass.Graph 1 represents the amino acid and nutritional composition of FM and TM as reported by Chen H *et al.*, in 2023, in which amino acids and nutritional composition are denoted on ordinate and their respective values in percentage are denoted on abscissa [26].Figure 1 illustrated the amino acid and nutritional composition of FM, highlighting its essential and non-essential amino acid profile along with key macronutrient and micronutrient contents. The figure provides a detailed breakdown of protein, carbohydrates, fats, vitamins, and minerals, emphasizing the nutritional value of FM. Essential amino acids such as lysine, leucine, and methionine are quantified, demonstrating their contribution to dietary requirements.Additionally, the figure compares these values with standard nutritional benchmarks, offering insights into the suitability of FM as a dietary protein source.This composition analysis plays a crucial role in evaluating the potential applications of FM in nutrition and health sciences.The amino acid and nutritional composition of fishmeal (FM), highlighting its protein content, essential amino acids, lipid profile, and micronutrient levels.This figure provides a comparative overview of FM's nutritional value, serving as a reference for evaluating alternative protein sources such as mealworms in aquaculture feed formulations (Figure 3).

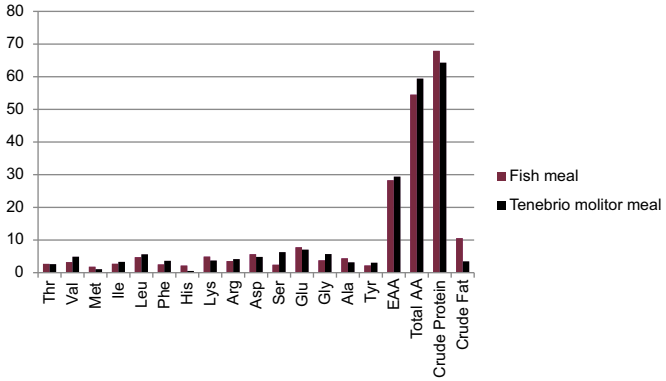

Figure 3: The Amino acid and Nutritional Composition of FM [26]

Substituting fishmeal with TM (mealworm) in fish diets can positively or negatively impact fish growth depending on the diet formulation and fish species Gu J *et al.*, in 2021 and Ido A *et al.*, in 2019 [27, 28].TM is rich in protein (50-60%), essential amino acids, and energy-dense lipids, making them a good alternative to fishmeal.However, they may lack certain amino acids like tryptophan and long-chain omega-3 fatty acids Zhang Z *et al.*, in 2022, which are crucial for fish health and growth Gu J *et al.*, in 2021 and Ido *et al.*, in 2019 [27-29].

Processing of TM Larvae

According to the International Platform of Insects for Food and Feed (IPIFF) has reported that insects, particularly TM larvae, are commonly processed for animal feed through slaughtering (heating or freezing) and post-slaughtering (drying and grinding) methods Hong J *et al.*, in 2020 [17]. For food safety and nutrient content preservation, these procedures are essential. Slaughtering process involves Blanching Vandeweyer D *et al.*, in 2017, Freezing, Chilling and Drying [30]. Blanching involves heating the larvae to kill vegetative cells Vandeweyer D *et al.*, in 2017 and prevent microbial growth during storage [30]. Freezing and chilling methods help in long-term storage and transportation. Drying is essential to reduce moisture content (around 68%) Kröncke N *et al.*, in 2019 to prevent enzymatic degradation, non-enzymatic degradation, and microbiological spoilage [31]. Common drying methods include oven drying Kröncke N *et al.*, in 2019, freeze-drying Bußler S *et al.*, in 2016, and microwave drying [31, 32]. Post-slaughtering process involves Grinding, Defatting and Hydrolysis.The dried larvae are ground into a fine powder for incorporation into animal feed. Defatting is the removal of excess fat to improve storage stability and prevent lipid oxidation.Methods include high-pressure pressing Thévenot A *et al.*, in 2018), organic solvent extraction, and supercritical CO2 extraction [33-35].Hydrolysis is the breaking down proteins into smaller peptides or amino

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acids to potentially improve digestibility and reduce anti-nutritional factors.

Schematic diagram illustrating the processing of Tenebrio molitor (TM) larvae for fish feed, detailing key steps such as harvesting, drying, grinding, and formulation. This diagram highlights the transformation of TM larvae into a nutrient-rich meal suitable for aquaculture, emphasizing its potential as a sustainable alternative to conventional fish feed ingredients (Figure 4).

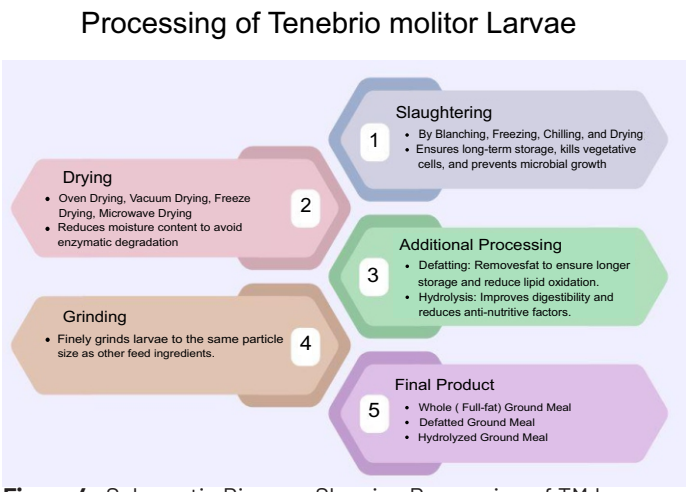

Figure 4: Schematic Diagram Showing Processing of TM Larvae for Fish Feed

CONCLUSIONS

In conclusion, mealworms present a sustainable and nutritionally rich alternative to traditional protein sources in aquaculture and fisheries. Their high protein content, essential nutrients, and minimal environmental impact make them an ideal substitute for fishmeal, addressing protein shortages and reducing reliance on conventional feed sources. Research has demonstrated their positive effects on fish growth, immunity, and feed efficiency, though careful formulation is necessary to prevent adverse outcomes. With growing global interest and regulatory approvals, incorporating mealworms into aquaculture can enhance sustainability, economic viability, and food security, paving the way for a more resilient and eco-friendly industry.

Authors Contribution

Conceptualization: MSK, MP, AS, AB, NS, HKY, MK
Methodology: MSK, MP, AS, AB, NS, HKY, MK
Formal analysis: MSK, MP, AS, AB, NS, HKY, MK
Writing, review and editing: MSK, MP, AS, AB, NS, HKY, MK

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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Original Article

Prevalence and Diversity of Ovine Gastrointestinal Parasites in the District Lower Dir

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ABSTRACT

Gastrointestinal parasitism is a key challenge to sheep production globally, These parasites result in weight loss, diarrhea, anemia, and higher production costs. **Objectives:** To find out the prevalence of gastrointestinal parasites in ovine of district lower Dir Khyber Pakhtunkhwa. **Methods:** A fecal sample was taken at random from the rectum of sheep (*Ovis aries*) using gloved fingers. The faecal components were then placed in sterile plastic bottles containing 10% formalin. A total number of 584 faecal samples of sheep were collected and then analyzed for the presence of parasites. Among them, 219 sheep were male, and 365 were female. **Results:** The Overall prevalence rate was 89.04%. Most commonly, parasites were *Haemonchus spp.*, *Strongyloides spp.*, *Trichuris spp.*, *Fasciola hepaticas spp.*, and *Moniezia spp.*, which were 43.27, 28.57, 15.59, 3.6, and 1.7% prevalences, respectively. Based on sex, there was a significant difference ($p<0.05$) in the overall incidence of gastrointestinal parasites between male (33.39%) and female (55.65%) sheep. The prevalence of gastrointestinal parasites in adult sheep was higher (69.18%) than in young sheep (19.86%). The highest infection was observed in the Balkhi breed (38.7%) and the Damani breed (32.53%) in comparison to the Lokhi breed (18.32%) ($p<0.05$). In contrast, in the tehsil-wise comparison, the maximum number of gastrointestinal parasites prevalence (17.46%) was recorded in tehsil Samar Bagh, followed by tehsil Munda 15.23%, Lal Qila 13.01%, Balambat 9.1%, and tehsil Khall 8.4%. **Conclusions:** It was concluded that parasitic spp, sex, age, breed, and different tehsils are vital factors that affect the prevalence of gastrointestinal parasites.

INTRODUCTION

Gastrointestinal Parasites (GIPs) presence is measured as one of the most hazardous physical health issues in cattle, which increase in pastures [1]. Gastrointestinal parasitism in sheep and cattle is mostly caused by helminths [2]. Trematodes gastrointestinal parasites are widely spread in ruminant animals and have a significant global presence [3]. The common gastrointestinal parasites present worldwide and can infect sheep and other small ruminant animals include *Fasciola* species, *Haemonchus* species, *Strongylus* species, and *Trichostrongylus* species [4]. It was identified that gastrointestinal parasites live inside the living host's alimentary canal, liver, gall bladder, lungs, body cavity, and intestinal tissues of blood [5, 3], which can badly infect the gastrointestinal tract of livestock [6]. A large number of helminthiasis infections have no symptoms [7]. Due to weak immunity, animals with gastrointestinal helminth infections have reduced rates of production and reproduction and are more vulnerable to infection by other pathogens [8]. Owners suffer losses in the form of decreased milk production, low fertility, diminished work capacity, involuntary culling, treatment expenses, mortality, and decreased market value of the diseased animal [9, 10]. However, predictable procedures of worm preventions comprise the whole flock treatment with artificial vermifuge, whereas in the present era, the universal problem of vermifuge resistance/tolerance in small ruminants safeguards that consideration was also necessary to be assumed to the sustainability of vermifuge

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treatment and their abrupt financial advantage [11]. Sheep parasite prevalence varies across Pakistan, with reports ranging from 25% to 92% [10]. Trematodes are one of the vital gastrointestinal parasites that can cause major infectious parasitic diseases of goats and sheep, which can produce a vital problem associated with a massive economic harm in domesticated animals by declining indirect and direct production [12]. Nevertheless, many regions of Pakistan still need to be selected for gastrointestinal parasites due to their high financial importance.

This study aims to determine the prevalence of ovine gastrointestinal parasites and to investigate the involved species and the association of several hazard factors of gastrointestinal parasites among sheep of the study area district, Lower Dir, Khyber Pakhtunkhwa, Pakistan.

METHODS

The current experimental study was restricted by the Upper Dir on the North side and East side by the district Swat. Similarly, on the West side, the district Lower Dir was bounded by Afghanistan and Bajaur Agency, while on the South side by district Malakand. The political division of the district Lower Dir and its geographic location were analyzed [13] (Figure 1).

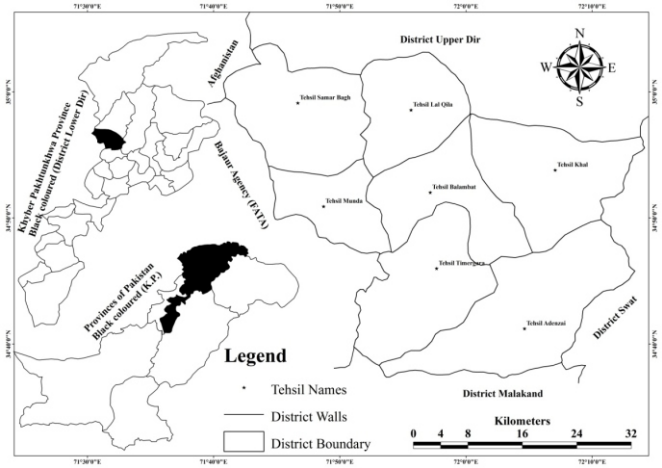

Figure 1: Political Division of the District Lower Dir and Its Geographic Location

The study population consisted of all sheep in seven tehsils of the district. Randomly fresh samples of sheep fecal samples were collected from both (male and female) sexes of sheep. The total sample size was 584 and was calculated by power analysis techniques. For the determination of age-wise prevalence, the sheep were categorized into young and adults. Sheep having age less than one year were categorized as young, while those having age more than one year were in the adults category. All ages of sheep from Lower Dir were included in this study. Besides this, all were excluded. From each sheep, the sample was collected in a sterilized polythene bag directly from the rectum.

Evidence concerning animal type, age, sex, date of animal and region from where the samples collection was noted. About 10 ml of 1% formalin was added into containers with samples and then stored for further analysis at 4°C in the refrigerator. The sedimentation technique was used for the microscopic investigation of faecal samples. The samples were centrifuged with a solution of zinc sulfate. To keep the sediment materials in the slide of glass, a pipette was used. A methylene blue drop was added on the slides and then was examined under a microscope at 4X and 10X, respectively. The eggs and larvae were properly identified based on morphological size with the help of a key developed [6, 14]. SPSS version 23.0 was used for the statistical analysis of data. Differences between host species, sex, and age groups were explored using a chi-square test. The confidence level was set at 97%, and $p \leq 0.05$ was considered significant. The relative occurrence of various helminths species or groups was designed by following the formula as used by [15]. Prevalance % = (Number of positive samples/Total number of samples examined)×100.

RESULTS

In the present work, 584 total fecal samples of the sheep were collected from different tehsils of Lower Dir and then analyzed for the incidence of gastrointestinal (GI) parasites. Out of these 584 sheep samples, 520 sheep were infected with GIP. By class-wise prevalence, the evaluation of the present study revealed the maximum percentage of prevalence for nematodes 83.90%, followed by trematodes (3.6%) and cestodes (1.7%). There were significant ($p \leq 0.05$) differences in the prevalence of nematodes, trematodes, and cestodes (Table 1).

Table 1: Overall and Class-Wise Prevalence of Sheep Parasites

Traits	n (%)	χ^2	p-Value
Positive Cases	520 (89.04%)	0.01	0.099
Negative Cases	64 (11%)		
Total Examine Cases	584 (100%)		
Class			
Nematodes	489 (83.90%)	60.65	0.000
Trematodes	21 (3.60%)		
Cestodes	10 (1.70%)		

Out of 584, 219 were male with 195 (33.39%) positive cases, and 365 were female with 325 (55.65%) positive cases. The present study indicated that the prevalence of gastrointestinal parasites in female sheep was higher as compared to the male sheep; however, their combined prevalence was 89.04% as presented in. The current study displayed significant ($p \leq 0.05$) differences in the prevalence in both male and female sheep. In the present study, out of the total examined 584 sheep animals, 130 were young, and 454 were adult. The infection rate in young animals was

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19.86%, while in adults, it was 69.18%. Thus, adults were found to be more infected with parasites as compared to young animals (Table 2).

Table 2: Sex-Wise and Age-Wise Prevalence of Sheep Parasites (n=584)

Risk Factor	Gender	No. Examine	No. Positive	Prevalence	X^2	p-Value
Sex	Male	219	195	33.390%	0.010	0.010
	Female	365	325	55.651%		
Total		584	520	89.041%		
Age	Young	130	116	19.86%	0.030	0.0000
	Adult	454	404	69.18%		
Total		584	520	89.04 %		

The present study demonstrated that six species of GIPs cause prevalence to sheep belonging to three classes (Nematodes, Trematodes, and Cestodes). Among these four GIPs of nematodes, the maximum prevalence (43.27%) was caused by Haemonchus, followed by Strongyloids (28.57%), Trichuris (15.59%), and Trichostrongylus (8.57%), respectively. However, only genera of class trematodes, namely Fasciola hepatica, cause 3.6%, and Cestode also only species Moniezia, which can have a 1.7% prevalence in sheep (Table 3).

Table 3: Species-Wise Prevalence of Parasites

Classes	Genera of Helminthes	Number of Infected	X^2	p-Value
Nematodes	Haemonchus	212 (43.27%)	1.71	0.9443
	Strongyloides	140 (28.57%)		
	Trichuris	95 (15.59%)		
	Trichostrongylus	42 (8.57%)		
Trematode	Fasciola hepatica	21 (3.6%)		
Cestode	Moniezia	10 (1.7%)		
Grant Total		520/584 (89.04%)		

Among the 584 sheep breed samples, Balkhi breed, total samples 250, positive 223, Damani breed total samples 214, positive 190, Lokhi breed total samples 120, and positive cases 107. The present study revealed that the maximum prevalence was in the Balkhi breed (38.7%), followed by the Damani breed (32.53%), and the minimum prevalence was in the Lokhi breed (18.32%). The study results revealed that the overall prevalence of gastrointestinal parasites was 89.04% in sheep (Table 4).

Table 4: Breed-Wise Prevalence of Helminths' Parasites

Breed	Total Number	Positive	Prevalence	X^2	p-Value
Balkhi Breed	250	223	38.7%	0.71	0.099
Damani Breed	214	190	32.53%		
Lokhi Breed	120	107	18.32%		
Total	584	520	89.04%		

The present study revealed that higher gastrointestinal parasites positive cases 114, prevalence (17.46%) was recorded in tehsil Samar Bagh, followed by tehsil Munda positive cases 89, prevalence (15.23%), Timergara positive cases 80, prevalence (13.69%), Lal Qilla positive cases 76, prevalence (13.01%), Adenzai positive cases 71, prevalence (12.16%), Balambat positive cases 53, prevalence (9.1%) and tehsil Khall positive cases 49 and prevalence (8.4%). The present study indicates that there were non-significant differences among all the tehsils presented (Table 5).

Table 5: Tehsil-Wise Prevalence of Gastrointestinal Parasites

Tehsil	Total Sample Size	Positive Sample Size	Negative Sample Size	X^2	p-Value
Adenzai	80	71	09 (12.16%)	0.004	1.0000
Balambat	60	53	07 (9.1%)		
Khall	55	49	06 (8.4%)		
Lal Qilla	85	76	26 (13.01%)		
Munda	100	89	11 (15.23%)		
Samar Bagh	114	102	12 (17.46%)		
Timergara	90	80	10 (13%)		
Total	584	520	64 (89.04%)		

A total number of 81 sheep was recorded in Tehsil Adenzai, in which the total number of Lokhi breed was 22, positive breed 20, Damani breed number 36, positive 31, Balkhi breed number 22 and positive Balkhi breed number 20. Similarly, a total number of 60 sheep was recorded in Tehsil Balambat, in which a total number of Lokhi breed 5, positive breed 4, Damani breed number 27, positive 24, Balkhi breed number 28 and positive Balkhi breed number 25. Similarly, a total number of 114 sheep was recorded in Tehsil Samar Bagh, comprising Lokhi breed number 8, positive breed 7, Damini breed number 16, positive 15, Balkhi breed number 90, and positive cases 90. Maximum number of breeds 114, positive cases 102 were recorded in Tehsil Samar Bagh followed by Tehsil Munda 100, positive cases 89, Timergara 90, positive cases 80, Lal Qilla 85, positive cases 76, Adenzai 81, positive cases 71, Balambat 60, positive cases 53, Khall 55 and positive cases 49 (Table 6).

Table 6: Breed-Wise and Tehsil-Wise Prevalence of Gips in Sheeps

Tehsil	Lokhi Breed		Damani Breed		Balkhi Breed		Total	
	Total	Positive	Total	Positive	Total	Positive	Total	Positive
Adenzai	22	20	36	31	22	20	80	71
Balambat	5	4	27	24	28	25	60	53
Khall	12	11	9	8	34	30	55	49
Lal Qilla	11	10	46	41	28	25	85	76
Munda	39	35	41	36	20	18	100	89
Samar Bagh	8	7	16	15	90	80	114	102
Timergara	23	20	39	35	28	25	90	80
Total	120	107	214	190	250	223	584	520

In the present study, a total number of six gastrointestinal parasites was recorded, which can infect 520 sheep. Among these, 520 were young sheep, numbered 116, and adult sheep numbered 404. The gastrointestinal parasite Haemonchus was found in 48 young and 164 adult sheep,

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while Strongyloides was found in 31 young sheep and 109 adult sheep. Similarly, Trichuris was found in 21 young and 74 adult sheep, while Trichostrongylus was found in 9 young and 33 adult sheep. In contrast, gastrointestinal parasites Fasciola hepatica was found in 5 young sheep and 16 adult sheep, while Moniezia was found in 2 young and 8 adult sheep. In the current study, mostly adult sheep 404 were infested by gastrointestinal parasites as compared to young, whose respective value was 116 (Table 7).

Table 7: Species-Wise and Age-Wise Prevalence of GIPs

GIPs	Young	Adult	Total	X^2	p-Value
Haemonchus	48	164	212		
Strongyloides	31	109	140		
Trichuris	21	74	95		
Trichostrongylus	9	33	42	0.090	1.000
Fasciola hepatica	5	16	21		
Moniezia	2	8	10		
06	116	404	520		

DISCUSSION

In the present study, 584 faecal samples were examined. A total of 520 samples were found to be infected with gastrointestinal parasites, with a high overall frequency of 89.04% in the Lower Dir district. Among the examined parasites, Haemonchus species displayed the highest prevalence (43.37%), followed by *Strongyloides* sp. (28.57%), *Trichuris* sp. (15.59%), *Trichostronngylus* sp. (8.57%), *Fasciola* sp. (3.6%), and *Moniezia* sp. (1.7%). The present work is compared with other investigations. In many other regions of Pakistan, nematodes had the highest prevalence, followed by trematodes and cestodes. Nevertheless, the current outcome differed from the findings of Kann *et al.*, who reported higher prevalence values (71.10%) for Strongyloides in cattle from the northern causal region of Colombia [16]. This result was also different from the findings of other researchers like Trinidad *et al.*, who reported 73.0% and 60.60% prevalence in cattle from Peru and Mexico, respectively [17]. In the present study, a high prevalence (89.04%) of infections was recorded in sheep. The current findings were in line with earlier research by Abebe *et al.*, which reported 80%, 83.24%, 82%, 81.1%, 77.4%, 86.6%, 87.5%, and 78.31% from Magadi division, south-western Kenya, Jammu district, India, Rawalpindi, Islamabad, Pakistan, Patiala, and its surrounding areas, respectively [18]. These studies also reported nearly identical prevalence in sheep with only small differences. Maximum prevalence was found in the country's diver region, which was linked to overstocking, inadequate nutrition (starvation), a lack of animal management skills (cleanliness), and frequent exposure to contaminated communal grazing areas [19]. Similarly, the prevalence of Strongyloides species in the present study area was 28.57%, and 26.2% Strongyloides species, which was the second prevalent parasite after Haemonchus species from Tulus study area [7]. The present study displayed a 15.59% prevalence of Trichuris in sheep. The prevalence of Trichuris species as compared to the previous finding, that 1.2% was reported by Rana & Subedi, and 8% by Acharya [20, 21]. Our study's the incidence of *Trichostrongylus* species was lower than that of previous investigations, which found that 48.8% of Bedelle, South-Western Ethiopia, Jimma town, Western Ethiopia, and Patiala had the species, 26.20% by Ibrahim *et al.*, and 3.30% by Kenea *et al.*, [22, 23]. Similar findings were previously reported for *Fasciola* species in Samba, Jammu district, India, Islamabad, and Rawalpindi, Pakistan, with prevalences of 0.5% by Ayvazoğlu *et al.*, and 4.56 by Mohammed *et al.*, respectively [24, 25]. However, the current study's findings on the prevalence of Fasciola species in sheep were lower than those of previous studies, which found that the prevalence of Fasciola gastrointestinal parasite species in sheep was 8.4% in Tangail district [26], 9% in Bako town Western Ethiopia Abebe *et al.*, [18], 15.18% in Pakistan [27], and 19.60% in India [22]. This could be due to the agroclimatic environments such as quality and quantity of humidity, temperature, pasture, and environmental conditions. In contrast to the previously recommended report of [28], which stated that the prevalence of *Moniezia* species in sheep was 0.96%, Bhat *et al.*, 3.0% [29] and 3.8% from the Bishnah, Kashmir valley of India, Nile-Delta, and Egypt, respectively, the current study found a 1.7% prevalence in sheep of this species. Although Abebe *et al.*, found a 7.30% prevalence [18], Mohammed *et al.*, found a 5.77% prevalence [25], Ibrahim *et al.*, found a 13.10% prevalence [22], and Acharya found an 11.70% prevalence in sheep from Bako town, Western Ethiopia, Indore, Patiala, and southern Rajasthan, India respectively [21]. Animal feed and drinking water become contaminated during the rainy season. As a result, helminthic and protozoan infections were likely to be most common during the rainy season [27]. Rainfall encourages pre-parasitic larval phases to grow. Gastrointestinal parasite prevalence depends on the availability of suitable transitional hosts, which might vary over time. Snails, which are carriers of several endoparasites, are typically at their highest during the mason season [30].

CONCLUSIONS

It was concluded that maximum gastrointestinal parasites prevalence in tehsil Samar Bagh followed by tehsil Munda, Timergara, Lal Qilla, Adenzai, Balambat and lower prevalence in tehsil Khall respectively. In these tehsils of the district Lower Dir, the recorded helminth parasites include nematodes, trematodes, and cestodes. Maximum

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gastrointestinal parasitic infection was caused by nematodes, while minimum by cestodes. It was also reported that the GIPs infection was more prevalent in female sheep as compared with male. Maximum infection of GIPs prevalence was recorded in the Balkhi breed, followed by the Damani breed, and minimum in the Lokhi breed. It was investigated that the adult sheep were more infected as compared to young sheep.

Authors Contribution

Conceptualization: RK
Methodology: RK, AS, MY
Formal analysis: RMK
Writing review and editing: SS, KK, AI

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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Original Article

Assessment of Egg Quality and Biochemical Parameters of Desi and Fayoumi Chicken Breeds of Kashmore Under Backyard Farming Conditions

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ABSTRACT

The egg quality factors and configuration are significant influences on grading and price. Hatching ability, weight, and consumer preference. **Objectives:** To evaluate and compare the egg quality criteria of two economically significant breeds of backyard-raised chickens such as Desi and Fayoumi. **Methods:** A variety of interior and exterior egg quality metrics were measured such as triacylglycerol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and cholesterol, which were the estimated biochemical characteristics of egg yolk. **Results:** Means for Desi egg's external characteristics, such as weight, length, width and thickness were 52.43 g, 5.16 cm, 3.85 cm, and 0.39 mm respectively. Fayoumi egg's equivalent values were 50.55g, 5.13 cm, 3.79 cm, 74.02%, 6.55 g and 0.34 mm. For Desi eggs, the yolk was 16.87 g, the albumin weight was 26.45 g, the breaking strength was 2.90, and the Haugh unit was 76.5. The Fayoumi chicken breed's egg had the following characteristics albumin weight of 26.27 g, breaking strength of 1.95, Haugh unit of 74.3, and yolk weight of 16.25 g. The calculated mean values for HDL, LDL, triacylglycerol, and egg yolk cholesterol were 9.38, 1.74, 0.17, and 1.94 mg/g for Desi eggs and 8.38, 1.84, 0.11, and 1.76 mg/g for Fayoumi eggs. **Conclusions:** It was concluded that Fayoumi eggs are heavier and better FCR than desi eggs due to breed, management conditions, nutrition, or environmental factors.

INTRODUCTION

Because of their superior molecular adaptation to local environmental circumstances, resistance to certain illnesses, high level of immunological competence, and strong and well-formed eggshells, local chicken breeds or strains are regarded as a priceless genetic gems. Even though indigenous strains often have poor productivity, several genetic modification techniques can increase their output. It's critical to strengthen eggshells for parents, grandparents, and egg-laying stocks. [1]. Fayoumi is an ancient breed that originated from Egypt in the place called Fayoumi and so they are also known as Egyptian Fayoumi. They are medium-sized chickens, the Fayoumi hen is the best layer among the various breeds. Fayoumi chickens have big black eyes and erect tails, despite their diminutive stature [2]. Their huge, single-comb silvery head, onyx-dark eyes and thin, black-speckled body are sometimes associated with roadrunners due to their forward-jutting neck and breasts as well as their erect tails. Fayoumi chickens are known for their dark horn-colored beaks and state blue skin [3]. Hen's head and neck are gleaming white, while the rest of their bodies are barred. The plumage of Fayoumi rooster is silver-white on the head, neck, saddle and back, while the remainder is black and white [4]. Desi eggs are the main Pakistani food and boast an unparalleled richness in flavor and nutritional value. These eggs produced by free-range chickens are prized for their superiority over commercially produced eggs in terms of taste and nutritional content. With a storied history in Pakistani culinary traditions. Desi eggs have been a fundamental ingredient in numerous iconic dishes. They

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are a cornerstone of breakfast recipes, elevating omelets, frittatas and quiches to new heights [5]. Moreover, Desi eggs seamlessly integrate into lunch and dinner staples such as biryani and korma adding depth and complexity to these beloved meals. Beyond their culinary significance, Desi eggs possess medicinal properties that alleviate various ailments [6]. This remarkable egg has woven itself into the fabric of Pakistani culture, transcending its role as a simple ingredient to become an integral part of the country's gastronomic heritage and traditional remedies [7]. The Desi egg is an exemplary nutrient-dense food, boasting an impressive array of essential vitamins, minerals and protein. Its nutritional profile is further enhanced by the presence of healthy fats and antioxidants which play a vital role in safeguarding the body against various diseases. In comparison to commercially produced eggs, the Desi egg stands out for its superior nutritional value, rendering it a healthier option for consumption. A single large Desi egg contains approximately 70 calories, complemented by 6 grams of protein and 5 grams of fat. Additionally, it serves as an excellent source of vital vitamins and minerals including vitamin A, vitamin D, and choline. For this reason, the Desi egg yolk is a very vital part of a healthy diet. [8].

This study aims to investigate and compare the egg quality criteria of two economically significant breeds of backyard-raised chickens such as Desi and Fayoumi.

METHODS

In this experimental search design, a total of 110 birds (32 weeks of age) were included. 50 female and 5 male from the Fayoumi and Desi chicken breeds were used in the study. Using the Power Analysis Formula: $n=2\sigma 2 (Z\alpha/2+Z\beta)2/\Delta 2$, data were collected. Where $Z\alpha/2=1.96$ (95% confidence level), $Z\beta = 0.84$ (80% power), σ=Standard deviation, and Δ=Expected difference between means. The sample size was calculated by open Epi software. The birds were divided into 10 duplicates, each consisting of 5 repetitions and consisting of 1 cock and 10 hens. For 13 weeks, from January to March 2022, each group was raised in a separate section with 5.8 square feet of floor area and maintained under comparable management settings. The temperature and relative humidity within the shed were kept constant and were found to range between 26°C and 28°C and 55% and 65%, respectively. Every day, the birds were exposed to 16 hours of light. The experimental birds were fed commercial L5 layer feed for the duration of the trial after receiving ethical permission. A computerized weighing scale was used to weigh the weekly leftover feed. Eggs were collected without delay every day at 12:00 pm. After subtracting the leftover feed, the feed consumption ratio (FCR) was measured. Every day, eggs were weighed, and after each week, the average weight of the eggs was noted. Yolk index: A Vernier caliper was utilized to identify the yolk's breadth and a tripod spherometer to determine its height. The yolk index was computed by multiplying the average yolk width by the average yolk height by 100. Haugh Unit: The log of albumin height times egg weight was the Haugh unit. Different formulas were used for measuring biological parameters. The weight of albumen=weight of an egg minus the weight of the yolk and shell, Albumen index=Albumen height (mm)/Albumen width (mm) × 100, Egg length (mm)=14.7 × (Egg weight) 0.341, Egg width (mm)=11.3 x(Egg weight)0.327, Shape Index(%)=(Egg Width / Egg Length) × 100, Shell weight (g) =0.0524 × (Egg Weight) 1.113, Shell thickness (mm)=0.0546 × (Egg Weight) 0.44, The yellow ratio (%)=0.346 × (egg weight)1.02. and Weight of egg yolk (g) = (weight of egg × yolk ratio) / 100. The Haugh Unit (HU) was determined according to the following formulation: HU = 100 log (H– 1.7W 0.37+7.57). where W was the egg's mass in grams and H was the thick albumen's height in millimetres. A 10 g egg yolk sample was homogenized in 40 mL of solvent (chloroform: methanol; 3:1) for 5 minutes to extract the samples' total lipids. Before being put on the Buchner suction filter, the mixture was kept for ten minutes. After being combined, the organic filtrates were sent into a separating funnel. After pouring two litres of 0.88% aqueous potassium chloride, the funnel was violently shaken. After the funnel was left undisturbed for 12 hours, the non-lipid material was separated into the upper aqueous phase. Using the Friedewald formula, the LDL-C was calculated as follows: HDL C TG / 5 LDL C TC. Where TG stands for triglycerides and TC for total cholesterol [9]. After removing the bottom layer, it had been dried over sodium sulfate. Methanol was added to clean the bottom phase after the top phase was removed. Lipid extract was dried in a water bath and then in a hot air oven at 60°C until it reached a consistent weight. TG, HDL, LDL, and cholesterol were calculated using the "ERBA System Pack" in an automated analyzer [10].

RESULTS

In contrast to Fayoumi chickens, Desi chickens had an elevated weekly feed consumption ratio per bird. Fayoumi birds were found to consume the least amount of feed each week. Both chicken breeds' average results are shown (Table 1).

Table 1: Mean Values for Various Fayoumi and Desi Layer Characteristics

Parameters	Desi Chicken Breed Mean ± SE	Fayoumi Chicken Breed Mean ± SE
FCR Eggs Dozen	2.453 ± 0.310	2.387 ± 0.137
Egg Weight	52.43 ± 3.401	50.55 ± 3.511
Production of Egg	3.65 ± 0.417	4.47 ± 0.259
FCR/Bird/Week Kg	0.739 ± 0.041	0.786 ± 0.055
FCR/ Egg Mass/ kg	4.215 ± 0.355	3.399 ± 0.251

The average values of all the parameters are shown.

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According to the current study's outcomes, Fayoumi chickens had larger albumin and egg yolk weights than Desi hens, and the week had a greater impact on the egg yolk weight. Fayoumi chickens had greater shell strength findings than Desi chickens, and the first week of the trial showed the highest breaking energy (Table 2).

Table 2: Various Traits of Quality of Eggs for Desi and Fayoumi Chicken Breeds

Parameters	Desi Chicken Breed Mean ± SE	Fayoumi Chicken Breed Mean ± SE
Yolk Weight (Grams)	16.87 ± 1.055	16.25 ± 1.91
Egg Length (cm)	5.16 ± 0.04	5.13 ± 0.03
Breaking Strength	2.90 ± 0.141	1.95 ± 0.075
Haugh Unit	76.5 ± 8.243	74.3 ± 6.876
Yolk Index	0.467 ± 0.031	0.437 ± 0.021
Shell Thickness	0.39 ± 0.013	0.34 ± 0.011
Shell Weight (Grams)	7.57 ± 0.645	6.55 ± 0611
Albumin Weight (Grams)	26.45 ± 3.317	26.27 ± 0.195
Shape Index (%)	74.75 ± 0.47	74.02 ± 0.46

The HDL levels in the egg yolks of Desi and Fayoumi hens were. Egg yolk LDL levels in Desi and Fayoumi hens were 0.17 ± 0.01 and 0.11 ± 0.01 mg/g, respectively. In Desi and Fayoumi hens, the levels of egg yolk TG were 1.94 ± 0.05 and 1.76 ± 0.04 mg/g, respectively (Table 3).

Table 3: Parameters of Desi and Fayoumi Chicken Breeds

Parameters	Desi Chicken Breed		Fayoumi Chicken Breed	
	Mean ± SE	CV (%)	Mean ± SE	CV (%)
TDC (mg/g)	9.38 ± 0.02	1.50	8.38 ± 0.01	1.05
HDL (mg/g)	1.74 ± 0.03	14.97	1.84 ± 0.03	12.50
LDL (mg/g)	0.17 ± 0.01	53.11	0.11 ± 0.01	52.97
TG (mg/g)	1.94 ± 0.05	18.36	1.76 ± 0.04	17.59

DISCUSSION

In comparison to Desi chickens, the birds in this research generated more eggs. Additionally, in comparison to Desi hens, Fayoumi delivered heavier eggs with a higher FCR per dozen. Fayoumi birds were found to consume the least amount of feed each week [11]. According to earlier research, Desi birds' feed consumption ratio was considerably higher than Rhode Fayoumi birds during the eighth week of the trial [12]. When compared to Desi chicken breeds, it was found that Fayoumi birds displayed the lowest feed consumption ratio values. Our results are consistent with earlier research showing that specific layer strains impacted feed consumption in comparison to other diets. It was generally accepted that FCR was a heritable trait and that the reason why Fayoumi and Desi birds consume the most feed may be because they engage in varied physical activities on the farm, where their bodies can efficiently use the most feed for a variety of purposes. In our investigation, we found that Fayoumi birds produced heavier and much higher ($p<0.05$) eggs than Desi birds [13]. Genetic potential and improved management circumstances with appropriate feeding, immunization, and treatment throughout the laying phase may be the cause of the variance in egg weight and production [14]. According to Świątkiewicz et al., under intensive circumstances, an average number of phenotypic traits such as the neck length of Desi and Fayoumi male recorded in cm (20.4 ± 1.9), the wingspan (23.7 ± 1.6) (18.3 ± 1.5), the shank length (12.5 ± 0.82) (8.3 ± 0.72). In contrast, both the Desi and Fayoumi male' neck length (18.7 ± 1.6) (16.4 ± 1.3) and body height (38.7 ± 3.2) (34.6 ± 3.1) were measured in the free range circumstances [15]. According to Ali S et al., the ratios associated with internal quality traits, such as albumen index (%), yolk index (%), the weight of albumen (g), the weight of yolk (g) were 0.058 ± 0.009, 35.15 ± 6.08, 43.60 ± 2.07, 56 ± 9.25 respectively [16]. The findings of a different study showed that the eggshell thickness (cm), egg yolk index (%), egg shape index (%), and were 0.381 ± 0.003, 47.25 4± 0.113, 77.003 ± 0.176, 11.915 ± 0.243, 6.001 ± 0.017, 3.791 ± 0.032 [17]. Other results showed that the average albumen elevation of Fayoumi bread was 6.6 and its weight was 26.0. The length of the egg was 5.0 and its width was 44.3 [18]. In this study, the average age and weight at sexual maturity were 1215 ± 11.12g and 183.5 ± 5.60 days, respectively. Hen day egg production was 41.23 ± 15.97%, while the average yearly egg output was 150.47 ± 3.15 eggs/hen/year. The mean values for the form index and egg weight were 75.95 ± 2.81 and 44.68 ± 3.63g, respectively, while the yolk colour, albumen weight, yolk weight, shell weight, and shell thickness were 5.89 ± 3.58, 14.54 ± 1.36g, 24.61 ± 2.67g, 5.63 ± 0.76g, and 0.36 ± 0.04mm, respectively. In terms of hatchability rate, 78.22% of the set of Fayoumi eggs hatched. In the Leta et al., investigation, a higher death rate of 54.85% was noted [19]. Fayoumi had the lowest average day-old weight, while Desi had the intermediate one. With 41%, the maximum generation of eggs was achieved, trailed by the Desi breed (29%), and Fayoumi (36%). The Desi breed has a lower feed effectiveness (g feed: g egg mass) (8.70) than the Fayoumi (6.79) [20].

CONCLUSIONS

It was concluded that the Fayoumi eggs are heavier and better FCR than desi eggs due to breed, management conditions, nutrition, or environmental factors.

Authors Contribution

Conceptualization: SAJ, JAU
Methodology: SAJ, SG, PA
Formal analysis: SAJ, SG
Writing review and editing: SAJ

All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

All the authors declare no conflict of interest.

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Original Article

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Epidemiological Analysis of Gastrointestinal Parasites in Various Breeds of Cattle in the Northern Region of Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Gastrointestinal (GI) parasites are major problem in cattle production worldwide. **Objectives:** To determine the common gastrointestinal parasites of cattle in district Lower Dir Khyber Pakhtunkhwa, Pakistan. **Methods:** A cross-sectional descriptive design was used. The cow fecal samples were taken and were examined under microscope by using the sedimentation and flotation techniques. To determine the impacts of breed, age, parity, eating habits, deworming status, and herd size. The data were subjected for statistical analysis via Chi-square test by using SPSS. **Results:** A total of (202/300), 67.3% of fecal samples were found positive for GI parasites. The parasites were more prevalent (41.6%, each) in the Friesian and Jersey breeds. Endo-parasitic infection was higher (92.6%; p=0.04) in female cattle than in male. Less than 2 years' cattle had a higher (75.2%; p=0.101) incidence of GI parasite. Cattle reared in a mixed feeding system had a higher (91.1%; p=0.245) incidence rate of parasitic infection. Herd of ≤5 animals had 72.3% infestation rate, and 6-8 animals per herd had 27.7% infestation. Cattle which are not treated with anthelmintic drugs had a higher (53%; p=0.988) incidence rate of GI parasites than those that are treated with anthelmintic drugs (47%). *Taxocara vitulorum* prevalence was high (46%) in single parasitic infestation, while *Taxocara vitulorum* + *Haemonchus contortus* were higher (34.6%) in double parasitic infection. *Haemonchus* + *Taxocara* + *Fasciola* spp were detected more (33.3%) in triple parasitic infection. **Conclusions:** It was concluded that the cattle population in district lower Dir had the highest prevalence of gastrointestinal parasites and need effective control measures to enhance productivity.

INTRODUCTION

Livestock play a crucial role in the economy of Pakistan, with a share of 62.68% in agriculture value added and 14.36% in GDP [1]. Almost eight million families in Pakistan with 30–35 million rural populations are involved in livestock production activities and derive 35–40% of their annual income from this sector [2]. Livestock is a crucial asset, a key source of food and a potential source of income for the poor and landless farmers in Khyber Pakhtunkhwa and has a significant role in the provincial economy through its contribution of 57.5 % to the GNP. However, diseases and parasites are among the major constraints that impact livestock productivity, particularly for small-scale farmers, due to poor disease diagnosis and limited understanding of the risk factors, hence affecting food supplies, commerce trade, and human health. Among parasites, the gastrointestinal parasites of different genera that inhabit the digestive tract of cattle, sheep and goats cause inappetence, poor feed utilization, anaemia, diarrhea, poor growth, decreased milk production, morbidity, mortality hence huge economic losses to the industry [3, 4]. In Pakistan, the prevalence of the parasitic infestation is very common and causes enormous losses to livestock [5, 6]. It has been observed that the prevalence of gastrointestinal helminthes is associated with agroclimatic conditions

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such as temperature, humidity, rainfall, quantity and quality of the feed and grazing behavior of animals [7]. Gastrointestinal parasites have been extensively studied in Pakistan with variable results concerning the type of parasite, species of animal, agroclimatic conditions and season [8, 9]. Hence, it is of prime importance to identify the burden and types of helminthes and the associated risk factors for specic areas for the effective control measures. Information on the prevalence of gastrointestinal parasites is scare in different livestock species, available in the districts of Lower Dir and Upper Dir [10, 11]. However, a precise investigation on the prevalence of gastrointestinal parasites and the associated risk factors is lacking in the study area.

This study aimed to explore the prevalence of gastrointestinal parasites and the associated risk factors to provide widespread information for designing effective control measures.

METHODS

A cross-sectional descriptive designed study was conducted at the district Lower Dir (KP) [13]. The district is located between 1200 and 2800 meters above sea level in the northern KP highlands, with latitudes of 34.35 and longitudes of 71.85. The Open Epi program was used to determine the sample size. The average annual rainfall was 1186 mm, and the average annual temperature was 16°C. During the wet and dry seasons, the relative humidity ranges from 70 to 81% and 40 to 50%, respectively. One fecal sample (~50 g) per cattle was directly collected from the rectum with gloved hands. The samples were immediately transferred to pre-labelled, hygienic plastic bottles. The sampling bottles were stored in screw caped container with ice packs and transported to laboratory. All samples were analyzed for the detection of parasites within 24 h of collection. Data on breed, sex, age, herd size, deworming status and feeding pattern, date and place of sampling were recorded for each sampled animal at the time of sampling. fecal samples were processed and examined by direct and indirect parasitological techniques (centrifugation, flotation and sedimentation) [12]. The GI parasites were identified using identification keys described by Otranto D and Wall R [13]. Briefly, fecal materials (1 g) were mixed with 0.9% normal saline wet mount solution in a mortar and a relatively homogenous preparation was obtained. The suspension was then filtered through a tea strainer. Finally, a drop of suspension was added to a glass slide and examined under a microscope. From each fecal sample, three direct smears were examined. For floatation technique, two grams of feces was put in sterile screw caped bottle, containing 5 ml of the saline solution and was mix and strained through a sieve. The mixed matters were riddled into a centrifuge tube or a walled test tube. Formalin was added to the test tube until a convex meniscus was formed. A cover slip was positioned on the top of the test tube carefully and left for 5 minutes. The cover slip was removed from the glass tube and placed on the slide, and was tested for helminthes eggs and oocysts under the microscope at 10X. In the sedimentation method, two g of feces samples were mixed with 50 ml of water and sieved. The suspension was centrifuged at 1000 rpm for 2-3 minutes with NaCl solution. The supernatant was decanted, and from the sediment, 1 drop was taken with a Pasteur's pipette and put on a slide and examined under the microscope at 10X for the presence of helminthes eggs. The data were presented in percentiles. Statistical Package for Social Sciences (SPSS) version 23.0 was used for data analysis, using the Chi-square test.

RESULTS

According to the present study, a total 67.3% samples were positive for parasitic infestation. Breed-wise analysis indicates highest (41.6% each breed) prevalence in Friesian and Jersey cow. Values are presented in percentages. The percentage value has been calculated from the total number of fecal samples examined (n=300). Data showing the overall prevalence of parasitic infestation in various breeds of cattle are presented in table 1.

Table 1: Prevalence (%) of Gastrointestinal Parasites in Various Breeds of Cattle (n=300

Breed	Sample Size	Positive	Prevalence	p-Value
Friesian	134	84	41.6%	0.116
Jersey	111	84	41.6%	
Achai	39	25	12.4%	
Non-descript	16	9	4.5%	
Total	300	202	67.3%	

Female cattle had a high (92.6%) prevalence of GI parasites. Cattle less than 2 years old had a high (75.2%) prevalence of GI parasites. Likewise, cattle reared under a mixed-type feeding system had a higher (91.1%) incidence of GI parasites than cattle under stall feeding. Notably, 72.3% of samples were detected positive in cattle with a herd size of ≤5 animals, while 6-8 animals per herd had 27.7% positive samples for GI parasites. Cattle having no history of anthelmintic use had a 53% detection rate of parasitic infection. Those partially dewormed had a 47% detection rate (Table 2).

Table 2: The Effect of Sex, Age, Feeding, Herd Size and Deworming Status on the Prevalence of GI Parasites in Cattle Breed

Breed	Sample Size	Positive	Prevalence	p-Value
Gender				
Male	17	15	7.4%	0.04
Female	283	187	92.6%	

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Age (Years)				
<2	214	152	75.2%	0.101
3-4	76	45	22.3%	
5-6	9	4	2%	
>6	1	1	0.5%	
Feeding				
Mixed Feeding	277	184	91.1%	0.245
Stall Feeding	23	18	8.9%	
Herd Size				
≤ 5	217	146	72.3%	0.975
6-8	83	56	27.7%	
Deworming				
Partially Deworm	141	95	47%	0.988
No	159	107	53%	

Data is in percentage and has been calculated from the total number of positive faecal samples observed (n=202)

Among the reported gastrointestinal nematodes, *Toxocara vitulorum* had a high (46%) prevalence rate, followed by *Haemonchus contortus* (19%), then other parasites. Among the gastrointestinal cestodes, *Moniezia spp* had a 17% prevalence rate. In the trematode parasite, *Fasciola hepatica* had 12% positive cases. Importantly, *Taxocara vitulorum + haemonchus contortus* were more (34.6%) prevalent in double parasitic infection. While *Haemonchus + Taxocara + Fasciola spp* were detected more (33.3%) in triple parasitic infection. The species-wise prevalence of parasites in various breeds of cattle is shown in table 3.

Table 3: Prevalence of Single, Double and Triple Parasites Species in Cattle (n=200)

Parasite Species		n (%)
Nematodes	*Taxocara vitulorum*	46 (46%)
	Haemonchus contortus	19 (19%)
	Trichuris	2 (2%)
	Monezia spp	17 (17%)
	Dictyocaulus viviparus	1 (1%)
	Ostertaigia	1 (1%)
Trematode	*Fasciola hepatica*	12 (12%)
	Trichostrongylus	1 (1%)
Protozoan	*Emeria bovis*	1 (1%)
Double Infection of Parasitic Species	*Taxocara vitulorum + Haemonchus contortus*	27 (34.6%)
	Taxocara vitulorum + Fasciola hepatica	12 (15.4%)
	Haemonchus contortus + Fasciola hepatica	11 (14.1%)
	Taxocara vitulorum + Monezia spp	8 (10.3%)
	Monezia spp + Fasciola hepatica	8 (10.3%)
	Haemonchus contortus + Monezia spp	7 (9.0%)
	Taxocara vitulorum + Trichuris	3 (3.8%)
	Haemonchus contortus + Emeria bovis	2 (2.6%)
Mixed Infection of Parasitic Species	*Taxocara vitulorum+ Haemonchus contortus+ Fasciola hepatica*	8 (33.3%)
	Taxocara vitulorum+ Monezia spp + Fasciola hepatica	5 (20.8%)
	Taxocara vitulorum + Haemonchus contortus + Monezia spp	3 (12.5%)
	Taxocara vitulorum + Haemonchus contortus + Teania	2 (8.3%)
	Hamonchus contortus + Monezia spp + Fasciola hepatica	2 (8.3%)
	Taxocara vitulorum + Trichuris + Fasciola hepatica	1 (4.2%)
	Hamonchus contortus + Fasciola hepatica + Ostertegia	1 (4.2%)
	Trichuris + Heamonchus contortus + Moniezia spp	1 (4.2%)
	Heamonchus contortus + Moniezia spp + Emeria bovis	1 (4.2%)

The microscopic appearance of different parasitic species is shown in figure 1.

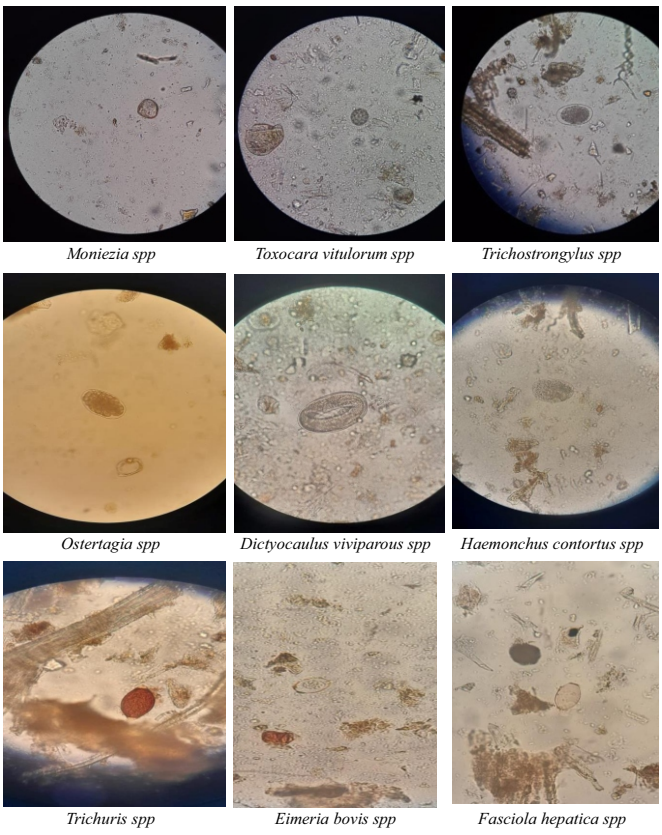

Figure 1: Different Parasitic Species

DISCUSSION

Parasitism is one of the most serious issues that the cattle population faces around the world. Parasitic diseases, particularly gastrointestinal nematode and trematode infections, pose a severe health risk to cattle and reduce output due to related morbidity, mortality, treatment costs, and control measures [14]. A thorough grasp of the disease epidemiology, pasture management, farm management techniques, and agroclimatic factors like rainfall and temperature is all necessar y for the control of gastrointestinal parasite infections in animals [15]. The kind of parasite, the extent of the infestation, and additional risk variables such as species, age, and season

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all are responsible for gastrointestinal parasite infections [16]. According to the present study, a total of 67.3% samples were positive for parasitic infestation. Breed-wise analysis indicates the highest (41.6% each breed) prevalence in Friesian and Jersey cows. In the previous study, Endo-parasites were detected in 43.96% of the total fecal samples, which was less than the 47.00% of Nigerian cattle housed at the study farm [17]. According to our ndings, nematodes (66.99%) were the most common helminthes infection, followed by cestodes (17%), and nally trematode (13%). The parasite infection ratio is only found in one protozoan (3%). In a prior study, nematodes (72.41%) were the most common helminth infection, followed by trematodes (25.00%) and cestodes (25.00%). Strongyles were found in larger numbers in cattle [17]. According to our analysis, parasite infestation was highest in the friesian cross (76.6%), followed by the Jersey cross (68.6%), sahiwal (50%) and achai (48.7%). In the nondescript, the parasite infection was the lowest. A significant difference (p 0.05) was also recorded with the infestation levels in different breeds in the prior study, local breed cattle having a higher infection rate than cross-breed cattle. In comparison to reports of GI nematode infection in cross-breed cattle, the prevalence of GI nematode infection in cross-breed cattle was lower.According to our analysis, parasitic infection was most common in animals aged 6 to 10, followed by animals aged 1 to 5, and finally, animals aged 16 to 20. The animals in the age group 11-15 had the lowest prevalence. The frequency of GI nematode infection was found to be higher in comparison to a previous study in those animals aged less than 1 year on Haramaya University dairy farm on the Holstein Friesian dairy breed followed by juvenile and mature [18]. According to our analysis, females have the highest parasite infestation followed by males. Furthermore, they had a larger endo-parasite infection percentage of *Haemonchus contortus* than males, according to [19]. The higher prevalence of gastrointestinal parasites could be linked to cattle management. The mixed feeding group had the highest parasitic infestation in the current study's grazing pattern. The animals with the 1-4 herd size had the highest prevalence ratio in the herd size category. Among terms of deworming techniques, the highest parasitic infestation was found in animals that had never been dewormed in their whole lives [20]. The lowest frequency rate of gastrointestinal parasites is due to deworming and care techniques. The majority of cattle are untreated, while other were grazing animals, who were rarely treated for GI diseases. Grazing animals has more chances for entry of various parasite stages into cattle's digestive tracts via oral ingestion.

CONCLUSIONS

It was concluded that numerous internal parasites are prevalent in cattle, with a greater infection incidence in the Friesian and Jersey crossbreed. Infestation of parasitic nematodes was high. On the other hand, the highest prevalence was recorded in female. In the age the highest prevalence was recorded in the age group of 6-10 years. Mixed feeding pattern, parasite infestation was common in diverse management approaches. The highest prevalence was recorded in those cattle which have parity level. The highest prevalence was recorded in those animals which do not properly dewormed. High parasite infestation were recorded in the small herd size as most of the people had a small herd size in the study area from where samples were collected. Risk factors had a close relation with parasite infestation.

Authors Contribution

Conceptualization: MS, IA
Methodology: MS, IA
Formal analysis: MH, MFK, FU
Writing review and editing: MZS, AJK, SB

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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Original Article

Early Passage Characterization of Canine Synovial Fluid-Derived Stem Cells Isolated from Stifle Joint

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ABSTRACT

Synovial Fluid-Derived Stem Cells (SFSCs) have emerged as a promising source of mesenchymal stem cells, offering a minimally invasive way of obtaining cells with high proliferative capacity and robust multilineage differentiation potential. Originating from the synovial membrane, SFSCs are believed to retain a cellular bias towards musculoskeletal tissue repair, positioning them as a valuable tool in treating musculoskeletal injuries and morbidities. **Objective:** To portray SFSCs differentiation behavior at early passage (P2) by evaluating their growth dynamics, immunophenotypic profile, and ability to differentiate into multilineages. **Methods:** In this experimental study, a typical MSC-like proliferation pattern was seen with distinct phases of lag, exponential and plateau growth curve. Immunohistochemistry revealed that CD73+, CD90+, and CD105+ lacking hematopoietic markers, further validated their MSC like nature. **Results:** SFSC showed bi-lineage differentiation into adipocytes and osteocytes validated by Oil O Red and Alizarin Red S staining respectively. **Conclusions:** In conclusion SFSC's possesses regenerative potential, which could be a future of regenerative medicine to repair bones and soft tissues. These findings contribute to MSC biology and its implementation as therapeutic role via SFSCs in musculoskeletal disorders.

INTRODUCTION

Synovial Fluid-Derived Stem Cells (SFSCs) have attracted attention in the field of regenerative medicine due to their undeniable ability of self-renewal, differentiation, and immune modulation [1]. These cells are found in the synovial joint milieu, offer a conveniently accessible and less intrusive source for tissue engineering and therapeutic applications, particularly for cartilage regeneration leading to osteoarthritis treatment [2]. SFSC's are more tolerant to mechanical stress contrasted to other Sources of Stem Cells (SCs), (bone marrow and adipose tissue) make them a viable approach among SC based therapies [3]. The ability of SCs to develop into many mesodermal lineages, including osteogenic, chondrogenic, and adipogenic, has resulted in promising results in regenerative therapies. Nevertheless, information is insufficient regarding the functional capacity and differentiation potential of SFSCs at early passages into osteogenic and adipogenic lineages [4]. Understanding the phenotypic stability and multipotency of SFSCs at passage 2 is crucial, since subsequent passages may result in senescence and may diminish its functionality, hence early passages are often preferred for therapeutic applications [5]. This study was conducted to describe the morphology, immunophenotype, proliferation capacity, and differentiating of SFSCs at P2. By focusing on P2, we want to know whether SFSCs at this early passage

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retain their stemness and ability to differentiate into osteogenic and adipogenic lineages since these properties are critical to use in regenerative medicine.

METHODS

Isolation and Culturing of SFSCs

This experimental study was conducted following ethical approval from the institutional ethical committee. Synovial fluid samples were collected (n = 3) from the stifle joints of dogs. After pelleting the cells at 1500 rpm for 10 minutes, they were resuspended in Dulbecco's Modified Eagle Medium (DMEM; Gibco) with 10% FBS (Invitrogen) and 1% penicillin-streptomycin. Seeded cells were incubated at 37°C in 5% CO_2 in 25 cm^2 culture flasks. Media was replaced after every 48 hours until cells achieved 80% confluence. Adherent cells were trypsinized and passaged at P0 using 0.25% trypsin-EDTA (Gibco). P2 cells were characterized further [6].

Cellular Viability Analysis

Cellular viability assay was conducted with trypan blue exclusion solution (Thermo Fisher Scientific) to stain the viable cells. The cell culture was pooled in 1/1 ratio with a 0.4% trypan blue working solution. Following the mixing process, the solution was allowed to rest for one minute at ambient temperature. To enumerate viable and non-viable cells, 10µL of solution was introduced into the Neubauer Improved Chamber. Viable cells appeared white under the microscope due to intact membranes, but dead cells exhibited a blue coloration resulting from membrane breakdown. In this way we confirmed cells viability percentage [7, 8].

Cellular Proliferation Analysis

The Cells at P2 were seeded on 96 well plates at a density of $5x10^3$ cells in each well and incubated for 24h, 48h, and 72h. MTT assay was conducted with MTT reagent (Sigma-Aldrich), 20 µL was added to each well and incubated for 4h and after blue formazan crystals, they were dissolved in 100 µL DMSO. To check the proliferation activity of cells at P2, absorbance was measured at 570 nm via microplate reader (BioTek 800TS, Linden Ave N Shoreline, WA, USA) [9, 10].

Growth Curve Analysis

To evaluate the proliferation behavior of SFSCs at P2, the growth curve was calculated. In 6-well cell culture plates (Costar®, USA), SFSCs were collected at a density of 1×10^4 cells/cm^2, using 2 mL of DMEM per well. The plates were kept in a humidified atmosphere with 5% CO2 at 37°C during the incubation process. For a total of 14 days, cells were trypsinized with 1 mL of Caisson Laboratories Inc., USA's 0.06% trypsin (in HBSS) every other day, and then neutralized with 2 mL of DMEM. Cell counting was done as described earlier. Throughout the experiment, the culture medium was changed every third day to guarantee that the SFSCs were growing in the best possible circumstances [11].

Immunophenotyping

SF-MSCs were cultured in 6-well plates with DMEM and 10% FBS at P2 until 80% confluent, with 5×10^4 cells per well on sterile 6-mm coverslips. The cells were given a quick was with DPBS following half-hour in 4% formaline and allowed to permeabilize for 15 minutes using 0.3% Triton X-100. Primary antibodies (CD73, CD90, CD105, FABP4, osteopontin; all diluted 1:50) were applied for one hour at 37°C and then incubated overnight at 4°C after 30 minutes of blocking with 10% normal goat serum. Secondary goat anti-rabbit IgG (1:100) was added and allowed to sit in the dark for an hour after being cleaned with DPBS. Under a fluorescence microscope, coverslips were coated with antifade medium, and nuclei were counterstained with DAPI (1:500) for five minutes [1, 12].

Differentiation Assay

P2 cells evaluated bi-lineage differentiation by stimulating them to differentiate into the osteogenic and adipogenic lineages. Osteogenic differentiation was induced by culturing the cells for 21 days in DMEM medium (Sigma Aldrich, Germany) enriched with 10 nM dexamethasone, 10 mM β-glycerophosphate, and 50 µM ascorbic acid. Calcium nodules were detected with Alizarin-Red staining as a marker of osteogenic differentiation [13]. Adipogenic differentiation was induced while culturing the cells for 14 days in DMEM medium (Sigma Aldrich, Germany) added with 1 µM dexamethasone, 0.5 mM IBMX, 200 µM indomethacin, with 10 µg/mL insulin and lipid droplets were marked with oil red O staining as an identifier of successful adipogenic differentiation [12, 14]. All experiments were performed in triplicate, and results are expressed as mean ± standard error of the mean (SEM). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. A p-value < 0.05 was considered statistically significant [15].

RESULTS

At P2 stage, SFSCs exhibited elongated spindle-shaped cells adherent to the culture surface (Figure 1A).

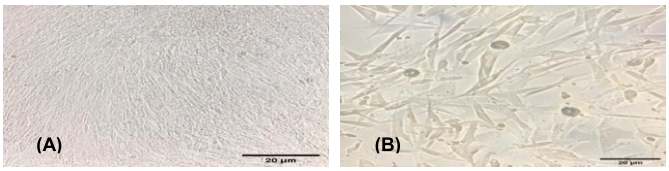

Figure 1A: Isolated Synovial Fluid-Derived Stem Cells (A) 10 X (B) 40 X Scale Bar= 20µm

Cells maintained uniform morphology without signs of senescence. The MTT assay demonstrated that P2 SFSCs proliferated actively over time, with a significant increase in cell viability from 24 to 72 h ($p < 0.05$). The absorbance values at 72 hours were 1.3-fold higher than at 24 hours, indicating robust proliferative capacity (Figure 1B).

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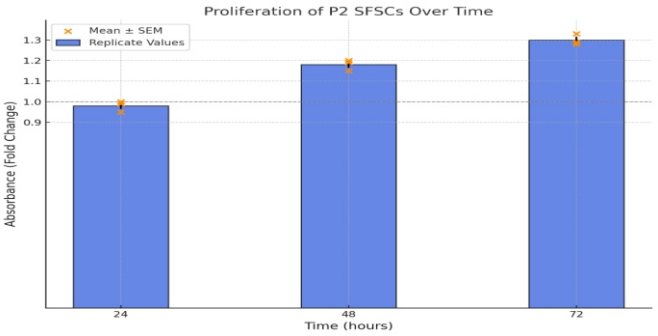

Figure 1B: Proliferation of passage 2 (P2) synovial fluid-derived stem cells (SFSCs) over time. Absorbance (fold change) was measured at 24, 48, and 72 hours to assess cell proliferation. Bars represent mean values of replicate measurements, while orange crosses indicate the mean ± standard error of the mean (SEM). The increase in absorbance over time reflects the proliferative potential of SFSCs.

The growth curve of SFSCs at passage 2 exhibited a typical sigmoidal proliferation pattern. An initial lag phase (Days 0–2) was followed by a rapid exponential growth phase (Days 2–8), where cell numbers increased significantly. By Day 10, the growth rate slowed, reaching a plateau phase at approximately 12.1 × 10^4 cells/cm^2, indicating contact inhibition or nutrient limitations figure 2.

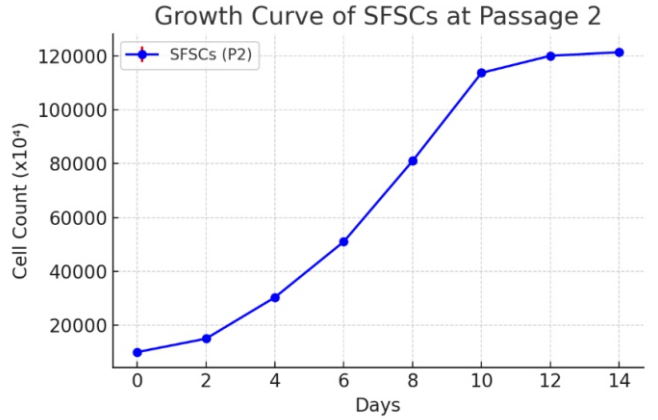

Figure 2: Growth curve of synovial fluid-derived stem cells (SFSCs) at (P2). The proliferation pattern was monitored over 14 days. An initial lag phase (Days 0–2) was followed by an exponential growth phase (Days 2–8), reaching a plateau phase after Day 10. Cell numbers were counted using a Neubauer Improved hemocytometer, and data presented as mean ± SEM (n=3)

SFSCs at P2 demonstrated a high clonogenic potential, forming visible colonies after 14 days in culture. The colony-forming efficiency was 15 ± 1.8%, indicating that a significant proportion of P2 cells retained their capacity for self-renewal and clonal expansion. Cell surface marker analysis confirmed that SFSCs at P2 retained the characteristic MSC immunophenotype. Most cells expressed CD73 (98.2% ± 0.6), CD90 (97.8% ± 0.7), and CD105 (95.6% ± 1.2), while negative for hematopoietic markers CD34 (1.2% ± 0.3) and CD45 (0.9% ± 0.2) (Figure 3).

These results confirm the mesenchymal origin of the isolated cells and their consistency at P2.

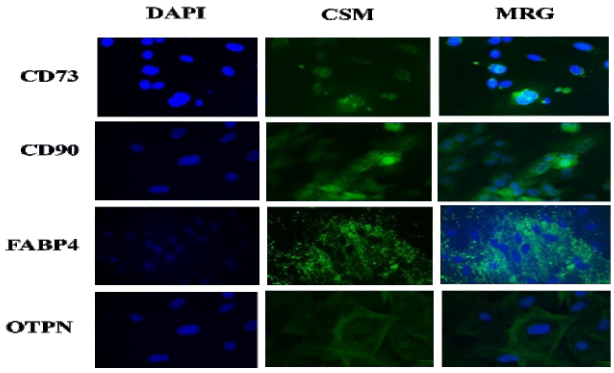

Figure 3: Expression of MSCs markers (CD73, CD90) on the synovial fluid derived stem cells and expression of special markers on differentiated cells (FABP4= Adipocytes, OTPN= Osteocytes). However, OTPN= Osteopontin, DAPI= 4′,6-diamidino-2-phenylindole., CSM=Cell Surface Marker, MRG= Merged.

SFSCs at P2 were differentiated into osteoblasts in a 3-week culturing in the osteogenic induction medium, evident by positive Alizarin Red staining for calcium deposits (Figure 4). Quantification of mineralized matrix formation showed a significant increase ($p < 0.01$) in osteogenic differentiation compared to the control group, confirming the osteogenic potential of P2 SFSCs. At P2, SFSCs successfully differentiated into adipocytes after 14 days of adipogenic induction confirmed by Oil Red O staining which indicated a cytoplasm full of with numerous lipid droplets of differentiated cells (Figure 4). Quantification of Oil Red O-positive areas showed a significant increase ($p < 0.01$) in lipid accumulation, indicating successful adipogenic differentiation of SFSCs at P2. Histological images reveal adipogenic lipid accumulation in AI and mineralized matrix in OI, confirming lineage-specific differentiation (Figure 4).

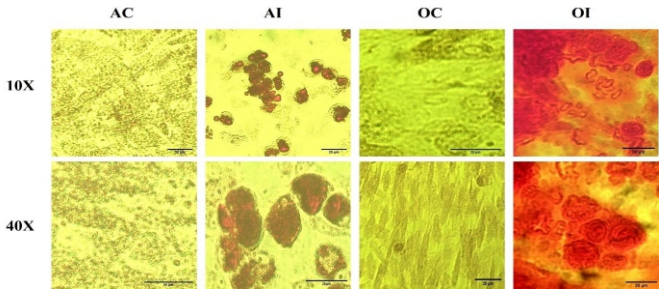

Figure 4: Representative microscopic images showing histological differences among the four experimental groups: AC (control adipogenic), AI (induced adipogenic), OC (control osteogenic), and OI (induced osteogenic). Images were captured at 10X and 40X magnifications. Lipid droplets (stained red) are prominent in the AI group, indicating successful adipogenic differentiation, whereas the OI group shows distinct mineralized extracellular matrix deposition. Scale bars = 20 µm

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DISCUSSION

This study aimed to characterize synovial fluid-derived stem cells (SFSCs) at early passage from canines and evaluate their potential for use in regenerative medicine. This study's findings indicate that SFSCs at P2 maintain their mesenchymal characteristics, which includes multipotent differentiation potential along with, proliferation making them a promising regenerative approach in tissue repair, particularly for osteoarthritis. In P2, SFSCs exhibited a spindle-shape morphology typical of stem cells derived from various sources, such as bone marrow and adipose tissue [16, 17]. The MTT values were indicated a substantial increase in proliferation of SFSC's at P2 with time. Similar results were previously described by Nantavisai *et al.*, where a significant increase in proliferative activity was seen in early stages of stem cells differentiation, making them suitable agents to be used in cell growth in clinical setting due to their increased differentiation or stemness as senescence was induced at later stages [18, 19]. The growth kinetics of SFSCs showed a sigmoidal growth pattern at P2 where a lag phase was observed at 0-2d moved to exponential growth phase (2-8d) and then decline in growth after 10 days which could be limited due to nutrients/growth factors deficiency or may their contact inhibition align with previous results reported by Garcia *et al.*, and Walczak *et al* [20, 21]. The small standard deviation across replicates reflects consistent proliferation patterns, reinforcing the reliability of these results. Such reproducibility is crucial for the scalability of SFSCs in therapeutic applications. Overall, these findings support the suitability of SFSCs for in vitro proliferation and their potential use in regenerative medicine, particularly for cartilage repair [20, 22]. Phenotypic study verified that P2 SFSCs exhibit essential MSC cell surface receptor CD73+, CD90+, and CD105+ which indicated SFSC's mesenchymal nature and not haematopoietic confirmed by lacking specific cell surface receptors CD34- and CD45-. This immunophenotypic profile aligns with the minimal criteria established by the International Society for Cellular Therapy (ISCT) for designating mesenchymal stem cells (MSCs) [23]. The higher proportion of positive cells for these markers at P2 substantiates the stemness and reliability of SFSCs, signifying their suitability for therapeutic applications where maintenance of an MSC phenotype is crucial for efficacy [11]. The colony-forming unit (CFU) assay indicated that SFSCs at P2 have a colony-forming efficiency of roughly 15%. The finding suggests that a significant fraction of the cell population maintains the capacity for self-renewal, a characteristic of MSCs. Prior studies indicated that the clonogenic capacity of MSCs diminishes with consecutive passages; nonetheless, early passages such as P2 retain a robust ability for self-renewal, hence endorsing its application in clinical studies [15, 24]. These findings indicated that SFSCs at P2 effectively differentiated into (i) osteogenic lineage validated by calcium nodules in differentiated osteocytes, and (ii) adipogenic lineages as validated lipid deposition in differentiated adipocyte cells which highlights multipotency of SFSCs at P2, makes their use in regenerative medicine. Differentiation capacity of SFSC's is crucial as osteocytes and adipocytes lineages could be a promising approach for cartilage and bone regeneration, rendering SFSCs a compelling option for addressing problems like osteoarthritis. These study stated that osteocyte lineage differentiation ability of SFSC's could be a promising option in the treatments of osteoarthritis by regenerating cartilage and bones. Previous studies also reported parallel findings of regenerating bone and cartilage using alternative sources of stem cells i.e. bone marrow due to their regenerative abilities at joint intrinsically subjected to mechanical stimuli [21, 25]. On the other hand, the adipogenic differentiation highlights their potential use in soft tissue regeneration. Jorgenson *et al.*, reported similar findings where synovial fluid derived mesenchymal stem cells showed multipotency at P2 [26]. This study provides valuable insights into the distinct ability of SFSCs at early passages. The use of canin derived SFSC's may not be directly relevant to human derived MSC's. This highlight a future research on human derived SFSC's to further verify the findings and to assess their therapeutic potential in pre-clinical models. As senescence induced at later passages would also provide forecast to study the stemness of SFSC's comprehensively at later stages. In conclusion, SFSC's possesses MSC's like characteristics of self-renewal, growth and differentiation at early passages which make them a suitable candidate in regenerative therapies.

CONCLUSIONS

SFSC's possesses regenerative potential, which could be a future of regenerative medicine to repair bones and soft tissues. These findings contribute to MSC biology and reinforce the therapeutic promise of SFSCs in musculoskeletal disorders.

Authors Contribution

Conceptualization: MUS
Methodology: US, HMA
Formal analysis: TI
Writing, review and editing: US, HMA, TI, RK, MA

All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

All the authors declare no conflict of interest.

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Original Article

In Vitro Antibacterial Activity of Ethanol Extracts of Neem, Papaya Leaves, and Garlic Compared to the Antimicrobial Agent Enrofloxacin against *Staphylococcus aureus*

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ABSTRACT

Mastitis is a significant health issue in buffaloes, commonly caused by *Staphylococcus aureus*, and is increasingly complicated by antimicrobial resistance. There is a growing interest in alternative therapies, particularly plant-based antimicrobials. **Objective:** To evaluate and compare the in vitro antibacterial activity of ethanol extracts of neem *(Azadirachta indica)*, papaya leaves *(Carica papaya)*, and garlic *(Allium sativum)* with enrofloxacin against S. aureus isolated from mastitis-infected buffalo milk. **Methods:** Fifty mastitis milk samples were collected and tested for the presence of *S. aureus* using cultural, staining, and biochemical techniques. Ethanol extracts were prepared from neem, papaya leaves, and garlic. The Minimum Inhibitory Concentration (MIC) of each extract and enrofloxacin was determined using serial dilution techniques. **Results:** *Staphylococcus aureus* was isolated from 24% of the samples. MIC values for neem, garlic, papaya, and enrofloxacin were 2.5 µg/µl, 0.312 µg/µl, 0.156 µg/µl, and 5 µg/µl, respectively. Papaya leaf extract showed the highest efficacy with the lowest MIC. All herbal extracts exhibited significant antibacterial activity, with statistically significant differences ($P<0.05$) among treatments. **Conclusions:** Ethanolic extracts of neem, garlic, and papaya demonstrated antibacterial activity against *S. aureus*, with papaya showing the highest potency. These findings suggest that herbal extracts could serve as effective alternatives or adjuncts to conventional antibiotics in managing buffalo mastitis.

INTRODUCTION

Bovine mastitis, an inflammatory response caused by physical damage or microbial infections in the udder tissue of the mammary gland, is a common disease that leads to substantial economic losses in the dairy industry due to decreased milk production and quality [1]. On average, mastitis costs are estimated at $147 per cow annually. Internal infections in the mammary glands caused by bacteria like streptococci, staphylococci, and coliforms are primary culprits. Bacterial invasion of the teat canal leads to toxin release, damaging milk-producing tissues

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and causing irritation. *Staphylococcus aureus (S. aureus)* is responsible for about 88% of these infections, significantly impacting dairy output. This issue is particularly severe in lactating buffalos, leading to substantial financial losses [2]. The most common mastitis-causing pathogens include *Streptococcus agalactiae, Staphylococcus aureus, Mycoplasma* spp., and others, with environmental pathogens such as *E. coli* and coagulase-negative staphylococci also playing a role [3].*Staphylococcus aureus,* a Gram-positive bacterium, is a significant zoonotic pathogen causing intramammary infections in dairy cattle [1]. Its increasing resistance to antimicrobial medications complicates treatment [4]. *S. aureus* resides on mucous membranes, skin, and in the noses of humans and animals [5]. The *Staphylococcus* genus includes both coagulase-positive and coagulase-negative species, with *S. aureus* being a notable coagulase-positive pathogen. Antibiotic resistance has escalated, necessitating alternatives to conventional treatments [6]. Contributing factors to this global issue include poor waste management, selection pressure, inadequate sanitation, overpopulation, extensive antibiotic use in agriculture, and wildlife dispersion [7]. The World Health Organization reports that over 80% of the global population relies on conventional medicine for various illnesses. However, multidrug-resistant bacteria's increasing resistance has intensified the search for effective alternatives [8]. Herbal plants play a crucial role in safeguarding health by combating harmful substances and oxidative stress [9]. *Azadirachta indica* (neem), a member of the Meliaceae family, is renowned for its therapeutic properties [10]. This tropical and subtropical tree has been used extensively in traditional medicine for various ailments. Neem contains numerous biologically active compounds with antioxidant, antifungal, and antibacterial properties [11]. Garlic *(Allium sativum* L.), from the Amaryllidaceae family, has well-documented health benefits and antimicrobial properties [12]. Its components, like allicin, exhibit broad-spectrum antibacterial activity [13]. *Carica papaya* L., known as papaya, has various therapeutic benefits and is used traditionally to treat multiple diseases. Its leaves contain bioactive compounds with antibacterial, antiviral, hypoglycemic, and anticancer properties [14]. Previous studies have demonstrated the antibacterial effects of ethanol extracts from papaya, neem, and garlic against various infections. Enrofloxacin, a second-generation fluoroquinolone, is widely used in veterinary medicine for its potent antibacterial activity against both Gram-positive and Gram-negative bacteria. It inhibits bacterial gyrase and topoisomerase IV enzymes. However, concerns over antibiotic resistance, particularly in intensive poultry production, have been raised [15].

This study aimed to compare the antibacterial effects of ethanol extracts from papaya, neem, and garlic with enrofloxacin against *Staphylococcus aureus* isolated from buffalo mastitis milk.

METHODS

This in vitro experimental comparative study was conducted to evaluate the antibacterial efficacy of ethanol extracts of neem, papaya leaves, and garlic against Staphylococcus aureus isolated from mastitis milk of buffaloes.

A total of 50 milk samples were selected based on availability and accessibility of clinically mastitic cases during the sampling period. Formal power analysis was not conducted due to practical constraints, but the sample size is comparable to previous similar studies. Samples were collected using a convenience sampling technique from buffaloes exhibiting clinical mastitis at various dairy farms in and around Tandojam. All samples were transported under cold chain conditions to the Central Veterinary Diagnostic Laboratory, Tandojam, for microbiological analysis.

Sterilization of Laboratory Equipment

Glassware, including Petri dishes, test tubes, pipettes, and conical flasks, was sterilized using standard procedures. Initially, all items were immersed overnight in a 1% hydrochloric acid (HCl) solution to eliminate grease and alkali residues. Subsequently, they were washed with a low-concentration solution of liquid detergent and disinfectant, followed by overnight rinsing in tap water and then four successive rinses in distilled water. The glassware was air-dried, wrapped in brown paper, and sealed with cotton plugs and aluminum foil. Sterilization was completed using a hot air oven at 165 °C for 2 h, followed by a 24 h cooling period. Plastic ware such as micropipette tips and Eppendorf tubes were sterilized in an autoclave at 121 °C under 15 psi for 20 min.

Culture Media Preparation

Blood agar was prepared by dissolving 4.8 g of dehydrated media in 100 mL of distilled water, followed by autoclaving at 121 °C for 15 min. After cooling, 5 mL of defibrinated sheep blood was added aseptically, and the mixture was dispensed into sterile Petri dishes. Plates were incubated at 37 °C for 24 h to confirm sterility. Nutrient agar was prepared using the Difco™ formula under similar autoclaving and plating procedures. Mueller-Hinton broth was prepared by dissolving 2.1 g of media in 100 mL of distilled water, autoclaved, and cooled for bacterial culture work.

Bacterial Isolation and Identification

Samples were initially cultured on nutrient agar and incubated at 37 °C for 24 h. Isolates were sub-cultured repeatedly on nutrient and blood agar plates to obtain pure colonies. Identification was performed based on colony morphology, Gram staining, and biochemical

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characteristics. Suspected Staphylococcus aureus isolates exhibited golden-yellow colonies, were Gram-positive cocci, and were confirmed using standard biochemical tests.

Gram Staining Procedure

A single bacterial colony was emulsified in a drop of distilled water on a glass slide and heat-fixed. The slide was sequentially stained with crystal violet (30 s), Lugol's iodine (30 s), and decolorized with 95% ethanol. A counterstain with carbol fuchsine was applied.Slides were rinsed between steps and air-dried before examination under an oil immersion objective.

Plant Material Collection and Extract Preparation

Leaves of *Carica papaya* (papaya) and *Azadirachta indica* (neem) were collected locally from Tandojam, while *Allium sativum* (garlic) bulbs were obtained from the local market. All materials were washed, shade-dried for approximately two weeks, and ground into a fine powder. For ethanol extraction, 20 g of powdered sample (dry weight basis) was soaked in 100 mL of 95% ethanol for 24 h at room temperature with intermittent shaking. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was evaporated to dryness under reduced pressure using a rotary evaporator. The dried extract residue was weighed to determine extraction yield and then reconstituted in distilled water to prepare a stock solution. Serial dilutions were prepared from this stock to achieve working concentrations ranging from 0.019 to 20 µg/µL. All concentrations reported represent micrograms of dry extract per microliter of solution. Enrofloxacin was used as a positive control and prepared by dissolving 20 mg in 20 mL of distilled water.

Antibacterial Susceptibility Testing

The antibacterial activity of the plant extracts and enrofloxacin was assessed using the microbroth dilution method in 96-well microtiter plates with Mueller-Hinton broth. Each well was inoculated with standardized bacterial suspensions and incubated at 37 °C for 24 h. Minimum inhibitory concentrations (MICs) were determined by visual inspection of turbidity, where the lowest concentration of extract or antibiotic that inhibited visible bacterial growth was recorded. All MIC values are expressed as µg of dry extract or drug per µL of solution. The bacterial inoculum was standardized to 0.5 McFarland turbidity standard (approximately 1.5×10^8 CFU/mL) before use. Before performing ANOVA, the data were assessed for normality using the Shapiro-Wilk test and for homogeneity of variances using Levene's test to validate the assumptions of parametric analysis. MIC values were statistically analyzed using a one-way analysis of variance (ANOVA) to determine significant differences between treatments. The analysis was performed using Statistix software version 8.1(Analytical Software, Tallahassee, FL, USA), with the significance level set at $p < 0.05$.

RESULTS

This study aimed to evaluate the antibacterial efficacy of ethanol extracts derived from neem, papaya leaves, and garlic, in comparison with the antimicrobial agent enrofloxacin, against Staphylococcus aureus isolated from the mastitis milk of buffaloes. A total of 50 mastitis milk samples were collected and subjected to pathogen testing (Table 2).

Table 2: Number and Percentage Prevalence of *Staphylococcus aureus* Isolated from Mastitis Milk Of Buffalo

Bacterial Species	Total sample	Frequency (%)
Staphylococcus aureus spp.	50	12 (24)
Mixed Population	50	38 (76)

Among these, 12 samples (24%) were positive for S. aureus, while the remaining 38 samples (76%) were negative (Figure 1). Pathogens were identified based on their morphology, cultural characteristics, and staining reactions, with further confirmation obtained through biochemical tests.

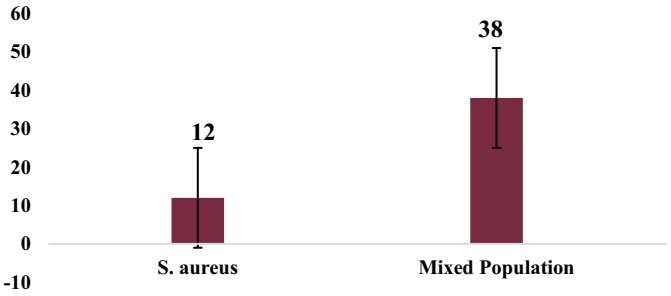

Figure 1: Number and Percentage Prevalence of *Staphylococcus aureus* Isolated from Mastitis milk of buffalo

Minimum inhibitory concentration of ethanol extract of papaya against *S. aureus*,

The susceptibility of Staphylococcus aureus isolated from buffalo mastitis milk was evaluated using a range of ethanol extract concentrations derived from papaya leaves. A concentration of 0.156 µg/µl was identified as the Minimum Inhibitory Concentration (MIC), as it effectively inhibited visible bacterial growth. Below this concentration, bacterial growth was observed, indicating reduced efficacy. At this MIC level (C8), 55.33% of the isolates showed susceptibility to the extract (Figure 2).

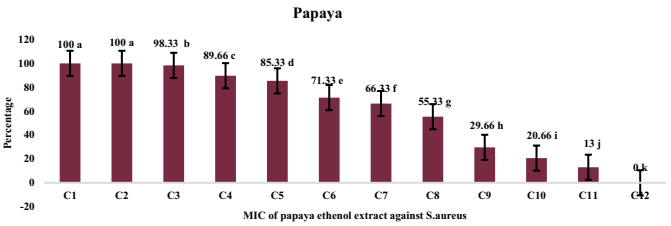

Figure 2: Shows MIC of isolated *Staphylococcus aureus* against different concentration of ethanol extract of papaya leaves

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Minimum inhibitory concentration of ethanolic extract of garlic against *S. aureus,* To ascertain the susceptibility of *Staphylococcus aureus* isolated from buffalo mastitis milk, a gradient of ethanol garlic extract concentrations (C1 = 20, C2 = 10, C3 = 5, C4 = 2.5, C5 = 1.25, C6 = 0.625, C7 = 0.312, C8 = 0.156, C9 = 0.078, C10 = 0.039, and C11 = 0.019 µg/µl) was employed. At lower concentrations, bacterial growth persisted, indicating resistance. However, at 0.312 µg/µl, visible growth inhibition was observed, and this concentration was identified as the minimum inhibitory concentration (MIC). At this MIC, 52.66% of the isolates exhibited susceptibility to the ethanol garlic extract (Figure 3).

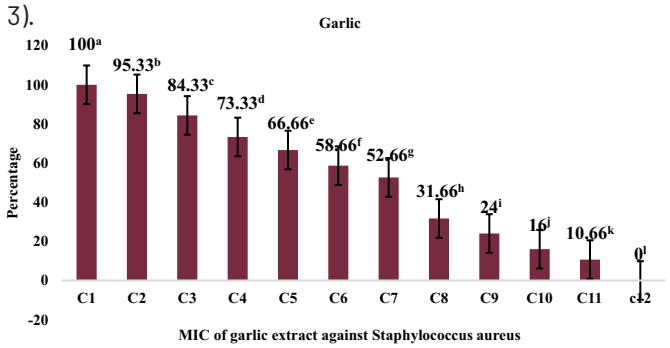

Figure 3: MIC of isolated *S. aureus* against different concentration of ethanol extract of garlic.

Minimum inhibitory concentration of ethanolic extract of neem against *S. aureus*, various concentrations of ethanol neem extract, ranging from C1 (20 µg/µl) to C11 (0.019 µg/µl), were employed to evaluate the susceptibility of *Staphylococcus aureus* isolated from buffalo mastitis milk. The extract inhibited visible bacterial growth at a concentration of 2.5 µg/µl, which was identified as the Minimum Inhibitory Concentration (MIC). Below this concentration, bacterial growth was observed, indicating resistance. At the MIC level, 51.33% of the isolates were found to be susceptible to the ethanol neem extract (Figure 4).

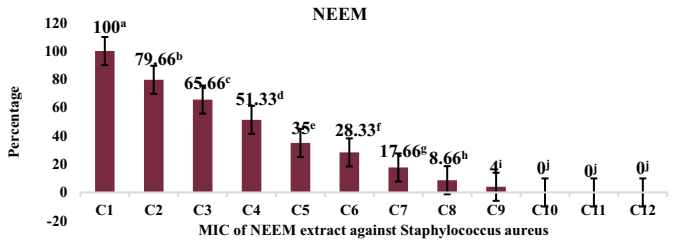

Figure 4: MIC of isolated *S. aureus* against different concentration of ethanol extract of neem leaves

Minimum inhibitory concentration of enrofloxacin against *S. aureus*, The susceptibility of Staphylococcus aureus isolated from buffalo mastitis milk was meticulously evaluated using enrofloxacin at a spectrum of concentrations, ranging from C1 (20 µg/µl) to C11 (0.019 µg/µl). The Minimum Inhibitory Concentration (MIC) was determined to be 5 µg/µl, at which visible bacterial growth was inhibited. According to the CLSI breakpoint for enrofloxacin, this MIC value indicates that 57% of the isolates were susceptible, while the remainder exhibited intermediate or resistant profiles. This threshold delineates the concentration required for effective bacterial inhibition (Figure 5).

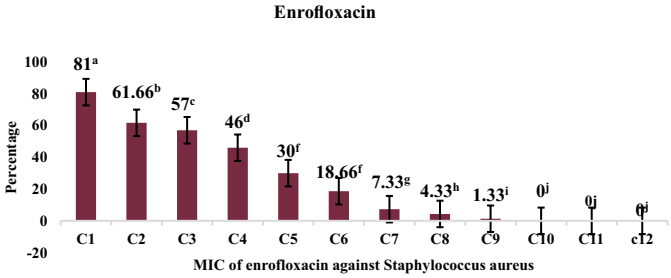

Figure 5: MIC of isolated *S. aureus* against different concentration of enrofloxacin.

Comparison of Minimum inhibitory concentration of ethanol extract of Neem, papaya leaves and garlic against *S. aureus*

To ascertain the comparative susceptibility of *Staphylococcus aureus* isolated from buffalo mastitis milk, various concentrations of ethanol extracts from neem, papaya leaves, and garlic (C1 = 20, C2 = 10, C3 = 5, C4 = 2.5, C5 = 1.25, C6 = 0.625, C7 = 0.312, C8 = 0.156, C9 = 0.078, C10 = 0.039, and C11 = 0.019 µg/µl) were employed. The MIC values for neem, papaya leaves, and garlic were determined to be 2.5, 0.156, and 0.312 µg/µl, respectively, with a statistically significant difference ($P<0.05$) observed among the concentrations. Resistance was noted at lower concentrations. The lowest MIC was recorded for the papaya leaf extract. ANOVA analysis revealed significant differences, further corroborated by LSD tests among the concentrations.

Comparison of Minimum inhibitory concentration of ethanol extract of Garlic, Neem and Papaya leaves with Enrofloxacin against *S. aureus*

The susceptibility of *Staphylococcus aureus* strains isolated from buffalo mastitis milk was assessed using ethanol extracts of neem, papaya leaves, and garlic at different concentrations, as well as enrofloxacin. The tested concentrations varied from C1 (20 µg/µl) to C11 (0.019 µg/µl). The minimum inhibitory concentration (MIC) values were determined to be 5 µg/µl for enrofloxacin, 2.5 µg/µl for neem, 0.312 µg/µl for garlic, and 0.156 µg/µl for papaya, as depicted in Figure 6.

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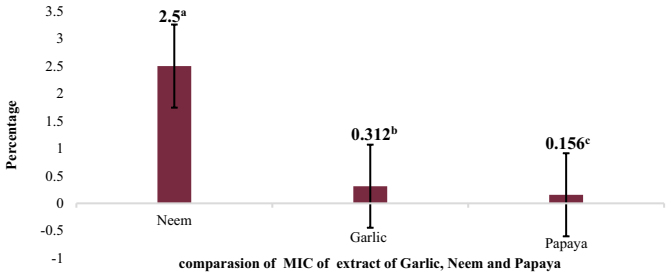

Figure 6: Comparative MIC of ethanol extract of neem, papaya and garlic against *S. aureus*

Herbal treatments demonstrated lower MIC values compared to enrofloxacin. Significant differences ($P < 0.05$) were observed among the concentrations tested, with MIC values of 5, 2.5, 0.156, and 0.312 µg/µl for enrofloxacin, neem, papaya leaf, and garlic extracts, respectively, and bacterial resistance noted at lower concentrations (Figure 7). Among the herbal extracts, papaya leaf extract exhibited the lowest MIC against S. aureus. Statistical analyses using ANOVA and LSD confirmed these significant differences ($p = 0.034$). Herbal treatments demonstrated significantly lower MIC values compared to enrofloxacin ($p = 0.021$), with papaya leaf extract exhibiting the highest efficacy ($p = 0.009$).

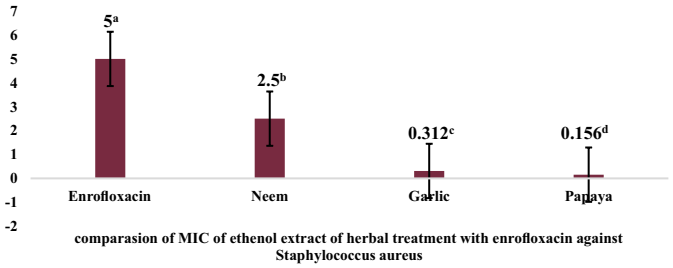

Figure 7: Comparative MIC of all herbal ethanol extract with enrofloxacin against *S. aureus*

DISCUSSION

Herbal medicine, an ancient medical practice, has been widely utilized across both developed and underdeveloped countries [16]. Literature indicates that approximately 50,000 plant species possess therapeutic properties [17]. Compounds found in plants like garlic, neem, and papaya have shown significant potential in treating infectious diseases. Their antibacterial properties make them valuable for addressing various medical conditions [18]. This study aimed to assess the antibacterial properties of ethanol extracts from neem, papaya leaves, and garlic at varying concentrations. These extracts were compared with enrofloxacin using the Minimum Inhibitory Concentration (MIC) method against *Staphylococcus aureus* isolated from buffalo mastitis milk. This study collected and analyzed 50 clinical mastitis milk samples from buffaloes under hygienic conditions. Among these, 12 samples (24%) were positive for *Staphylococcus aureus (S. aureus),* highlighting its prevalence as a mastitis-causing pathogen. The transmission of *S. aureus* is often linked to poor milking hygiene, particularly through the hands of milkers and towels used during milking. *S. aureus* is also a public health concern due to its entero-toxigenic strains, which can cause food poisoning in humans. Additionally, *Streptococcus* species, including *Streptococcus agalactiae,* have been identified as mastitis-triggering microorganisms, further emphasizing the need for effective control measures [19]. The Minimum Inhibitory Concentration (MIC) of various ethanol papaya leaf extract concentrations against *Staphylococcus aureus* was assessed. Resistance was observed at concentrations below 0.156 µg/µl, while the MIC of 0.156 µg/µl showed sensitivity in 55.33% of isolates. This supports previous findings that Carica papaya extracts possess antibacterial properties against S. aureus and *E. coli,* indicating potential for broad-spectrum therapeutic applications. Papaya leaf extract contains several bioactive constituents such as papain, alkaloids, and flavonoids, which contribute to its antibacterial activity. Papain, a proteolytic enzyme, is known to degrade bacterial proteins and disrupt microbial cell walls, while flavonoids can interfere with DNA gyrase and other essential bacterial enzymes [20]. The MIC of various ethanol garlic extract concentrations was evaluated against *Staphylococcus aureus*. Resistance was observed at doses below 0.312 µg/µl, while the MIC of 0.312 µg/µl showed sensitivity in 52.66% of isolates. This aligns with prior studies that found 2.5 mg/ml ethanol garlic extracts effectively inhibited S. aureus [21]. Previous studies noted MICs ranging from 0.14 to 0.63 µg/ml for garlic, demonstrating its potent antibacterial properties [22]. The key antibacterial compound in garlic is allicin, a sulfur-containing compound formed when garlic is crushed or chopped. Allicin inhibits thiol-containing enzymes in bacteria, disrupts metabolic functions, and interferes with quorum sensing and biofilm formation, thereby weakening the pathogen's resistance mechanisms. Similarly, ethanol neem leaf extract was tested for MIC against *S. aureus*, showing resistance at concentrations below 2.5 µg/µl. The MIC of 2.5 µg/µl was effective against 51.33% of isolates.Earlier research reported MIC values of 4–8 mg/ml for neem, with high efficacy at 8 mg/ml against *S. aureus* [23]. However, it is important to interpret the antibacterial efficacy of herbal extracts cautiously. Although the MIC values of plant extracts appear lower compared to enrofloxacin, this does not directly translate into superior clinical efficacy. Plant extracts are complex mixtures containing multiple bioactive compounds with variable concentrations, unlike purified antibiotics with defined pharmacokinetic and pharmacodynamic profiles. The variability in extract composition, potential interactions among constituents, and limited data on absorption, distribution, metabolism,

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and excretion limit the ability to predict in vivo effectiveness solely based on in vitro MICs.The findings indicate that both garlic and neem ethanol extracts possess significant antibacterial properties against S. aureus, corroborating previous research and highlighting their potential as alternative treatments for bacterial infections. Further exploration of these herbal extracts could lead to the development of effective antimicrobial therapies, especially in regions with limited access to conventional antibiotics. The inclusion of mechanistic insights into their action helps bridge the gap between ethnopharmacology and modern drug discovery. The MIC of enrofloxacin against Staphylococcus aureus was evaluated, revealing resistance at doses below 5 µg/µl. Enrofloxacin, with a MIC of 5 µg/µl, was sensitive to 57% of isolated organisms. Findings indicated dried leaf extract's efficacy against certain *S. aureus* and *Streptococcus pyogenes* strains resistant to common antibiotics like ofloxacin and nalidixic acid [24]. Enrofloxacin, frequently used in poultry, shows antibacterial activity similar to garlic's aqueous extract, effective against enrofloxacin-resistant *S. aureus* and E. coli [25].

CONCLUSIONS

All ethanol herbal extracts (neem, papaya leaves and garlic) yielded antibacterial activity against *S. aureus*. Papaya produced lowest minimum inhibitory concentration against isolated organisms. In comparison to herbal extracts, enrofloxacin exhibited higher MIC than all other treatments.

Authors Contribution

Conceptualization: BY, SB, RB, SA, VK
Methodology: JS, LK, QA
Formal analysis: MK
Writing, review and editing: AK

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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