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Microbial Infections of Pet Animals Urinary Tract: Review Article

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Abstract

Microbial urinary tract infections (UTIs) are reported to be between the second and third most common reasons for antimicrobial use in pet animals, dogs and cats, representing 12% of all antibiotic prescriptions. UTI refers to microbial colonization of any portion of the urinary system that is normally sterile. The distal urethra is not sterile; it has a normal flora. UTIs are often caused by bacterial organisms that are part of the microflora of the intestinal tract. UTI is usually caused by one single bacterial species. Predominant bacterial species were Gram-ve bacteria, as *Escherichia coli* constituted the major isolated species (about 40-50%), followed by *Proteus* spp., *Pseudomonas aureogenosa, Klebsiella* spp., and *Enterobacter* spp. Gram-positive bacteria such as *Staphylococcus* spp., *Enterococcus* spp., and *Streptococcus* spp., *Leptospira* and *Mycoplasma* spp. Additionally, fungal and viral causes play a reside role to some extent in UTIs. Diagnosis of UTIs in pets is based on clinical signs, urine analysis, and bacterial culture results obtained from urine samples collected, preferably using cystocentesis. The empiric antimicrobial treatments are often administered in the presence of clinical signs as they affect a broad spectrum of bacterial etiology associated with UTIs. Antimicrobial therapy is indicated in most cases while awaiting culture and susceptibility results to overcome the condition. Multidrug-resistant bacteria are an alarming development with significant public and pet health ramifications. Natural alternative methods can be useful as supplemental therapy choices and are much required. Cranberry is frequently used to prevent UTIs in older male dogs, but more research is needed. Prophylactic antibiotic medication, particularly for non-neutered male dogs, has not yet been shown to be significant; however, it may be of help in some cases. This work aimed to supply the researchers and veterinarians with a wider point of view about urinary tract infections in pet animals.

Keywords: Pet animals, Urinary tract infections, UTIs, Companian animals, Diagnosis, Treatment and follow up.

INTRODUCTION

Urinary tract infections (UTIs) are a common concern in pet animal practice as sequelae of compromised host defense mechanisms and a virulent microbe adhere, multiply, and persist in a portion of the urinary tract. The prevalence of UTIs in the dog over its lifetime has been reported to be 14%, and in cats, it has been reported to be between 3% to 19% (Pereira *et al*., 2024).

There are numerous factors thought to impact the risk of UTI in species, comprising sex, age, comorbidities, and functional abnormalities of the urinary tract. Host defenses include normal micturition, anatomic structures, the mucosal barrier, properties of urine, and systemic immunocompetence. (Amphaiphan *et al*., 2021).

UTIs may involve more than one anatomic location, and the infection should be categorized as upper urinary tract (kidneys and ureters) versus lower urinary tract (bladder and urethra). Most bacterial UTIs occur as a consequence of ascending migration of

pathogens through the genital tract and urethra to the bladder, ureters, and one or both kidneys (Tigabie *et al*., 2025).

Frequently, UTIs are induced by bacteria, fungi and viruses. Most of the UTIs in dogs and cats (~75%) involve a sole agent, 20% two co-infecting species, and approximately 5% are caused by 3 mixed species, with *Escherichia coli* (*E. coli*)being responsible for up to half of the infections in dogs. This Gram -ve organisms are also the most common pathogen in cats (60%), with *Staphylococcus felis* (*S. felis*)being the most common Gram +ve in that species, followed by 20% of other Gram-positive cocci, in addition to *Leptospira* spp. (Deprey *et al*., 2021) and *Mycoplasma* spp. (Alves *et al*., 2023). Fungal UTIs is uncommon and occurs usually because of temporary or permanent breaches in immunity of the lower UTIs. *Candida albicans* is the most commonly followed by *Candida glabrata* and *Candida tropicalis*. Also, other fungi may include as *Aspergillus* spp., *Blastomysis* spp., and *Cryptococcus* spp. (Reagan *et al*., 2019; Sender *et al*., 2024). Viral-induced diseases in pets are increasingly determined, particularly of the upper urinary tract, as canine adenovirus type I, herpesvirus, as well as feline coronavirus and leukemia virus (Kruger *et al*., 2011). A better understanding of the defense mechanisms of the urinary tract, the behavior of uropathogenic bacteria, and a rising awareness of the dangers of antimicrobial resistance have led to alterations in the recommendations for diagnosis and treatment of UTI in dogs and cats (Grant *et al*., 2021). So the emergence of multidrug-resistant bacteria (MDR) isolates resistant to three or more antimicrobial categories' implemented in UTI in dogs and cats creates questions about the role of companion animals as potential reservoirs of resistant bacteria and has been reported as a serious public health problem (Smoglica *et al*., 2022).

Given these challenges, there is a critical need to explore alternative treatments that can effectively combat MDR urinary pathogens. These types of treatments are receiving increasing attention in the treatment of UTIs, especially uncomplicated clinical conditions (Biasibetti *et al*., 2019).

The current article aimed to discusse the microbial urinary tract infections in pet animals; their types, the risk factors for complicated conditions, the major associated species, the standards for diagnosis, and different antimicrobial and non-antimicrobial remedy approaches. The zoonotic risks and public health implications associated with MDR UTIs in dogs and cats are addressed.

TYPES OF UTI IN DOGS AND CATS

UTIs in pets can be either: i) Simple or uncomplicated (sporadic cystitis): No predisposing factors or other diseases present; ii) Complicated or recurrent: Seen in pets with underlying medical conditions or predisposing causes; pets with more than three UTIs in the past 12 months (Smee *et al*., 2013). The 2019, International Society for Companion Animal Infectious Diseases revised the classifications of UTI. The revised classification has 3 diagnoses: subclinical bacteriuria, sporadic cystitis, and recurrent UTI (RUTI) (Weese *et al*., 2019).

Predisposing or risk factors for different types of UTIs were correlated very often to several urinary disorders; with the occurrence and the development of urinary bladder stones, insufficient protection of the urogenital tract against external influences due to immunosuppressive therapy (e.g. hyperadrenocorticism and diabetes mellitus) are important risk factors contributing to UTIs in pets. From the anatomical point of view, it is necessary to mention that the urethra in the females of dogs and cats is shorter and wider in comparison to males and this is the reason why urethritis develops more often in females than in males. UTIs are more common in older female dogs (>7 years) (Kocúreková *et al*., 2021).

The significant association of breeds with the presence of UTIs was reported, where Golden Retrievers were found to be more likely to be positive for *E. coli*/*K. pneumoniae* than other pure breeds or mixed breeds, according to a statistical analysis. (Facchin *et al*., 2025).

2. BACTERIAL CAUSES OF UTIS IN DOGS AND CATS

Bacterial urinary tract infections are one of the more common infections in dogs. Approximately 14% of the canine population afflicted by a UTIs. Although they often affect older female canines (>7 years) **(Hernando *et al*., 2021)**.

2.1. *Escherichia coli*

E. coli is the most frequently isolated pathogen, with a prevalence ranging from 35% to 64%, fundamentally, most UTIs are caused by what is termed "extraintestinal pathogenic *E.* coli" (ExPEC) which are belong to phylogenetic group B2 (generally containing more virulence factors or genes) and to a lesser extent group D. These groups of organisms are phylogenetically distinct from commensal and intestinal *E. coli*, which predominantly belong to groups A and B1 (Govindarajan *et al*., 2024).

Specific uropathogenic characteristics and virulence factors (VFs) are required for bacterial strains to initiate an infection, regardless of the presence of a competitive colonization advantage in the gut. VFs of importance to uropathogenic *E. coli* (UPEC) include capsular factors, cytotoxins, invasion factors, siderophores and related transport systems, as well as adhesins that mediate binding to the renal tubule (P, S and F1C fimbriae) and bladder urothelium (Type I fimbriae). This is likely to be of particular importance in dogs with intact urinary tract defense mechanisms (Fig. 1). However, phylogenetic group A and B1 *E. coli*, whilst generally possessing fewer VFs than group B2 and D *E. coli*, may also initiate UTI in dogs if host defenses are compromised (Halaji *et al*., 2022).

E. coli that colonize the urinary tract can protect themselves from the harsh bladder environment by forming biofilms. These biofilms promote persistence leads to chronic and recurrent UTIs (Ballash *et al*., 2022). *E. coli* are assumed a global threat because of the lowering options for antimicrobial therapy. Pets could be a reservoir of multidrug resistanate (MDR) *E. coli*, and the households owning pets had increased (Teng *et al*., 2023).

In a recent Italian study, urine samples collected from 133 dogs manifested at least one of UTI clinical signs and admitted to the Veterinary Teaching Hospital of Milan. Out of these, 28 *E. coli* strains were found, with 60. 7% producing biofilms, 25% being multidrug-resistant, and 3. 6% harboring ESBL (Facchin *et al*., 2025). An investigation study across 12 European countries supported *E. coli* as the most frequently isolated bacterium in dogs; 46.9% and 61.2% in cats (Temmerman *et al*., 2024). The incidence of *E. coli* was 45.58% in study included 2,583 urine samples from dogs suspected of UTIs with high prevalence of resistance to ampicillin (31.42%) (Yudhanto *et al*., 2022). Hernando *et al*., (2021) determined *E. coli* in UTI dogs as (1333/2942; 45.3%) in Spanish survey. While, Punia *et al*., (2018) reported less incidence of *E. coli*; (29.62%) in urine samples collected from 35 dogs suspected of UTI, in India. In Egypt, Hakim *et al*., (2024a) determined 34 and 8 *E. coli* isolates out of 81canine (41.97%) and 38 feline (21.05%) urine samples with notable resistance against imipenem and loading of *bla*NDM-1gene was responsible for resistance against carbapenems. Farag *et al*., (2024) mentioned that *E. coli* was the most common pathogen isolated from 146 dogs (46.4 %) and 162 cats (66.7 %) suffered from lower urinary tract disorders.

2.2. *Leptospira* spp.

Leptospirosis is caused by pathogenic spirochetes of the genus *Leptospira,* which colonize the renal tubules where they reproduce before being excreted via urine. Contaminated water with infected urine is the source of leptospirosis infection, where

Leptospira can enter the bodies of mammalian hosts via lacerations in the skin, contact with mucosa, conjunctiva, and inhalation of aerosols as shown in figure (1). Dogs may have an asymptomatic form or may suffer from a wide range of clinical manifestations, including hepatic, renal failure and severe pulmonary hemorrhage (Schuller *et al*., 2015). Formerly, it was thought that domestic cats were resistant to leptospirosis infection. However, published reports on feline leptospirosis concluded that cats are exposed to Leptospira and may play a role in the epidemiology of the disease (Palerme *et al*., 2019). A study investigated *Leptospira* spp. prevalence in 112 cats from southern Italy, the data revealed detection of 6 serovars in 15.3% (17/112) of tested cats so can represent an additional reservoir or sentinel for a risk of infection (Donato *et al*., 2022).

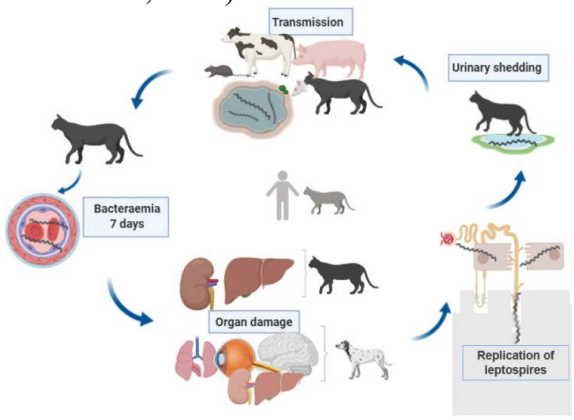


Figure (1) Proposed pathogenesis of leptospirosis in cats and dogs. Replication of leptospires occurs in the kidney leading to urinary bacterial shedding (Murillo *et al*., 2020).

A cross-sectional study in Reunion Island determined cats as a part of the maintenance community of different strains of *Leptospira* spp. The prevalence of *Leptospira* infection in 92 samples of stray and domestic cats has been studied using serological and molecular detection. The results revealed a seroprevalence of 37.0% (34/92) (cut-off 1:40), while using PCR; 28.6% (12/42) of stray cats were tested positive and Leptospiral DNA was detected in renal tissue, urine and blood. The study confirmed that renal carriage and urinary shedding are possible, especially in stray cats which can be considered potential actors within the maintenance community of *Leptospira* in Reunion Island (Holzapfel *et al*., 2021). Also, a longitudinal study was performed using a multidisciplinary approach for the identification of chronically infected stray and sheltered dog populations in São Paulo, Brazil. A total of 123 dogs from three populations were included. Asymptomatic *L. santarosai* infection was observed in all populations studied, suggesting a possible role of dogs in the chain of transmission of this leptospiral species (Miotto *et al*., 2018). Another study of pet leptospirosis detection in Algeria, was conducted in the urines of stray dogs and cats. The results revealed that 5/104 (4.8%) canine urine samples (asymptomatic mixed-breed dogs) were positive while all of the 107 cat urine samples were negative. The confirmed *L. interrogans* prevalence was significantly higher in dogs aged < one year (16.46% - 29.41%) than in adults (Zaidi *et al*., 2018). On the other hand, Delaude *et al*. (2017) stated that in spite of human leptospirosis remains rare in Switzerland, the incidence of canine leptospirosis is unusually high compared to other European countries.

Leptospirosis pulmonary hemorrhage syndrome (LPHS) in dogs and cats has poor prognosis due to acute respiratory failure and dyspnea, leading to death. Cats with mild clinical signs respond well to antimicrobial therapy, while those with chronic leptospirosis developed permanent renal damage (Murillo *et al*., 2020).

2.3. *Mycoplasma* spp.

Mycoplasmas are considered to be part of the normal microbial flora of canine mucosal membranes. However, some of these mycoplasmas may causes UTI by ascending migration from the lower urinary or genital tract. Hemmatzadeh (2019) reported the first report of detection of *Ureaplasma canigenitalium* in an English Cocker Spaniel dog with the history of UTIs and chronic renal insufficiency in Australia. *Mycoplasma canis* was isolated from four of 100 (4%) urine samples obtained by cystocentesis from 100 dogs with symptoms of lower urinary tract disease. Also, Mycoplasmas identified as *M. canis* were isolated from nine dogs with clinical signs of urogenital disease in Norway over 20 months (L'Abée-Lund *et al*., 2003).

2.4. Other Bacterial Species

In Italy, urine samples collected from 133 dogs at Veterinary Teaching Hospital of Milan showed positive microbiological culture of *K. pneumoniae* isolates, (4.51%) (Facchin *et al*., 2025).

European survey determined the causative bacteria of canine UTIs next to *E. coli*; *Staphylococcus intermedius* and *Proteus mirabilis* (13.1%). The frequent Gram +ve isolates were *Streptococcus* spp. (8.3%), *Enterococcus* spp. (8.0%) and *S. aureus* was (0.9%). Gram-negative bacteria included *Klebsiella* spp. (4.0%), *Pseudomonas aeruginosa* (3.6%) and finally *Pasteurella* spp. In second period (0.5%). On the other hand, among examined feline urine samples, coagulase-negative *Staphylococci* were the second-most frequently isolated species (13.2%), including the specific pathogen *Staphylococcus felis*. *Enterococcus* spp. was (17.7%) and *S. aureus* (4.2%). The other Gram-negative pathogens; *Proteus* spp., *Pseudomonas* spp., *Klebsiella* spp., and *Pasteurella* spp. were 5.3%, 4.2%, 1.6% and 1.6%, respectively (Temmerman *et al*., 2024).

A study aimed to bring new insights into the current bacterial urinary tract infections in companion animals scenario of Portugal showed 5306/17472 (30.4%) +ve bacterial culture. Of the culture-positive samples, 5224 (96.6%) were pure cultures and 82 (3.2%) had mixed growth. Other *E. coli* bacteria were *Proteus mirabilis* (11%), *Enterococcus faecium* (5.2%) and *Staphylococcus pseudintermedius* (4.3%), as shown in Figure (2) **(Garcês, *et al*., 2022)**.

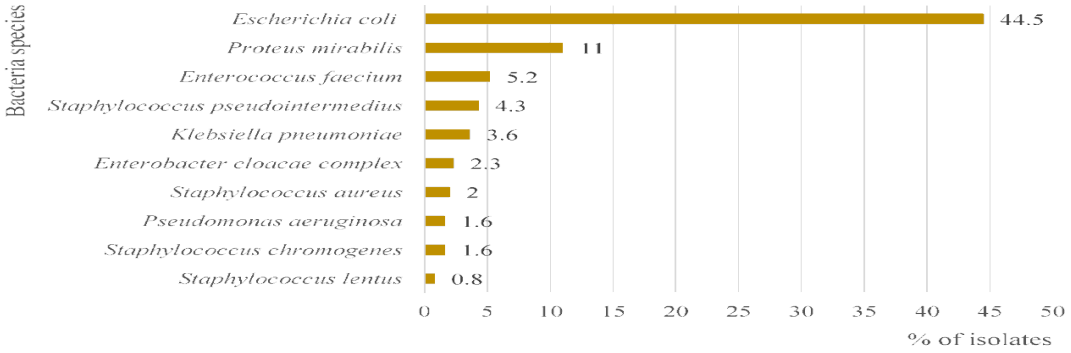


Figure 2: Bacteria species that predominate in the 5306 isolates with positive culture grown from urine samples from dogs and cats submitted to the INNO veterinary laboratory between 2017 and 2021 (Garcês *et al*., 2022).

In Egypt, hypervirulent type *K. pneumoniae* (hvKp) isolates were recovered from canine urinary samples in Al Qalyubia and Giza Governorates by a rate of 2% (Soliman *et al*., 2024). While. Farag *et al*., (2024) recorded that the second prevalent species isolated were *Proteus* spp. in canine isolates (16.1 %) and *Klebsiella* spp. in feline isolates (14.3 %). *Staphylococcus* spp. was isolated from canine cases only with the detection of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) strains at 3.6 %.

3. MYCOTIC URINARY TRACT INFECTIONS

Mycotic infections that frequently affect the urinary tract of dogs and cats are generally flourished when animal's immunological state become lower. Most of fungal UTIs are caused by an overgrowth of *Candida* spp. Although candida yeasts are normally present in the body as well as on the skin circumstances, it can disrupt the natural functioning of the body, when this occurs in the lower urinary tract it causes an infection which can become progressively. Fungal infections of the lower urinary tract often are asymptomatic, and may be uncovered when diagnosing another issue or during regular veterinary check-ups. Opportunistic mycoses in dogs and cats can result in a wide variety of symptoms, from localized infections to catastrophic systemic illnesses. Such fungi like *Microsporum canis* and *Sporothrix brasiliensis* may be important zoonotic agents (Eissa, 2023).

3.1. *Candida* species

Candiduria is the most commonly reported manifestation of candidiasis in the veterinary literature as shown in figure (3). In a retrospective study of urinary tract infections in dogs; risk factors for candiduria in pet animals were reported; comorbidities involved diabetes mellitus, antibiotic use in the preceding 30 days, immunosuppression, and lower urinary tract illness (Sykes *et al*., 2014).

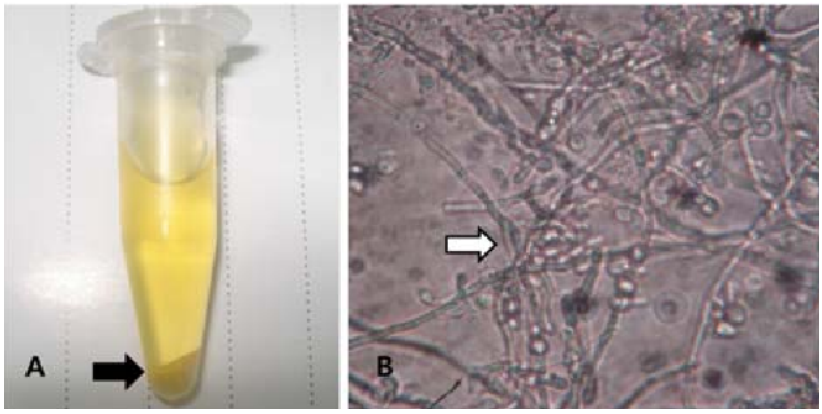


Figure 3: Urine sample (A) Cloudy sediment (black arrow) after centrifugation of urine. (B) Microscopic examination found pseudohyphae and budding yeast (white arrow) indicating *Candida* infection. 1,000×g (Sung *et al*., 2017).

Eighteen dogs belonged to 4 mixed breeds with candiduria without suspected systemic infection were identified. Three species of *Candida* were isolated, *C. albicans,* and *C. tropicalis*, and then *C. glabrata*. Ages ranged from 1-14 years. of age (median 7 years.). Antibacterial drug administration within the 30 days before diagnosis was recorded in 15 (83%) dogs. Potential causes of immunosuppression were recorded in 10 (55%) dogs. Lower urinary tract disease or urinary catheter placement was in the history of 6 (33%) dogs. Other urinary abnormalities include urethral tear during a cystoscopy, intermittent urinary catheterization, cystotomy, and obstructive prostatic cyst. One dog had been diagnosed with diabetes mellitus. On the other side, four *Candida* isolates were reported: *C. albicans, C. glabrata, C. tropicalis,* and *C. parapsilosis* in eight cats had candiduria. The risk factors comprised other lower urinary tract disorders, urethral avulsion secondary to trauma, perineal urethrostomy, and bladder rupture secondary to urethral obstruction. Four cats had received immunosuppressive medications. Administration of antibacterial drugs in the last 30 days was reported for 7 of the 8 cats (Reagan *et al.,* 2019).

In April 2011, a 3-year-old male Yorkshire terrier dog was referred to the Clinical Veterinary Hospital de Madrid, Spain, with a diagnosis of relapsing UTI. The dog had a history of ammonium urate bladder stones and had been treated previously with

marbofloxacin. Microscopic examination and culturing of urine specimens revealed the presence of yeasts. Yeast isolates recovered from clinical specimens were identified as *Candida tropicalis* on the basis of the morphology and pigmentation of their colonies on ChromAgar medium and also by sequencing the D1/D2 domains of the large subunit (LSU) rRNA gene (Álvarez-Pérez *et al.,* 2016).

3.2. Other Fungal Species

In October 2020, a 10-year-old male intact Border Collie dog presented to a specialty veterinary hospital at Michigan State University for a two-month history mural ureteral and bladder granuloma. The diagnosis, culture followed by MALDIToF, PCR, and sequencing was performed and identified *Scedosporium apiospermum* which is an opportunistic mold that is an emerging disease in humans and animals (Tsoi *et al.,* 2021). A nine-year-old female Labrador retriever suffered from urinary tract infection and was in the 14th day of a 21-day course of oral antibiotics amoxicillin-clavulanic acid in Sydney, Australia, in March 2017. The signs were hematuria, sanguinous vulval discharge, and urinary incontinence. The main compliance was the ultrasonographic observation of two intra-abdominal masses, "eumycetomas" which are chronic pyogranulomatous lesions caused by molds. Antifungal therapy was started on day 3 with oral itraconazole at 5mg/kg SID for 90 days. However, the dog continued to have urinary tract infections and urinary incontinence for the whole ninety days after treatment commenced. Due to cost constraints, euthanasia was elected on day 97. Amplification and sequence analysis of internal transcribed spacers and the partial large subunit of the 25–28s ribosomal RNA regions of fungus cultured was performed, identifying this as belonging to the *Curvularia* species (Herbert *et al.,* 2019). The *Blastomyces* spp. organism was detected in urine sediment obtained from a 2-year-old castrated male Doberman pinscher (Reagan *et al.,* 2019). A 2.5-year-old female spayed GSD dog was presented to the University Veterinary Teaching Hospital, Sydney, Australia, for investigation of polyuria, polydipsia, and urinary incontinence at night of at least four months duration. Fungal colonies were grown from both urine and lymph node aspirates. Molecular identification targeting the partial beta-tubulin gene revealed these colonies to be *Aspergillus deflectus* (Bennett *et al*., 2018). Cryptococcal UTI was diagnosed cytologically and via fungal culture in a male domestic shorthaired cat with stranguria and pollakiuria.

4. VIRAL URINARY TRACT INFECTION

Viral-induced disease is recognized, particularly of the upper urinary tract. However, it can be difficult to determine cause-and-effect relationships because viral-induced illness may occur in the absence of detectable replicating virus. Several viruses have been implicated in canine and feline disease, as shown in table(1) by Olin and Bartges, (2015).

Table 1: Viruses associated with urinary tract disease in dogs and cats. (Olin and Bartges, 2015).

Species	Upper Urinary Tract Disease	Lower Urinary Tract Disease
Canine	Canine adenovirus type I	
	Canine herpesvirus	
Feline	Feline coronavirus	Feline calicivirus
	Feline immunodeficiency virus	Bovine herpesvirus-4
	Feline leukemia virus	
	Feline foamy (syncytium-forming) virus	Feline foamy (syncytium-forming) virus

DIAGNOSIS

In 2019, the International Society for Companion Animal Infectious Diseases (ISCAID) released revised guidelines for the diagnosis and treatment of bacterial UTIs in pets. The

recommended diagnostic method for UTIs combines clinical signs, urinalysis; including dipstick testing, specific gravity measurement, and sediment cytology, haemato-biochemical analysis, radiography, and ultrasonography as well as bacterial culture results obtained from urine samples collected using cystocentesis (Barot *et al*., 2022).

4.1. Clinical examination

Clinical signs associated with UTI are variable and depend on the interaction of (1) virulence and numbers of the uropathogen, (2) presence or absence of predisposing causes, (3) the body's compensatory response to infection, (4) duration of infection, and (5) site (s) of infection. Pollakiuria, stranguria, dysuria, hematuria and inappropriate urination may be observed with lower UTI (Bartges, 2004).

4.2. Sampling

Free-catch urine sampling

When collecting a free-catch urine sample, a mid-stream sample is preferred. The initial urine that is voided contain cells, bacteria, and debris from the urethra and vulva or prepuce, and may not be a representative sample. Because female dogs are usually positioned with the vulva close to the ground when urinating, a shallow container (such as a pie pan) may be helpful. Quantitative analysis should be performed on all free-catch samples submitted for bacterial culture.

Obtaining a free-catch midstream urine sample from a cat is a difficult task. Removing the litter from the litter box or replacing it with nonabsorbent material, plastic packing material, etc. may allow collection of a suitable sample. Plastic wrap can be loosely placed over the litter to collect urine from declawed cats. Generally, the use of voided urine specimens for bacteriological culture is discouraged because contamination from external genitalia led to misinterpretation of laboratory results (Olin and Bartges 2015).

A study addressed the usefulness of voided specimens and determined veterinary cut-off values for significant bacteriuria. The results showed false negative culture results. False negative results are concerning, as they led to under-treatment, thereby posing a risk of complications to individual dogs. The veterinary cut-off yielded an accuracy of 95% with a sensitivity and specificity of 94%. (Sørensen *et al*., 2016).

Urinary catheterization

Male dogs are placed in lateral or sternal recumbency for catheterization. The penis is extruded from the prepuce, and the tip is cleaned with a dilute disinfectant solution to remove any debris or discharge. Sterile lubricant is placed on the tip of the urinary catheter, which is then placed in the urethral orifice, and the catheter is advanced into the bladder (Fig. 4). It is helpful to create a sleeve from the catheter packaging to maintain sterility while passing the catheter. Most female dogs need chemical restraint, such as lidocaine gel, to allow urinary catheterization (Figure 4).

Both male and female cats usually need chemical restraint to permit urinary catheterization. Generally, it is not routinely used for urine collection in cats unless it has been performed for another reason such as treating urethral obstructions (Reppas and Foster, 2016). After passing a urinary catheter to obtain a urine sample, the first few milliliters of urine should be discarded, as that is the portion most likely to have debris from the urethra. A second aliquot can then be obtained for urinalysis and quantitative culture if desired.

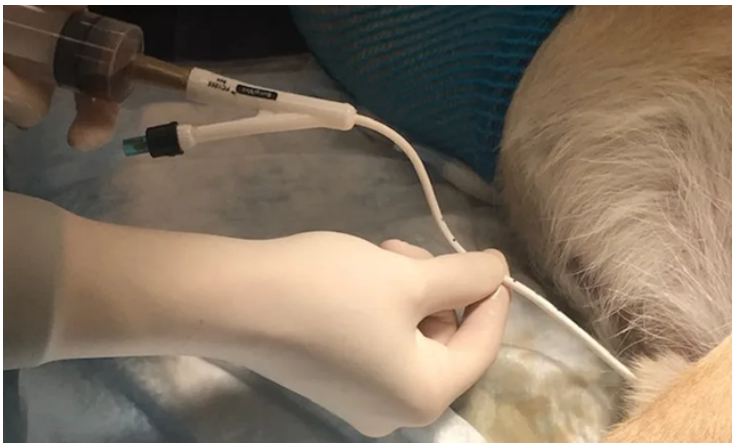


Figure 4: Urethral catheterization placement in male dog.
https://www.cliniciansbrief.com/article/urinary-catheter-placement-dogs

Cystocentesis

Cystocentesis allows the collection of an uncontaminated urine sample ideal for bacterial or fungal culture and can be performed on awake animals. It also allows appropriate timing of collection when timing is important.

The dog is usually positioned in dorsal recumbence. Palpating the bladder with one hand helps immobilize it while acquiring the sample. The needle should be angled caudally, toward the pelvic inlet, so that as the bladder empties, the needle is still in the lumen of the bladder (Fig. 5). It is also possible to use a lateral or standing approach when the bladder is palpable. A 1-inch, 22-gauge needle is preferred, and for very large or obese dogs, a 1.5 to 2-inch needle may be required.

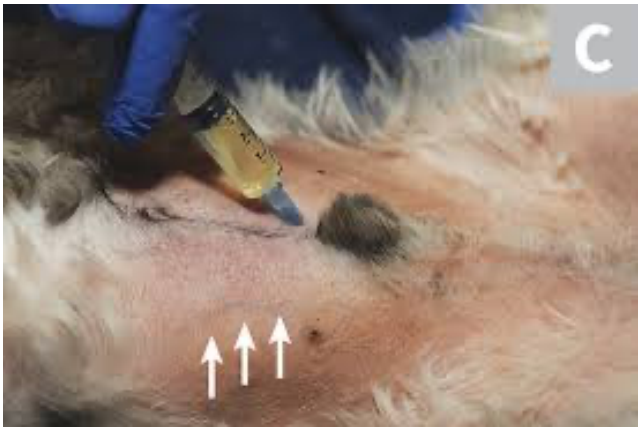


Figure 5: Cystocentesis (ie, obtaining urine directly from the urinary bladder by inserting a needle through abdominal wall).
https://www.cliniciansbrief.com/article/cystocentesis

In cats, cystocentesis is usually performed relatively easily unless they have idiopathic feline lower urinary tract disease (iFLUTD). These cats typically have a small bladder, and

any handling results in voiding; ultrasound-guided sampling may be more rewarding in these situations (Reppas and Foster, 2016).

Notably, with both cystocentesis and urinary catheterization, there is a risk of iatrogenic hematuria. Iatrogenic hematuria can occur if the needle is in contact with the opposite bladder wall. Other complications include puncture of the colon, laceration of the bladder, and laceration of the major blood vessels dorsal to the bladder. Inadvertent puncture of the colon caused bacterial contamination of the urine sample, and a mixed population of bacteria on the urine culture (Esparaz et al., 2016).

Storage of samples

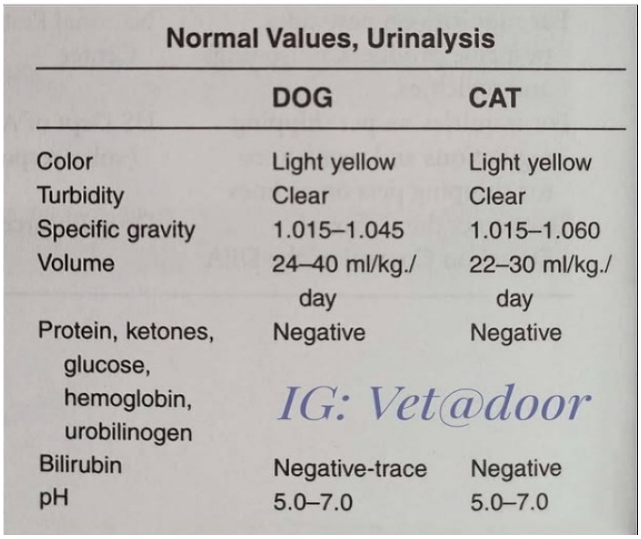
The storage condition was vital to maintaining the quality and accuracy of the urine samples. A study assessed different storage conditions of urine samples obtained from 30 dogs and 49 cats. The study showed that storing conditions at room temperature or refrigeration for 24 h do not impact the results of culture count in cat urine samples. For dog samples, chilled samples have a higher accuracy rate than room temperature samples, although the overall agreement was still satisfactory (Lien and Wang, 2023).

Additionally, Hedström, et al. (2021) declared that boric acid sponge preservation is a useful alternative to refrigeration of urine samples during transport. Reliable quantitative bacterial culture results can be obtained from canine urine up to 48 h after collection if urine is refrigerated and for at least 24 h if urine is stored using a boric acid-containing urine transport system.

4.3. Urinalysis

A urinalysis should be performed routinely as part of a minimum database and is an essential part of the diagnostic evaluation for all urinary and many metabolic diseases (Bartges, 2004).

A complete urinalysis assessment includes determining urine specific gravity (USPG) using a refractometer, evaluation of physical characteristics (color, clarity, and volume), biochemical parameters (urine pH, blood, glucose, ketones, bilirubin, urobilinogen, and protein) using analytic test pads on dipsticks, and microscopic sediment evaluation (RBC, WBC, organisms, epithelial cells, crystals, and casts) (figure 6). Collection of urine by cystocentesis is the preferred method when evaluating patients for UTI. If infectious prostatitis or vaginitis is suspected, different techniques were indicated (Reine and Langston, 2005).



Normal Values, Urinalysis		
	DOG	CAT
Color	Light yellow	Light yellow
Turbidity	Clear	Clear
Specific gravity	1.015–1.045	1.015–1.060
Volume	24–40 ml/kg./ day	22–30 ml/kg./ day
Protein, ketones, glucose, hemoglobin, urobilinogen	Negative	Negative
Bilirubin	Negative-trace	Negative
pH	5.0–7.0	5.0–7.0

Figure (6): Normal values, urinalysis in dog and cat (Bartges, 2004).

The physical characteristics of urine should be inspected when conducting urinalysis on every sample. These features are easily observed by both client and clinician alike and, as such, add no expense to case management. Urine volume, color, clarity, and odor are often overlooked. The veterinary team would do well to reconsider these urine properties because they provide unique insight about pet health and ongoing disease processes. Physical properties of urine also prompt pattern recognition. For example, observing turbid urine in canine or feline cases generates a list of the most common differentials, including pyuria, crystalluria, and bacteriuria. These clues guide decision-making as clinicians determine what is most likely ongoing in the affected case and how best to achieve a definitive diagnosis (Englar, 2022).

The dipstick was proven useful for rapid urinalysis to evaluate urine specific gravity (USG), pH, leukocytes, nitrites, glucose, proteins, ketones, urobilinogen, bilirubin, and blood. The USG and pH significantly changed during the neonatal period. Other parameters did not vary significantly in relation to age (Melandri *et al*., 2020).

Dipstick urinalysis is easily performable under every condition and is a cheap and repeatable diagnostic test, providing immediate results. A drop of urine can be put on each field of the strip to obtain reliable results using small volumes of urine. After dripping, the results were read after 60 seconds for all the parameters, except for leukocytes that were read at 120 seconds (Balogh *et al*., 2017).

Urinary pH is linked to alimentary habits; in carnivores, including dogs, it normally ranges between 6 and 6.5, being slightly acidic, and the urinary pH is influenced by alterations in systemic homeostasis.

Sediment evaluation

Normal urine should contain very few red blood cells. Owners may observe macroscopic hematuria, but microscopic hematuria will go undetected without sediment evaluation. Hematuria resulted from pyelonephritis, bladder infection, genitourinary tract inflammation, neoplasia, bleeding disorders, or trauma (McGuire *et al*., 2002). The existence of increased WBC indicated the existence of urinary tract inflammation, typically greater than 5 WBC/high power field (HPF). The presence of pyuria certainly raises concern about the presence of a bacterial urinary tract infection. Pyuria in a sample obtained via cystocentesis

indicated infection or inflammation of the kidneys, ureters, urinary bladder, or prostate. Any case with pyuria, particularly those with signs referable to the urinary tract (i.e., stranguria, pollakiuria, hematuria, polyuria), should have a urine culture and sensitivity performed. Dogs with diabetes mellitus are predisposed to urinary tract infection and many times do not have significant pyuria (McGuire *et al*., 2002).

Normal urine is sterile. Because bacteria can be found in the distal urethra and genital tract, samples obtained via free catch or catheterization may have some degree of contamination. Cystocentesis is the method of choice for obtaining the urine sample when bacterial urinary tract infection is suspected. Although bacteria can be visualized on microscopic examination, it is sometimes difficult to distinguish bacteria from debris. The presence of pyuria in the same sample would support the finding of bacteriuria. Microscopic examination of modified Wright's stained urine samples has been shown to be superior to traditional wet mounts when attempting to identify bacterial urinary infections in dogs. A bacterial culture and sensitivity should be performed on urine samples with microscopic evidence of bacteriuria (Swenson *et al*., 2004).

Occasionally, yeast or fungal hyphae may be seen in a urine sample and often represent contamination. In cats, true fungal urinary tract infections are most commonly seen associated with prolonged antibiotic and/or glucocorticoid therapy, aciduria, and the use of indwelling transurethral catheters. Fungal organisms can also be identified in the urinary bladder of pets with systemic mycoses (i.e., blastomycosis) (Werner and Norton, 2011).

Increased numbers of epithelial cells can be seen in association with infection, inflammation, irritation, and neoplasia. It is regarded as the gold standard method to attain an accurate diagnosis of bacteriuria and to gain the best course of treatment based on a decision-making process to limit the spread of resistance. Importantly, cultures should be repeated three to five days after the termination of antimicrobial therapy to ensure elimination of infection (Rampacci *et al*., 2018).

The quantitative bacterial culture (QBC) using the Calibrated Loop/Surface Streak Method (Fig. 7) is considered the gold standard for determining UTIs before initiation of antimicrobial therapy. It is necessary for differentiation of harmless bacterial contaminants from bacterial pathogens that facilitating accurate identification of specific bacterial species aids in selection of antimicrobial drugs. It also facilitates differentiation of recurrent UTIs caused by relapses from recurrent UTIs caused by reinfections (Strachan *et al*., 2022). One µL of urine sample was inoculated using a sterile inoculating loop at the surface of the standard nutrient agar plate with a single streak across the center. Then, spread the inoculum in a cross-zigzag manner (Fig. 7). After overnight incubation at 37°C, colonies were counted, and the number of colony-forming units (CFUs) per milliliter of urine was calculated. A quantitative urine culture demonstrated significant bacterial infection in the case of ≥ 103 CFU/ml in cystocentesis samples (Karah *et al*., 2020).

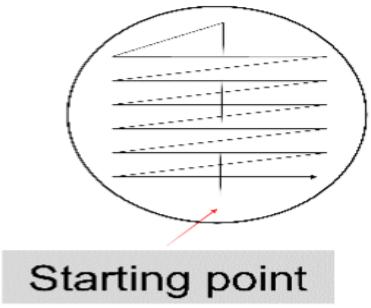


Figure 7. Urine culture using the calibrated loop/surface streak method.

The general-purpose media are sufficient for urine culture in low-resource settings; according to traditional guidelines, blood agar (non-selective medium) and MacConkey agar (selective and differential for Gram-negative rods) are probably the most commonly recommended and used media for routine urine cultures. As an alternative, cysteine lactose electrolyte-deficient (CLED) agar or chromogenic agar has been proposed as standard media for urine culture. Eosin methylene blue agar (EMB) was used as a differential for suspected *E. coli* colonies that were subcultured with the formation of a characteristic green metallic sheen. Mannitol salt agar is used as a selective medium for the isolation of *Staphylococcus* spp. Sabouraud agar should be added, in addition to the usual bacterial media, to culture the urine sample in particular care units or if yeasts have been seen by microscopic examination. The choice of media for routine urine culture should be made locally based on available resources and the desired approach of identification. Blood agar is replaced by nutrient agar in order to keep the costs low and since Gram-negatives have frequently accounted for the majority of anticipated pathogens. Isolates were identified based on colony morphology and characteristics (Public Health, 2020).

In freshly voided urine, the culture of ≥ 100 colonies of one type (number of bacteria is ≥ 105 cfu/mL) has usually been regarded as a cutoff for UTI. If 10–100 colonies of one type are counted (number of bacteria is between 104 and 105 cfu/mL), the result should be evaluated according to the clinical status. On the other hand, the probability of UTI is low if the number of colonies is <10 (number of bacteria is < 104 cfu/mL). For cultures containing two types of colonies, UTI is likely if ≥100 colonies are counted for at least one of the two types. Subcultures, for further identification and antimicrobial susceptibility testing, should be performed for each type counting ≥ 100 colonies, but it is also recommended to request a new sample. Notably, if both types have <100 colonies, for each one, UTI is not likely, and the sample is often contaminated. If there are more than two types of colonies, the sample is often contaminated. A new sample should be requested (CDC, 2020).

The sensitivity and specificity of Gram stain were evaluated on 103 canine urine samples acquired via cystocentesis and suspected UTIs. The centrifuged urine from the urine sediment preparation was used to make slides for Gram staining. These slides were allowed to air dry and then heat-fixed and stained with a commercially available Gram stain kit. A quantitative assessment of bacteria (none <3, 3–6, 6–10, 10–20, 20–50, 50–100, 100–200, 200–500, >500 bacteria/HPF) was recorded for each sample. Any slide with bacteria was

subsequently assessed for bacterial morphology (rod or cocci) and whether these bacteria stained Gram positive or Gram negative. Slides were considered positive for bacteriuria if ≥1 bacteria per HPF was observed and parallel to positive urine culture. The results revealed that Gram stain demonstrated 96% sensitivity, 100% specificity, 100% positive predictive values (PPV), and 93% negative predictive values (NPV) in the detection of bacteriuria for all dogs. Gram staining should be considered when bacteriuria is highly suspected and requires rapid identification while bacterial culture is pending (Way *et al*., 2013).

A number of 459 urine samples collected by cystocentesis from dogs suffered from UTIs; signs were prepared as unstained wet-mounted air-dried urine sediment. The preparations were subjected directly to modified Wright stain and examined for the presence of bacteria. Compared with the results of quantitative bacteriologic culture, modified Wright-stained preparations had a sensitivity of 93.2%, a specificity of 99.0%, PPV of 94.5%, NPV of 98.7%, and a test efficiency of 98.0%. So examination of urine sediment preparations by modified Wright-stained appeared to be a rapid, cost-effective method that significantly improved the sensitivity, specificity, PPV, NPV, and test efficiency of light microscopic detection of bacteriuria (Swenson *et al*., 2004)**.**

4.4. Biochemical Characterization

Positive urine culture is usually followed by a variety of biochemical identification tests to determine the species/genus of the implicated bacterium. The identification of bacterial pathogens is divided into two levels The Basic (level 1)" shall be available one day after receiving the sample and the advanced (level 2)." shall be ready in two days after receiving the sample (Table 2&3) (Karah *et al*., 2020).

Table 2. Basic biochemical identification of common uropathogens. (Karah et al., 2020).

Bacterium	Mac 1	Gram Stained Bacterial Cell Morphology	Glu 1	Oxi 1	Cat 1	PYR 1	Lanc 1
Enterobacterales	+	Red or pink rod-shaped	+	–	NA	NA	NA
***Pseudomonas*-like glucose-non-fermenter Gram-negative rods**	+	Red or pink rod-shaped	–	+	NA	NA	NA
***Acinetobacter*-like glucose-non-fermenter Gram-negative rods**	+	Red or pink rod-shaped	–	–	NA	NA	NA
Staphylococci	–	Clusters of purple or mauve sphere-shaped	NA	NA	+	NA	NA
Enterococci	–	Pairs or short chains of purple sphere-shaped	NA	NA	–	+	D
Streptococci	–	Chains of purple or mauve sphere-shaped	NA	NA	–	– *	B or D

Mac, MacConkey Agar; Glu, glucose fermentation; Oxi, oxidase; Cat, catalase; PYR, Pyrrolidone arylamidase; Lanc, Lancefield grouping; NA, Not Applicable. * Except for streptococci group A, which is not a common uropathogen.

Table 3. Advanced biochemical identification of common uropathogens (Karah et al., 2020).

Enterobacterales	Lac [1]	Ind [1]	Cit [1]	VP [1]	Ure [1]	Mot [1]	H_2S [1]	LDC [1]	Nit [1]
Escherichia coli	+	+	–	–	–	+	–	+	+
Klebsiella pneumoniae	+	–	+	+	+	–	–	+	+
Klebsiella oxytoca	+	+	+	+	+	–	–	+	+
Enterobacter cloacae	+	–	+	+	V	+	–	–	+
Enterobacter aerogenes	+	–	+	+	–	+	–	+	+
Citrobacter freundii	V	–	+	–	V	+	(+)	–	+
Citrobacter koseri	V	+	+	–	V	+	–	–	+
Proteus mirabilis	–	–	V	V	+	+	+	–	+
Proteus vulgaris	–	+	(–)	–	+	+	+	–	+
Providencia stuartii	–	+	+	–	V	(+)	–	–	+
Morganella morganii	–	+	–	–	+	+	–	–	+
Serratia marcescens	–	–	+	+	(–)	+	–	+	+

Glucose-non-fermenting Gram-negative rods	Oxi [1]	Lac [1]	Ind [1]	Cit [1]	VP [1]	Ure [1]	Mot [1]	H2S [1]	LDC [1]	Nit [1]
P. aeruginosa	+	-	-	V	-	(-)	+	-	-	V
A. baumannii	-	-	-	+	-	-	-	-	-	-

Staphylococci (catalase-positive)	Slide Agg [1]	Tube Coag [1]	Hemolysis	Salt Tol [1]	Mann [1]	Nov [1]
Staphylococcus aureus	+	+	V	+	+	S
S. saprophyticus	-	-	None *	+	+ * or -	R
S. epidermidis group	-	-	None *	+	-	S

Streptococci (catalase-negative)	Lanc [1]	Hemolysis	Bile esc [1]	6.5% NaCl tol [1]
Enterococci	D	No hemolysis *	+	+
Group D streptococci other than enterococci	D	β-, α-, or no hemolysis	+	-
Streptococcus agalactiae	B	β-hemolysis *	-	-

Lac, lactose fermentation; Ind, indole; Cit, citrate utilization; VP, Voges-Proskauer; Ure, urease; Mot, motility; H_2S, hydrogen sulfide production; LDC, lysine decarboxylase; Nit, nitrates reduction; Oxi, oxidase; Agg, agglutination; Coag, coagulase; Tol, tolerance; Mann, mannitol fermentation; Nov, novobiocin susceptibility; Bile esc, bile esculin hydrolysis; +, 90–100% Positive; -, 0–10% positive; (+), 76–89% positive; (-), 11–25% positive; V, variable.* Most strains.

4.5. Serological Identification

Smith *et al*. (2020a) evaluated urine myeloperoxidase (uMPO) as a rapidly available, accurate marker to predict urine culture results. They hypothesized that uMPO would be higher in dogs with a positive urine culture than in dogs with a negative urine culture and that uMPO could be used to aid in the accurate diagnosis of significant bacteriuria. The authors measured uMPO using a commercially available canine myeloperoxidase ELISA on urine samples from 98 dogs (forty-seven dogs had a negative urine culture and fifty-one dogs had a positive urine culture). The given results indicated that uMPO levels were significantly higher in samples that had a positive culture (median 2.13 ng/ml) versus samples that had a negative culture (median 1.07 ng/ml) ($p < 0.005$). Using a cutoff of 0.55 ng/ml, uMPO had a sensitivity of 70% and specificity of 69% to determine the presence of a positive culture.

A rapid immunoassay (RIA; BacVet) test utilizes a cocktail of monoclonal antibodies targeting a panel of bacterial surface proteins. It was carried out on twenty-one freely voided urine specimens obtained from dogs, which showed many signs of LUTIs according to the manufacturer's guidelines in the USA. The sensitivity of the RIA was 89%, specificity 100%, PPV 100%, and NPV 92%. The study revealed that the simple point-of-care RIA test can be performed in-office, rapidly, at low-cost, and without specialized training **(Grant et al., 2021)**.

Thereafter, Grant *et al*. (2023) evaluated the previous rapid immunoassay (RIA: BacVet) for the diagnostic performance immediately after urine collection and after refrigeration at 4 and 24 hours. The study was conducted on 40 voided urine samples from dogs with clinical signs of LUTIs. The results showed sensitivity, specificity, PPV, and NPV of the RIA were 100%, 88%, 82%, and 100%, respectively, and results were not different after 4 and 24 hours of refrigeration. The test is inexpensive, rapid, and accurate. Similarly, in an Australian study, a rapid immunoassay (RIA; RapidBac) was performed on forty-four urine specimens obtained by cystocentesis from 44 dogs according to the manufacturer's guidelines for diagnosis of bacteriuria. The results determined good sensitivity and excellent specificity, 81.8% and 95.5%, respectively, compared to urine culture (Sutter *et al*., 2023).

Microscopic Agglutination Test is the recommended technique for leptospirosis diagnosis, as reactivity to a serovar indicates exposure to a corresponding serogroup. Antibodies (IgM and IgG) were detected around 15 days post-infection, but little information was available on their duration in pets' blood. Clinical interpretation relies on paired serum titres, and some infected animals may produce lower results than the accepted 1:100. It is even possible that seroconversion in cats was expressed at a lower titre compared with dogs (Shropshire *et al*., 2016)**.**

The serum of 112 cats was investigated by MAT, detecting anti-Leptospira antibodies against 14 pathogenic serovars. Antibodies against 6 serovars—Poi, Bratislava, Arborea, Ballum, Pomona, and Lora—were detected in 15.3% (17/111) of cats (titers range: 20-320) as shown in figure (8) **(Donato et al., 2022).** A study in Indian Ocean islands addressed stray and domestic cats (n = 92) using a serological MAT. The results revealed a seroprevalence of 37.0% (34/92) (cut-off 1:40) without a significant difference in the living conditions of animals. The predominant serogroup was Icterohaemorrhagiae, but Ballum, Cynopteri, and Australis were also detected (Holzapfel *et al*., 2021).

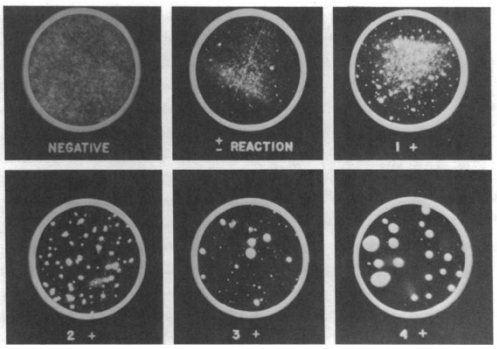


Figure (8): Microscopic Agglutination Test (MAT) (Donato et al., 2022).

Delaude *et al*. (2017) tested canine urine samples by MAT for antibodies against a panel of 12 serovars. Seropositivity (MAT ≥1:100) was most common to serovars Australis

(14.9%) and Bratislava (8.8%), followed by Copenhageni (6.1%), Canicola (5%), Grippotyphosa (4.5%), Pomona (4%), Autumnalis (2.7%), and Icterohaemorrhagiae (1.6%).

Microscopic Agglutination Test (MAT) test interpretation may be more reliable in cats than in dogs because no interference with vaccine antibodies exists as cats are not vaccinated. Furthermore, laboratory-reared young adult specific pathogen-free cats infected with *Borrelia burgdorferi* did not form antibodies against *Leptospira* species as a cross-reaction. The authors of that study suggest that positive *Leptospira* species MAT results from cats in the field are likely to reflect antibodies against *Leptospires* and not *B. burgdorferi* (Murillo *et al*., 2020).

ELISA used for leptospirosis identified the presence of specific IgM leptospiral antibodies earlier than MAT, 4–6 days post-infection. The main advantages of ELISA compared with MAT are the stability of antigenic preparations and the genus specificity, meaning all types of *Leptospires* can be diagnosed with a single antigenic preparation, irrespective of the causal serovar. In dogs, a combination of ELISA and MAT were recommended for leptospirosis diagnosis (Murillo *et al*., 2020). An IgM immunoblot assay was 88% sensitive in the first 3 days of human leptospirosis compared with 2% for the MAT. Use of rapid, broadly reactive antibody assays as screening tests before performing the more specific and cumbersome MAT may help decrease false negative test results relating to inadequate serovar inclusion in the MAT. Ideally, they should detect antibodies that react only with pathogenic serovars. Recombinant LipL32-based assays recently were evaluated and was found to be sensitive and specific in dogs and humans compared with MAT testing (Sykes *et al*., 2011). Rapid patient-side tests for leptospirosis diagnosis were developed. Curtis *et al*. (2015) performed a recombinant LipL32-based rapid in-clinic ELISA (SNAP Lepto) for the detection of antibodies against *Leptospira* species in dogs. Neither of the tests distinguishes between serovars, nor do they provide a titre magnitude. The first test, 66, is based on the detection of *Leptospira*-specific IgM and has demonstrated a sensitivity and specificity of 100% and 95.3%, respectively. It can therefore detect dogs with clinically suspected acute leptospirosis. Dogs previously vaccinated or suffering from an acute but subclinical infection can also produce positive results. A LipL32-based in-clinic ELISA for the rapid detection of *Leptospira* specific antibodies in dogs is not IgM specific, but the study authors considered it a convenient tool to assess *Leptospira* antibody status in dogs. Neither rapid test techniques nor ELISA to diagnose leptospirosis in cats have yet been developed.

4.6. Molecular Identification

Generally, the significance of molecular-based techniques is relied on that they elucidate a reliable, quick, precise, and low-cost diagnosis, especially in non-culturable or fastidious pathogens.

Melgarejo (2021) used next-generation sequencing (NGS) and stated that the diversity and abundance of bacterial and fungal communities varied between urine samples from different dogs.

Pulsed-field gel electrophoresis (PFGE) identified 31 PFGE patterns among the 43 *E. coli* isolates, which could be classified into nine and four groups showing > 80 % and > 95 % similarity. Homology ranged from 25% to 100%, indicating that canine urinary *E. coli* isolates exhibited a high degree of genetic polymorphism (Yu *et al*., 2020).

A quantitative real-time PCR assay was developed for the diagnosis and monitoring of mycoplasma UTIs in an English Cocker Spaniel dog. Attempts to culture organisms from purulent urine failed, and empirical antibiotic therapy did not resolve the pyuria. A mycoplasma species most closely resembling *Ureaplasma canigenitalium* was identified in urine samples by conventional PCR and sequencing. The qPCR method has provided rapid results and achieved verified successful treatment (Hemmatzadeh *et al*., 2019). In *Leptospira* diagnosis, PCR-based techniques played a very crucial role for detection; partial rrs gene (16S rRNA) sequencing identified *Leptospira interrogans* in all PCR-positive samples, and Leptospira DNA was detected in renal tissue, urine, and blood (Holzapfel et al., 2021). A real-time polymerase chain reaction targeting the lipL32 gene of pathogenic *Leptospira* was conducted on feline blood and urine samples. *Leptospira* spp. DNA was found in 3% (4/109) of blood and 9% (10/111) of urine samples (Donato *et al*., 2022).

Positive qPCR canine samples were subjected to 16S rRNA and secY gene phylogenetic analysis. The recovered strains were characterized by multilocus sequence typing. Phylogenetic analysis revealed that 10 dogs had *L. interrogans* infection. Three dogs (3/13) had *L. santarosai* infection. The secY phylogenetic analysis revealed that the *L. santarosai* sequences clustered separately from those obtained from other hosts. The results suggested a genetic distinction between lineages of Brazilian *L. santarosai* maintained by dogs and other animal hosts (Miotto *et al*., 2018).

PCR directly identifies leptospiral DNA. It does not determine the infecting serogroup or serovar, but it can indicate the *Leptospira* species. The test can be performed on blood, urine, cerebrospinal fluid, and body tissues. In cases of acute leptospirosis, this would be the test of choice to perform on blood and urine in cats. Compared with culture, PCR gives fast results, contributing to an early diagnosis. Real-time PCR techniques were recommended due to their greater sensitivity and specificity. Genes that have more than one copy in the genome, such as lig or rrs, were selected for increasing the sensitivity of the technique. Genes present only in the pathogenic species can also be added, as they will increase the specificity of the test. A positive PCR result means that leptospiral DNA is present in the sample. In acute infections or in chronic carriers, the test would be positive in urine, indicating that bacterial DNA is being shed. However, negative results in blood and urine do not rule out leptospirosis, as leptospiraemia is transient (only occurring in the initial phases of the disease); also, results are usually negative if the pet has received antibiotic therapy, and shedding in urine can be intermittent (Dorsch *et al*., 2017). MicroRNAs (miRNAs), classified as non-coding RNAs, regulate various metabolic systems and viral life cycles. Feline foamy virus (FFV) was identified in feline urine samples using expression of miRNAs and confirmed by application of dual-luciferase reporter assay. It was found that the seed sequences of the miRNAs identified in the study were conserved among all previously reported FFV isolates. These obtained results suggest that FFV-derived miRNAs played a pivotal role in FFV infection (Aso *et al*., 2021)**.**

4.7. Ultrasonography for pet animals

It is a significant diagnostic tool and an ideal noninvasive approach to evaluate urinary tract disorders because it is easy to implicate, low cost, and provides high real-time contrast resolution. Ultrasonography is commonly utilized as the first diagnostic technique in

instances of hematuria or dysuria (Fig. 9), as it enhances the diagnosis of cystolith, nephroliths, renal mass, cystitis, and hydronephrosis (Barot *et al*., 2022).

For ultrasonographic examination, the animals were restrained and positioned in lateral or dorsal recumbence. The abdomen was shaved and coated with an ultrasonic coupling gel, then examined with a probe of 9–3 MHz. Ultrasonography was used to inspect the urinary bladder's wall thickness, urine content, and turbidity, or the presence of any abnormalities such as cystic calculi, polyps, or tumors. The kidneys were examined for the presence of cysts, abscesses, or any abnormalities, and the male animals were inspected for any prostatic cyst or abscess to be excluded (Mantis, 2008).

Figure (9): Ultrasonography for dogs (Barot et al., 2022).

4.8. Clinical diagnostic approaches

Sporadic bacterial cystitis

Sporadic bacterial cystitis is a common condition in dogs and less encountered in cats, in which a bacterial infection of the bladder results in inflammation and corresponding clinical symptoms, which can involve dysuria, pollakiuria, stranguria, hematuria, or a combination of these symptoms. Previously, 'simple uncomplicated' or 'complicated' urinary tract infection (UTI) has been used to describe bacterial cystitis in dogs and cats (Jessen *et al*., 2015)**.**

Diagnosis is based on the presence of lower urinary tract signs, ideally with concurrent evidence supporting bacterial cystitis (e.g., hematuria, pyuria, cytologically evident bacteriuria) and bacterial culture results.

Urinalysis (dipstick, urine specific gravity, and cytological examination of the sediment) should be performed in all cases to provide supporting evidence and detect potential comorbidities (e.g., glucosuria, crystalluria).

Specimens for culture should be collected by cystocentesis unless there is a contraindication (which would rarely be present in animals with sporadic cystitis) or significant difficulties in sample collection are anticipated (e.g., from a large, morbidly obese dog). Ultrasound guidance facilitates cystocentesis and assess the bladder for abnormalities such as uroliths or masses (Patterson *et al*., 2016).

Culture of voided samples should only be performed when cystocentesis is contraindicated because of the potential for both false positive and false negative cultures.

Voided samples should only be cultured if they are refrigerated and processed by the diagnostic laboratory within a few hours or cultured in-house (Sørensen *et al*., 2016). The level of growth (105 CFU/mL), bacterial species (i.e., and whether pure growth is present) are important factors to assess when evaluating culture results from voided samples, along with urine cytology and clinical signs.

Recurrent bacterial cystitis

Recurrent bacterial cystitis definition in veterinary medicine is similar to that in human medicine. A diagnosis of three or more episodes of clinical bacterial cystitis in the preceding 12 months or two or more episodes in the preceding 6 months (Arnold *et al*., 2016). Recurrent cystitis may result from relapsing or persistent infection or reinfection. Consideration of which of these is likely present can be useful for determining the diagnostic plan (e.g., evaluation of a nidus of infection vs. reasons for susceptibility to repeated infections). Diagnosis since recurrent cystitis can be associated with ultrasound, plain radiography, contrast imaging, or possibly cystoscopy, they may be considered for refractory clinical recurrent cystitis cases to investigate further for underlying comorbidities and obtain a biopsy of the bladder mucosa, if clinically indicated. If clinical signs persist despite negative urine cultures, biopsies of bladder mucosa can be obtained during cystoscopy and submitted for culture and histological examination to evaluate for deep-seated bladder infections or other causes.

1) Urine culture, ideally from a sample collected via cystocentesis, should be performed in all animals with recurrent cystitis.

2) A diagnostic plan should be established for every animal with recurrent cystitis.

3) If the pathogen isolated from the animal with recurrent infection is different from previous organisms isolated, reinfection is likely, and efforts should be undertaken to identify and address any predisposing factors.

Upper urinary tract infections (pyelonephritis)

Pyelonephritis is an infection of the renal parenchyma that can occur from ascending infection or bacteremia, often with *Enterobacteriaceae* causing the majority of infections (Wong *et al*., 2015). In human medicine, acute pyelonephritis is classified as 'uncomplicated' or 'complicated'. Uncomplicated implies there is no underlying comorbidity; complicated suggested the presence of a systemic disease such as diabetes mellitus or neoplasia or an anatomical/obstructive disorder such as urinary stone disease or ectopic ureter**.** Ascending infection can result from clinically evident lower urinary tract disease. Additionally, leptospirosis must be considered in endemic regions (Sykes *et al*., 2011).

A definitive diagnosis is difficult, and signs attributable to pyelonephritis can be vague. As opposed to bacterial cystitis, where morbidity is relatively low, pyelonephritis can result in severe and rapid kidney injury. Thus, rapid diagnosis is important for proper treatment. Diagnosis of acute pyelonephritis can be suspected based on positive aerobic bacterial urine culture when accompanied by systemic signs such as fever, lethargy, and/or polyuria/polydipsia or renal pain on abdominal palpation. Laboratory findings of azotemia, casts, and peripheral neutrophilia with or without left shift. However, animals with acute pyelonephritis may be oliguric or anuric or have vague clinical signs. Imaging findings such as renal pelvic dilation and/or blunting of the renal papilla on ultrasound examination may be noted but are nonspecific (D'Anjou *et al*., 2011).

Increased concentrations of biomarkers such as serum creatinine or serum symmetric dimethylarginine (SDMA) can also support the presence of renal injury (Dahlem *et al*., 2017)

in association with bacteriuria, but are indicators of glomerular filtration rate and are not specific for bacterial pyelonephritis as the cause of kidney injury.

- Culture cystocentesis specimens and susceptibility testing should always be performed.
- Obtaining a urine specimen for cytology and culture by pyelocentesis should be considered, particularly if results of culture of a cystocentesis specimen are negative, or when a cystocentesis specimen can not be obtained.
- Blood cultures are recommended at the same time as urine cultures in immunosuppressed or febrile animals.
- It is important that culture specimen submissions indicate that pyelonephritis is suspected to ensure that urine breakpoints are not applied.
- If multiple organisms are isolated from urine, the suspected relative relevance of these should be considered. This assessment would include the bacterial species and colony counts.
- Evaluation for leptospirosis should be considered in culture-negative dogs by use of serological testing and PCR (Sykes *et al*., 2011).

Subclinical bacteriuria

Subclinical bacteriuria is defined as the presence of bacteria in urine as determined by positive bacterial culture from a properly collected urine specimen, in the absence of clinical evidence of infectious urinary tract disease. Terminology such as 'urinary tract infection' or 'occult infections' has been used in reference to animals with positive bacterial cultures but no clinical signs of lower urinary tract disease (Peterson *et al*., 2012); however, this terminology now should be avoided.

The term bacteriuria has been used to describe cases where bacteria are visible cytologically, irrespective of culture results (Way *et al*., 2013); however, diagnosis of bacteriuria should be based on culture (Nicolle *et al*., 2005). Cytological evaluation is an important part of urinalysis in animals with suspected urinary tract disease. An increased urine sediment white blood cell count has been associated with increased odds of a positive culture (O'Neil *et al*., 2013), but this has not been a consistent finding (McGuire *et al*., 2002). Poor agreement between cytological detection of bacteria and positive urine culture has been reported in dogs (McGhie *et al*., 2014). Increased urine sediment red blood cell count is also not predictive of positive cultures (O'Neil *et al*., 2013). Thus, cytological data are useful adjunctive data to assess animals with potential urinary tract disease but may not be highly predictive of culture results, infectious disease, or correlate well with clinical signs of upper or lower urinary tract disease. Similarly, proteinuria is not predictive of subclinical bacteriuria (Lippi *et al*., 2022).

Subclinical bacteriuria is common, even in individuals with no known predisposing factors. Rates of 2.1–12% have been reported in healthy dogs (McGhie *et al*., 2014), with higher rates (15–74%) in groups such as dogs with diabetes mellitus, morbidly obese dogs, puppies with parvoviral enteritis, dogs with acute disk herniation, chronically paralyzed dogs, and dogs treated with cyclosporine or glucocorticoids (Baigi *et al*., 2017). Study of subclinical bacteriuria has been limited in cats, and the prevalence may be lower than reported in dogs; however, rates of 1–13% have been reported in healthy cats (White *et al*., 2016; Puchot *et al*., 2017).

No evidence of an association between subclinical bacteriuria and risk of development of cystitis or other infectious complications has been reported in dogs or cats (Wan *et al*., 2014; White *et al*., 2016).

Culture of urine from animals with no evidence of urinary tract disease should not be performed when there would be no indication to treat based on a positive culture result (McGuire *et al*., 2002).

A diagnosis of subclinical bacteriuria is made based on identification of bacteria by culture of urine collected via cystocentesis in an animal without clinical signs attributable to bacterial cystitis.

Cystocentesis is the preferred method for urine collection, and urine should not be collected by other methods unless there are contraindications to cystocentesis.

Bacterial cell count, typically expressed as CFU/mL, can not differentiate subclinical bacteriuria from bacterial cystitis. Subclinical bacteriuria is differentiated from bacterial cystitis by the absence of clinical signs and not by the bacterial load. There is no evidence that high CFU counts indicate a greater risk of disease development. Subclinical bacteriuria is also not defined by the presence or absence of pyuria on urine sediment examination.

Re-testing of bacteriuric animals is not recommended.

6. TREATMENT

Generally according to ISCAID guidelines, UTIs are treated with antibiotics. Initially, an antibiotic 'empirical' may be prescribed that targets the most likely bacteria causing infection. After the culture finalizes (which may take a few days), the veterinarian specialist may need to change the antibiotic if the results indicate the first antibiotic is not ideal. Antimicrobial therapy is indicated in most cases while awaiting culture and susceptibility results to relieve patient discomfort. In most situations, amoxicillin (11–15 mg/kg PO q8h), amoxicillin-clavulanic acid, and trimethoprim-sulphonamides (15 mg/kg PO q12h) are considered as the first empirical antimicrobial choices for UTI treatment in pets. Meanwhile, nitrofurantoin, fluoroquinolones, and 3rd generation cephalosporins are only recommended if resistance to first-line antimicrobials is detected or the condition of the pet warrants it (Weese *et al*., 2019).

The intention of antimicrobial therapy is to eliminate the bacterial growth in the urinary tract utilizing an antimicrobial agent in a cost-effective manner. The degree of infection is dependent on the susceptibility of the bacteria to the concentration of the antimicrobial agent reached in the urine. Antimicrobial agents can eliminate the bacterial growth in the urinary tract within an hour. An effective antimicrobial agent generally attains minimal inhibitory concentration (MIC) both in the serum and urine of healthy adults.

The urinary levels are frequently manyfold larger than the serum levels. However, the serum levels are critical in cases with urinary infections. Traditionally, antimicrobial therapy has been used for the treatment of UTIs using either a prophylactic or therapeutic approach.

Antibiotics such as penicillins, sulfanilamide, and cephalexin have been used in the RUTI therapy (Bader *et al*., 2020). Following the last update, treatment of subclinical bacteriuria is not indicated for humans, dogs, or cats". Up to 12% of healthy dogs and 13% of healthy cats have subclinical bacteriuria. Even if pyuria is present along with bacteriuria, antibiotic therapy is not recommended in the absence of clinical signs of cystitis **(Acierno *et al*., 2024)**.

6.1. Antimicrobial Susceptibility Test (AST)

The AST is usually done by the Mueller-Hinton broth micro-dilution method.

Assays were performed according to CLSI guidelines in triplicate, with the exception of the indicated procedural variations and use of nonstandard AST media. The experimental parameters are varied one at a time during testing, and results were compared with those obtained using the standard reference Broth microdilution (BMD) method or Cation Adjusted Mueller Hinton Broth (CAMHB). Falcon 96-well polystyrene plates were used, except for indicated analyses using Falcon 96-well polypropylene plates. Assay plates were incubated for 18 h at 35 ± 2°C in ambient air, and MICs are determined visually unless otherwise indicated. MIC50 and MIC90 values were defined as the minimum concentration of an antimicrobial agent necessary to inhibit the growth of ≥ 50 % and ≥ 90 % of the isolates, respectively. MIC50 and MIC90 determinations are important for assessing the efficacy of a given antimicrobial. Reference strains of tested bacteria were used as quality control strains. Quality control standards and test results were interpreted with reference to CLSI documents (CLSI, 2023).

6.2. Clinical Therapeutic Approach

Sporadic bacterial cystitis

Clinical signs are a result of inflammation. In dogs, a decision to start antimicrobial therapy while awaiting culture results (if samples are submitted) is reasonable. However, there is evidence from humans that analgesics alone may be as effective as antimicrobials in uncomplicated cases (Gágyor *et al*., 2015; Bleidorn *et al*., 2016), which could be applied to sporadic cystitis in cats and dogs. Consideration can be given to prescribing an initial course of analgesics (e.g., NSAIDs) and adding antimicrobials 3–4 days later if clinical signs persist or worsen. Regardless, NSAIDs (use with caution in cats) should be considered during the initial treatment period to help ameliorate clinical signs. To avoid unnecessary antimicrobial use in cats, withholding antimicrobial treatment pending the result of aerobic urine culture is reasonable.

Optimal empirical choices vary based on the pathogen and resistance patterns in the region. However, amoxicillin is a reasonable first choice in most areas. If amoxicillin without clavulanic acid is not readily available, use of amoxicillin/clavulanic acid is reasonable. Evidence of a need for clavulanic acid is lacking, and it may not be necessary, even in infections with beta-lactamase-producing bacteria, because of the high amoxicillin concentrations that are achieved in urine. Trimethoprim-sulfonamides (trimethoprim-sulfadiazine, trimethoprim-sulfamethoxazole) are other first-tier options but may be associated with greater adverse effects. However, the likelihood of adverse effects is low with short courses of therapy as are recommended below (Weese *et al*., 2019). The recommended

duration of therapy is 3–5 days. The short end of that dosing period may be optimal, but veterinary research to support this is currently limited (ACADI, 2019).

Nitrofurantoin, fluoroquinolones, and 3rd generation cephalosporins should be reserved for sporadic cystitis where amoxicillin (±clavulanic acid) and trimethoprim-sulfonamide are not appropriate based on culture and susceptibility testing results or case factors. These drugs can be effective but uncommonly needed, and their use in animals is scrutinized because of concerns regarding antimicrobial resistance and public health. Rarely, the dosing regimens that some of these drugs offer (e.g., once daily administration or single injection) may be required for proper treatment, and owner compliance is an important consideration. However, clinicians must differentiate between need and convenience when choosing one of these drugs over recommended first line options. Additionally, the US FDA has discouraged routine use of fluoroquinolones in humans for uncomplicated infections because of adverse effects on joint, tendon, and nerve damage (Weese *et al*., 2022). It is not necessary to administer fluoroquinolones in most cases of sporadic cystitis when other alternatives exist. Treatment of intact male dogs with no evidence of prostatitis, as well as dogs with comorbidities not involving the urinary tract and with non-recurrent infections, should be approached as described above, with the understanding the underlying factors might increase the likelihood of recurrence.

Veterinarians should be aware of local (ideally clinic-level) antimicrobial susceptibility patterns to help guide empirical choices. If the expected incidence of treatment failure to a given antimicrobial increases, an alternate antimicrobial should be considered. However, care must be taken when interpreting potentially biased data, such as culture data obtained predominantly from specimens submitted from animals with refractory or recurrent cystitis. Veterinarians are encouraged to collect surveillance data on pathogen susceptibility patterns and clinical response to guide optimal empirical therapy. Consultations with their laboratory microbiologists are encouraged (Weese *et al*., 2019).

Infusion of substances (e.g., antimicrobials, anti-inflammatories, biocides) into the bladder via urinary catheter is not recommended because of a lack of evidence of efficacy and the potential for iatrogenic infection, trauma from catheterization, or irritation of the bladder from infusates. There is currently no evidence that adjunctive treatment measures (e.g., cranberry extract, D-mannose) are useful for treatment of sporadic cystitis (Stapleton *et al*., 2011).

Follow up

Lack of clinical response within 48 h of starting appropriate antimicrobials should prompt further investigation to determine whether cystitis is actually present and identify complicating factors.

If initial culture results indicate resistance to the empirical antimicrobial that was chosen, the drug should be changed unless there has been a good clinical response.

Empirically changing antimicrobials in response to poor initial response to treatment is not recommended. If clinical failure has been documented, the cause must be determined, as it may be unlikely that a different drug will result in a better outcome. Animals with partial or

complete clinical failure to treatment should be re-examined. Unless the initial culture results indicated resistance to the antimicrobial that was used empirically, or poor owner compliance is documented, prescribing a new course of antimicrobials in the absence of further investigation of the reason for clinical failure is not recommended (Weese *et al*., 2011).

Post-treatment urinalysis or urine culture is not recommended for sporadic cystitis when clinical signs have resolved.

Recurrent bacterial cystitis

Previously, guidelines supported long durations (4 weeks) of antimicrobials for recurrent cystitis (Weese *et al*., 2011). However, recurrent cystitis encompasses a broad range of conditions, including repeated and relatively uncomplicated infections that likely respond quickly to antimicrobials and others with marked bladder pathology that complicates treatment. Broad recommendations for treatment duration are difficult because of this variation.

The goals of treatment must be considered. The primary objective is clinical cure with minimal risk of adverse effects (including antimicrobial resistance). Microbiological cure (elimination of the offending organism) is desirable but not necessarily achievable or required for short- or long-term clinical resolution.

Depending on the severity of clinical signs and the owner's ability to observe the animal, treatment with analgesics (e.g., NSAIDs) alone could be considered while awaiting urine culture results. However, empirical therapy is reasonable and should be approached as described for 'sporadic bacterial cystitis'.

If empirical antimicrobials are initially prescribed, antimicrobial choice should be reassessed when culture results are available. If the bacterial strains isolated are reported to be susceptible to the antimicrobial drug selected, no change in treatment plan is required. If one or more isolated strains are not susceptible, the animal's response should be considered. If clinical cure is documented, it is acceptable to continue with the initial antimicrobial that was chosen. If clinical failure is documented, an antimicrobial change is indicated.

Long-term therapy is not automatically warranted for recurrent cystitis, even in dogs with underlying comorbidities such as diabetes mellitus, and this is especially true if recurrent disease appears to be caused by re-infection. Short (3–5 days) durations should be considered for reinfection. Longer courses (7–14 days duration) may be reasonable in persistent and potentially relapsing infections if factors that inhibit response to antimicrobials, such as bladder wall invasion, are suspected to be present. In those situations, drugs that are ineffective against *Escherichia coli* in tissue (e.g., amoxicillin/clavulanic acid) should be avoided (CLSI, 2023).

Intra-vesicular administration of antimicrobials or biocides is not recommended.

Follow up

Clinical cure rates are poorly established for recurrent cystitis. Most monitoring is based on clinical response, as data on the expected or desired microbiological, cytological, or hematological response to treatment are lacking.

When short (3–5 days) durations of treatment are being used, culture during treatment is not recommended. When longer durations of treatment are being used, the benefit of intra-treatment culture is unclear (Weese *et al*., 2011).

When longer durations of treatment are being used, urine culture is reasonable to consider after 5–7 days of treatment; however, the approach to a positive or negative result should be considered in advance. Positive cultures indicate the need for evaluation of compliance and further diagnostic testing to determine why the bacterium has not been eliminated, not simply a change in antimicrobial, particularly if clinical cure has been documented. Negative results could be used to help determine when to stop therapy if a long course of treatment is being used but are not a guarantee of microbiological cure.

Culture of urine specimens, ideally collected by cystocentesis, can be considered 5–7 days after cessation of antimicrobials in animals where clinical cure is documented. However, this should be used as part of the diagnostic process to help differentiate relapse, re-infection, and persistent infection, and to guide potential future diagnostic testing, not as an indication of a need to treat. The presence of bacteriuria post-treatment should be approached as described under 'Subclinical bacteriuria'. If client compliance is deemed to have been adequate, referral to a specialist should be considered to explore reasons for microbial persistence or rapid re-infection (CLSI, 2023).

Prevention

Balancing potential efficacy, resistance, and adverse effects is a challenge, and there are few published studies in dogs or cats. Single nightly dose nitrofurantoin has been anecdotally used in dogs to prevent recurrent cystitis, but efficacy data are lacking. Furthermore, adverse effects of these drugs exist, and there is concern for the selection of resistant bacteria. Alternative approaches for prevention and treatment of recurrent cystitis that have been investigated in human beings as well as animal UTI models include the use of cranberry extract (McMurdo *et al*., 2009), cranberry juice (Stapleton *et al*., 2011), probiotics (Rodrigues *et al*., 2014), live biotherapeutic products (such as asymptomatic strains of *E. coli*) (Segev *et al*., 2018), vaccines (Billips et al., 2009), and various other alternative therapies, such as methenamine, D-mannose, and intravesicular or orally administered glycosaminoglycans (Mansour *et al*., 2014). There has been limited study in dogs. In one study, cranberry extract prevented adherence of *E. coli coli*strains isolated from dogs to canine kidney cells, and six dogs with recurrent UTI treated with cranberry extract did not develop UTI when monitored for 2 months (Chou *et al*., 2016), but a placebo-treated control population was not studied. In another prospective, randomized, placebo-controlled study of 94 dogs with thoracolumbar disk herniation, cranberry extract did not appear to reduce the prevalence of bacteriuria, with six dogs in the placebo and 11 dogs in the cranberry extract group developing bacteriuria over a 6-week period (Olby *et al*., 2017). Live biotherapeutic products appear promising for treatment of recurrent cystitis, with a preliminary study reporting complete or nearly complete clinical cures in four out of nine dogs with recurrent cystitis in response to instillation of *E. coli* 2-12 (Segev *et al*., 2018).

Prophylactic antimicrobial therapy for dogs and cats is not recommended.

Treatment with a short course (3–5 days duration) therapy ideally based on susceptibility testing, is most appropriate to alleviate clinical signs, with a focus on clinical rather than microbiological cure.

There is insufficient evidence to recommend the administration of cranberry extract products and other alternative therapies at this time.

There is insufficient evidence to recommend administration of methenamine. Data from human medicine suggest it may be effective in some (but not all) human populations with recurrent cystitis (Lee *et al*., 2012); however**,** evidence of efficacy and safety in dogs and cats is lacking. Conversion to the active form (formaldehyde) requires low pH, which is not always assured in dogs and cats with recurrent cystitis.

Upper urinary tract infections (pyelonephritis)

Treatment should be initiated immediately, while awaiting culture and susceptibility results.

Initial treatment should involve antimicrobial drugs known to have local or regional efficacy against *Enterobacteriaceae*. If regional data are supportive, a veterinary fluoroquinolone or cefpodoxime are reasonable first choices. Cefotaxime and ceftazidime are options for IV administration (Table 4).

If ascending infection is suspected, recently obtained urine culture results should be the basis of initial therapy (remembering that serum breakpoints must be considered). If hematogenous spread is suspected, initial therapy should be based on cultures of blood or the infected site whenever available (CLSI, 2023)**.**

Oral antimicrobial therapy is recommended in animals that otherwise appear systemically well and have normal appetites. Intravenous therapy is recommended for animals that are dehydrated, hyporexic, anorexic, or lethargic as recommended in humans (Strohmeier *et al*., 2014).

- Culture and susceptibility data should be reviewed when results are received.
- If combination therapy was initiated empirically and the isolate is susceptible to both drugs, one might be discontinued if supported by evidence of clinical response.
- If resistance is reported to one of the drugs, that antimicrobial should be discontinued. A second drug to which the isolate is susceptible should be substituted if the patient has not responded sufficiently; substitution is not necessary if the patient response has been sufficient (Strohmeier *et al*., 2014).
- If resistance is reported to both antimicrobials and clinical evidence of improvement is not evident, antimicrobial treatment should be changed to a drug to which the offending organism is susceptible *in vitro*.
- If resistance to the drug(s) that are used is reported but there has been good clinical response, continuation with the initial therapy could be considered, provided there are not

other reasons (such as fluid therapy) that might explain clinical improvement. Otherwise, a change in antimicrobial is indicated.

- Consultation with a specialist (veterinary clinical microbiologist and/or veterinary pharmacologist/pharmacist) is indicated with multidrug-resistant organisms (Strohmeier *et al*., 2014).

A diagnosis other than bacterial pyelonephritis should be considered if there is no improvement in systemic signs, hematology, or serum biochemistry (e.g., azotemia, acute phase proteins) within 72 h of antimicrobial therapy and the results of culture and susceptibility indicate susceptibility to the antimicrobial used and there is confidence in client compliance. At that time, consideration should be given to a diagnosis of subclinical bacteriuria (with discontinuation of antimicrobial therapy) or for the presence of uncontrolled underlying factors (e.g., ureteroliths, neoplasia) that would need to be addressed to resolve the underlying infection (CLSI, 2023).

Treatment for 4–6 weeks has previously been recommended for pets (Weese *et al*., 2011). However, the recommended duration of therapy for acute bacterial pyelonephritis in humans is 7–14 days (Morello *et al*., 2016; Ren *et al*., 2017). There is no reason to suspect that a longer duration would be necessary for dogs and cats. In the absence of veterinary-specific data, the Working Group recommends 10–14 days of treatment.

Subclinical bacteriuria

Treatment of subclinical bacteriuria with antimicrobials is rarely indicated and is discouraged. In animals where it is unclear whether clinical signs are attributable to cystitis, a short course (e.g., 3–5 days duration) of antimicrobials as recommended for sporadic cystitis could be considered. If there is no clinical response, antimicrobials should be discontinued, as an infectious process is unlikely. Treatment of animals with pyuria or other cytological abnormalities without lower urinary tract signs is not recommended (Johnstone, 2020).

The isolation of a multidrug-resistant bacterial species should not affect the decision whether to treat subclinical bacteriuria. Antimicrobial resistance genes are not virulence factors, and resistant organisms are not more likely to cause disease than their susceptible counterparts.

Treatment of subclinical bacteriuria caused by multidrug-resistant pathogens for infection control purposes (e.g., to eliminate urine shedding of a possible pathogen) is not recommended. It is reasonable to assume that the bacterial strain in the bladder is also present in the gastrointestinal tract; therefore, even if bacteria are eliminated from the bladder with antimicrobials, it would likely have limited impact on the overall risk .

In rare circumstances, treatment of subclinical bacteriuria may be considered if there is concern that there is a particularly high risk of ascending or systemic infection or that the bladder may be a focus of extra-urinary infection.

In cases that are unable to display clinical signs of cystitis (e.g., spinal cord injury), a clinical judgment must be made, ensuring that consideration of the need and potential adverse impacts (e.g., adverse drug effects, antimicrobial resistance) are balanced. The relevance of changes in urine appearance and odor (e.g., gross discoloration, malodor) to differentiate infection from subclinical bacteriuria is unclear. However, sometimes a short course of treatment (e.g., 3–5 days duration) could be considered for the quality of life issues if bacteriuria may be playing a role (Johnstone, 2020).

Table 4. Drugs for the management of bacterial urinary tract infection in dogs and cats (Weese *et al.*, 2011).

Drug (WHO category)[a]	Dose	Comments
Amikacin (CIA)	Dogs: 15–30 mg/kg IV/IM/SC every 24 h Cats: 10–14 mg/kg IV/IM/SC every 24 h	Not recommended for routine use but may be useful for treatment of MDR organisms. Potentially nephrotoxic.
Amoxicillin (CIA)	11–15 mg/kg PO every 8–12 h	Good first-line option for sporadic bacterial cystitis. Excreted in urine predominantly in active. *Klebsiella* spp. are resistant. Not recommended for pyelonephritis.
Amoxicillin/clavulanic acid (CIA)	12.5–25 mg/kg PO every 12 h Note: dose of total product (amoxicillin + clavulanic acid)	Reasonable empiric choice for cystitis. Not recommended for pyelonephritis.
Ampicillin (CIA)	N/A	Not recommended because of poor oral bioavailability.
Cefazolin (HIA)	22 mg/kg IV ~30 min prior to the procedure.	Main use is for peri-procedure prophylaxis as a single pre-procedure dose
Cefpodoxime proxetil (HP-CIA)	Dogs: 5–10 mg/kg every 24 h PO days. Cats: no dose established.	More active than cephalexin or cefadroxil against Enterobacteria except *Enterococcus* spp.
Ceftiofur (HP-CIA)	Dogs: 2 mg/kg every 12–24 h SC Cats: no dose established	Approved for treatment of bacterial cystitis in dogs in some regions. *Enterococcus* spp. are resistant.
Cefuroxime (HIA)	Peri-operative prophylaxis: 20–50 mg/kg slow IV	2nd generation cephalosporin that can be used perioperatively. *Enterococcus* are resistant.
Cephalexin, cefadroxil (HIA)	12–25 mg/kg PO every 12 h	Narrow-spectrum activity; not active against Enterobacterials. *Enterococcus* spp. are resistant.
Chloramphenicol (HIA)	Dogs: 40–50 mg/kg PO every 8 h Cats: 12.5–20 mg/kg (to a maximum of 50 mg/cat) PO every 12 h	Reserved for multidrug resistant infections with few other options. Myelosuppression can occur, particularly in cats and with long-term (e.g. >28 days) therapy. Not a first line treatment for pyelonephritis.
Ciprofloxacin (HP-CIA)	25–30 mg/kg PO every 24 h	Sometimes used because of lower cost than fluoroquinolones. Variable oral bioavailability.
Doxycycline (HIA)	5 mg/kg PO every 12 h	Not excreted in urine at high levels but can achieve levels that are effective against some pathogens. Care should be taken with cats to reduce the risk of esophageal ulceration.
Enrofloxacin (HP-CIA)	5–20 mg/kg every 24 h (dogs) 5 mg/kg PO every 24 h (cats)	Excreted in urine predominantly in active form. Reserve for MDR infections but initial/empirical choice for pyelonephritis in dogs. Not recommended for *Enterococcus* spp. It is recommended in cats.
Fosfomycin (CIA)	40 mg/kg PO (with food) every 12 h	Should be reserved for multidrug resistant infections. Do not use in cats. Potential option for pyelonephritis.

Imipenem-cilastatin (CIA)	5 mg/kg IV/IM every 6–8 h	Reserve for treatment of MDR infections, particularly those caused by ESBL-producing *Enterobacterials* or *P. aeruginosa*, while *E. faecium* is inherently resistant.
Levofloxacin (HP-CIA)	25 mg/kg PO every 24 h (dogs)	Sometimes used as a lower cost fluoroquinolone. High oral bioavailability in dogs.
Marbofloxacin (HP-CIA)	2.7–5.5 mg/kg PO every 24 h	Excreted in urine predominantly in active form. Good first line choice for pyelonephritis. Not recommended for *Enterococcus* spp.
Meropenem (CIA)	Dogs: 8.5 mg/kg every 12 h (SC) or every 8 h (IV) Cats: 10 mg/kg every 12 h IV, SC, IM	Reserve for treatment of MDR infections, particularly those caused by ESBL-producing *Enterobacterials* or *P. aeruginosa*, while *E. faecium* is inherently resistant.
Nitrofurantoin (IA)	4.4–5 mg/kg PO every 8 h	Option for sporadic bacterial cystitis, particularly when MDR pathogens are involved. Excreted in urine predominantly in active form.
Orbifloxacin (HP-CIA	Tablets: 2.5–7.5 mg/kg PO every 24 h Suspension (cats): 7.5 mg/kg every 24 h	Reserve for documented MDR but good first choice for pyelonephritis. Not recommended for *Enterococcus* spp.
Pradofloxacin (HP-CIA)	Dogs: 3–5 mg/kg PO every 24 h. Cats: 3–5 mg/kg once daily (tablets) or 5–7.5 mg/kg every 24 h (suspension)	Bacterial cystitis in dogs and cats. Greater activity than older fluoroquinolones. Theoretically a good 1st line choice for pyelonephritis, especially in cats.
Trimethoprim-sulfadiazine/Trimethoprim-sulfamethoxazole/Ormetoprim-sulfadimethoxine (HIA)	15–30 mg/kg PO every 12 h	Appropriate initial or empirical option. Concerns regarding idiosyncratic and immune-mediated adverse effects in some patients; however, this is most relevant with long-term therapy. If prolonged (>7 days) therapy is anticipated. Avoid in dogs that may be sensitive to potential adverse effects such as hepatopathy, hypersensitivity and skin eruptions. Activity against *Enterococcus* spp. in urine is controversial and should be avoided.

HP-CIA: highest priority critically important antimicrobial. CIA: Critically important antimicrobial.
HIA: highly important antimicrobial. IA: Important antimicrobial.
CLSI: Clinical Laboratory Standards Institute. ESBL: Extended spectrum β-lactamase.
a Drug category per World Health Organization guidelines.

Treatment of subclinical bacteriuria caused by plaque-forming (*Corynebacterium urealyticum*) and urease-producing (e.g., *Staphylococci*) organisms could be considered because of their associations with encrusting cystitis and struvite urolith formation, respectively (Biegen *et al*., 2013, Raab *et al*., 2015). Because of the potential difficulties in treating these conditions, consideration of a single short course (3–5 days duration) of treatment, as per 'Sporadic bacterial cystitis,' could be considered after confirming that

bladder wall plaque or uroliths are not present. However, it is unknown whether this is a necessary or effective approach. Continued treatment of subclinical bacteriuria with these strains is likely not warranted.

There is currently no evidence that screening bacterial isolates for urovirulence factors should impact decision-making for subclinical bacteria, as there are currently no data that indicate the isolation of a bacterial strain that possesses urovirulence genes is of greater clinical relevance to an individual patient or that treatment will reduce the risk of disease (Johnstone, 2020).

There is currently no evidence that the use of adjunctive treatments (e.g., cranberry extracts or probiotics) for the prevention of cystitis or subclinical bacteriuria is effective, but there is no contraindication to the use of treatments and supplements that are known to be safe.

If an animal that was recently diagnosed with subclinical bacteriuria subsequently develops signs consistent with cystitis or pyelonephritis, treatment designed to target the organism isolated while clinical signs were absent can be considered. However, the likelihood that a subsequent infection is caused by a previous bacteriuria isolate is not known and probably decreases with time from the last culture. Repeat culture is indicated to determine the optimal treatment if bacteriuria with a multidrug-resistant bacterial species was previously diagnosed. If pyelonephritis is suspected, treatment targeting the previously identified resistant bacterial isolate should be considered, with a plan to de-escalate once culture results are available if a susceptible organism is identified (Johnstone, 2020).

6.3. Antifungal Drugs

Fluconazole is recommended as initial treatment in most cases because of the high margin of safety, sensitivity of most strains of *Candida* spp., and excretion of active drug into urine in high concentrations. On the other side, 19 *Candida* spp. other than *C. albicans* are more likely to be resistant to fluconazole, and antifungal sensitivity testing is recommended to determine if a higher dose of fluconazole is appropriate or if another drug should be used. Although amphotericin B is renally excreted and achieves high concentrations in urine, it is not often used because it is parenterally administered and nephrotoxic (Reagan *et al*., 2019). Other commonly used antifungal drugs, including itraconazole and ketoconazole, are not renally excreted in active form (Hizlisoy *et al*., 2025). Secondary fungal UTI occurs because of shedding of organisms into urine in cases with systemic infections. Organisms most commonly associated with urine shedding are *Aspergillus* spp. in dogs (particularly German shepherd dogs) and *Cryptococcus* spp. in cats. These cases should be treated with antifungal agents standardly recommended for systemic infections (Grassi *et al*., 2024).

6.4. Non Antimicrobial UTIs Therapeutic Approaches

Multidrug-resistant bacterial isolates have significantly increased in recent years as a cause of bacterial UTIs. This increasing resistance to antimicrobial drugs leaves fewer therapeutic options for effective treatment. Urinary tract infections with multidrug-resistant (MDR) bacteria increase morbidity, treatment failures, and therapeutic costs. The emergence of antibiotic-resistant bacteria in the community is necessitating the need to explore non-pharmacologic treatment options to reduce the spread and proliferation of these species (Amphaiphan *et al*., 2021).

Ozone therapy

Ozone is an emerging therapy with both potent antibacterial properties and the ability to modulate the immune system, reduce oxidative stress induced by chronic infection, and upregulate the endogenous antioxidant system, providing further protection from free radical injury.

A 14-year-old female dog, paraplegic, suffered from recurrent bacterial cystitis caused by *Proteus* spp. The case has been treated for two months with enrofloxacin, amoxicillin, and potassium clavulanate. Because of the development of adverse effects such as pharmacodermia and corneal ulcers, the dog was referred to the ozone therapy sector of the Santa Maria Veterinary Hospital, Brazil. The ozone therapy consisted of bladder instillations of ozonized saline solution (O_3SS); (59 µg/mL), once a day for 3 consecutive days. After antisepsis of the external genitalia with 1% chlorhexidine gluconate, a bladder catheterization was performed. Next, within a 20-minute interval, the bladder was washed with 1 L of O_3SS at a concentration of 59 µg/mL. The data extracted from the study proposed removal of the biofilm that covered the bladder mucosa, marked reduction in the suspended echogenic content (sediment), and small hyperechogenic, punctiform, and linear structures suggestive of crystals or small clots. Furthermore, it seemed that O_3SS had changed the pattern of susceptibility to cephalothin and neomycin, which could expand the therapeutic options (Engelmann *et al*., 2023).

Probiotic therapy

Several probiotic bacteria have been proposed as an alternative to combat MDR UTI. Lactic acid bacteria in the genus *Lactobacillus* are some of the most studied and used probiotics (Gupta *et al*., 2024).

L. reuteri KUB-AC5 (AC5), isolated from the chicken gut, has been investigated for both its direct and indirect effects against uropathogenic *E. coli* (UPEC) isolates in vitro using a spot-on lawn, agar-well diffusion, and competitive growth assays. It is found that viable AC5 cells and cell-free components of this probiotic significantly reduced the UPEC growth of all strains tested. The data showed that AC5 can attach to the examined cell line and decrease UPEC attachment in a dose-dependent manner. Pretreatment of UPEC-infected murine macrophage RAW264.7 cells with viable AC5 (multiplicity of infection, MOI = 1) for 24 hours enhanced macrophage-killing activity and increased proinflammatory and anti-inflammatory gene expression. These findings indicate the gut-derived AC5 probiotic could be a potential urogenital probiotic against MDR UTI (Tantibhadrasapa *et al*., 2024).

Snell *et al*. (2022) assessed the in vitro effects of *E. coli* strain Nissle 1917 as a probiotic on UPEC isolated from 40 cats with clinical UTI and subclinical bacteriuria. The results revealed that 52% of isolates were found to be resistant to antimicrobials, with 19% of these being multidrug resistant (MDR). Nissle 1917 adversely affected the growth of 82.5% of all isolates and 100% of MDR isolates in vitro. The median zone of inhibition was 3.33 mm (range, 1.67 to 10.67 mm). Thirteen isolates were affected via competitive overgrowth and 20 via growth inhibition.

The importance of dietary modifications in medical protocols designed to treat and prevent LUTS in pets was postulated. The main goals of dietary modifications to prevent LUTS are 1) promoting large dilute volumes of urine, 2) decreasing the relative supersaturation of urine for specific stone types, and 3) promoting healthy bacterial populations in the gastrointestinal and urogenital tracts. The impact of dietary composition, including dietary moisture, protein concentration and digestibility, mineral concentrations, inclusion of acidifiers and alkalinizing agents, inclusion of vitamin B6, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and γ-linolenic acid, fiber concentration and characteristics, and oxalate degrading probiotics (Kerr, 2013).

Bacteriophage

A study evaluated the therapeutic efficacy of phage P2-71 against *P. mirabilis,* "a particular urinary tract pathogen," in vivo and in vitro environments. The results demonstrated that in vitro, bacteriophage P2-71 achieved significant reductions in *P. mirabilis* concentrations, with log reductions of 1.537 and 0.7009 CFU/mL in laboratory and urine environments, respectively ($p < 0.001$). The phage also decreased biofilm formation by 34-49% and lysed 15-25% of mature biofilms at various multiplicities of infection (MOIs) ($p < 0.001$). In vivo, phage treatment significantly lowered bacterial concentrations in the urine on Days 1 and 3 ($p < 0.0001$), achieving a maximum reduction of 4.602 $\log_{10}$ CFU/mL; however, its effectiveness diminished by Day 5 ($p > 0.05$). Concurrently, phage titers decreased over time. Importantly, phage treatment notably reduced bacterial load in the bladder, kidneys, and spleen ($p < 0.001$). Inflammatory markers such as IL-6, IL-1β, and TNF-α were significantly lower in the treatment group, especially in the bladder ($p < 0.0001$), indicating an effective reduction in inflammation. So it was concluded that bacteriophage P2-71 is a promising alternative therapy for UTIs caused by MDR *Proteus mirabilis* (Wu *et al*., 2024).

6.5. Ancillary Therapies and Prevention

Methenamine

Methenamine salt is a urinary antiseptic that is converted to bacteriostatic formaldehyde in an acidic environment (urine pH <5.5). There is controversy in human medicine as to whether methenamine prevents UTI, although there is some evidence that it may be effective for short-term prophylaxis. It is unknown if the 2 salts described in the literature, hippurate and mandelate, are equally effective; the mandelate salt is difficult to find. There is limited veterinary literature on the use of methenamine in small animals, although there is a theoretic benefit. Studies of safety, efficacy, and appropriate dosing are lacking. Commonly recommended doses are 10 to 20 mg/kg orally every 12 hours (dog) and 250 mg per cat orally every 12 hours. Gastrointestinal upset and dysuria are the most commonly reported adverse events; methenamine is poorly tolerated by feline patients. Methenamine should not be used in cases of renal failure. Concurrent use of a urinary acidifier, such as dl-methionine, is usually required for maximal effect (Weese *et al*., 2011).

Cranberry

Proanthocyanidin, the "active ingredient" in cranberry, alters the genotypic or phenotypic expression of fimbriae, which subsequently inhibits *E. coli* adherence to human bladder and vaginal epithelial cells. There are few veterinary studies in healthy dogs and no feline studies. In addition, quality and potency are variable among over-the-counter products; ideally, each formulation would be tested in the species of interest. The consensus of the Antimicrobial Guidelines Working Group of the International Society for Companion Animal Infectious Diseases is that there is insufficient evidence to support the use of cranberry extract to prevent recurrent UTIs in dogs and cats (Weese *et al*., 2011).

Anti-biofilms

Some bacteria have the capacity for biofilm formation, which facilitates colonization. A biofilm is composed of organisms adhered together by a self-produced polysaccharide matrix. It has been suggested that the bacteria within the biofilm become sessile; they are protected from the immune system, are antimicrobial, and inherently are resistant to shear forces of removal. Biofilms are also implemented in the development of catheter-associated UTIs.

Strategies to prevent catheter-associated biofilms include using (1) materials that are less amendable to biofilm formation and (2) coatings or surface modifications that decrease biofilm formation. For example, silicone catheters are preferred over latex because scanning electron microscope imaging reveals that latex surfaces are more irregular and promote microbial adherence (Olin and Bartges 2015). In a veterinary prospective study (n = 26 dogs) evaluating biofilm formation on indwelling urinary catheters, sustained-release varnish of chlorhexidine-coated urinary catheters statistically decreased biofilm formation (Segev *et al*., 2013). There are an array of other catheter coatings and modifications to decrease bacterial adherence and biofilm formation that have primarily been studied in a research setting, including silver coating, nanoparticles, iontophoresis, antimicrobials, urease and other enzyme inhibitors, liposomes, and bacteriophages. Other novel strategies include quorum sensing inhibitors and vibroacoustic stimulation (Siddiq and Darouiche, 2012).

Vaccination

Vaccination is the most logical way to try and control infection and reduce the prevalence of clinical disease and shedding. Bivalent vaccines containing the serogroups *L. icterohaemorrhagiae* and *L. canicola* have been in widespread use for many years, and infection with *L. canicola* is now uncommon based upon the MAT. Leptospirosis has been reported in dogs in Europe vaccinated with bivalent vaccines. Quadrivalent leptospirosis vaccines, targeting *L. canicola*, *L. icterohaemorrhagiae*, *L. pomona,* and *L. grippotyphosa*, were introduced in the USA in 2001 and are now available in Europe. The current European vaccines contain either three serogroups (*L. canicola*, *L. icterohaemorrhagiae*, and *L. grippotyphosa*) or four serogroups (*L. canicola*, *L. icterohaemorrhagiae*, *L. grippotyphosa,* and *L. bratislava*). The European consensus statement recommends the use of quadrivalent vaccines (Murphy, 2018).

Vaccination is recommended as soon as clinical recovery is seen. There have been concerns raised about reactions to quadrivalent vaccines and about reactions to leptospirosis vaccination, particularly in small dogs. Veterinarians should report any suspected adverse

drug reactions to the authorities to increase the evidence available to confirm or refute these claims. Current literature does not show vaccines with leptospiral antigen to be associated with more significant reactions than other vaccines (Murphy, 2018). There is no commercial vaccine available for cats. However, one study has shown that cats can produce antibodies (of lower titre magnitude than vaccinated dogs) when experimentally inoculated with a commercial dog vaccine (containing four different serovars). The follow-up time for the animals was 42 days, at which point only one animal maintained antibody levels. The authors of that study suggest further work is needed before a vaccine against *Leptospira* species for cats can be considered. Given the current lack of a vaccine, the best way to avoid infection in cats is via prevention of exposure. Cats that are kept indoors have a lower risk of being infected. Prevention of predation opportunities and avoidance of contact with stagnant water, urine from infected animals, and dogs at risk of clinical leptospirosis is recommended. For cats that share an environment with a positively diagnosed animal, doxycycline can be given at 5 mg/kg PO q12h or at 10 mg/kg PO q24h for 2 weeks. 19,60 **(Murillo *et al*., 2020)**.

TRANSMISSION OF UROPATHOGENS BETWEEN HUMANS AND PETS: A PUBLIC HEALTH IMPORTANCE

Since the close relationship and direct contact between humans and pets, the potential for transmission of antimicrobial resistant bacteria or their resistance determinants from companion animals to vulnerable populations, especially children and immunocompromised persons, poses a public health concern (Amphaiphan *et al*., 2021). Given the possibility of zoonotic transmission of antimicrobial-resistant bacteria, veterinarians, when treating UTI cases, should inform pet owners of the potential transmission risk (Yudhanto *et al*., 2022). Often, major urinary bacterial pathogens of dogs can be resistant to antimicrobials commonly used to treat UTIs or to antimicrobials important for human medicine. Previous studies described resistance to carbapenems in *E. coli* isolates that have carried the carbapenem resistance gene blaNDM-5. Carbapenems are critically important antibiotics used to treat serious bacterial infections in humans and are considered one of the human use last resorts (Tyson *et al*., 2019). A recent study in Egypt declared the prevalence of shared UPEC serotypes isolated from companion animals and humans, as well as sharing in the presence of virulence determinants and the blaNDM-1 gene, which is responsible for carbapenem resistance, proposing a potential public health concern (Hakim *et al*., 2024a). In Australia, there is a particular significance with the detection of the human UTI pandemic MDR *E. coli* strain O25b:H4-ST131 in dogs. The significantly higher prevalence of this clonal lineage among fluoroquinolone-resistant *E. coli* isolates from humans compared to dogs suggests that human-to-dog transmission may currently predominate (Johnstone, 2020).

Similarly, resistance to fluoroquinolones in *Pseudomonas aeruginosa* isolates has been mentioned (Harada *et al*., 2012) and against cephalosporins (Hakim *et al*., 2024b). Also, an increase in MDR methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) isolated from dogs with UTI has been reported (Grönthal *et al*., 2017; Smith *et al*., 2020b).

The zoonosis, leptospirosis, is caused by pathogenic spirochetes of the genus *Leptospira,* which colonize the renal tubules where they reproduce before being excreted via the urine. Infected urine or contaminated water are sources of leptospirosis infection; asymptomatic and chronic carrier dogs can be maintenance hosts, acting as sources of infection and therefore causing a public health problem. Formerly, it was thought that domestic cats were resistant to leptospirosis infection; however, recently published reports on feline leptospirosis conclude that cats may play a role in the epidemiology of this disease (Donato *et al*., 2022).

CONCLUSION

The illnesses of the urinary tract in small animal, particularly in dogs and cats, occur frequently with different etiologies. Many factors can predispose pets to UTIs, including bladder stones, diabetes mellitus, anatomical abnormalities, and weakened immune systems. The most common causes of UTIs are bacteria that go upwards through the urethral opening. Bacterial UTIs are reported to occur in about 14% of all dogs at some time during their lives, and the infection rate was higher in females compared to males because of differences in anatomy. The infection rate was highest in dogs younger than 2 years and older than 6 years, reaching 50% in females older than 10 years. Due to their cleanliness and high urine osmolarity, cats have a lower prevalence. The UTIs in dogs and cats are caused by *E. coli*, *P. aeruginosa*, *Klebsiella* spp., *Proteus* spp., *Staphylococcus* spp., *Streptococcus* spp., and *Enterococci*. In addition to bacteria, fungi or viruses may also infect the urinary tracts. Cystocentesis is the proper aseptic urine sampling for culture diagnostics. Diagnosis is based on microbial culture as a gold standard method, besides direct microscopy examination of urine sediment, biochemical examinations, urinalysis, serological, and molecular techniques used for species identification. The foundation of effective treatment is the disc-diffusion method, which determines a pathogen's susceptibility to antibiotics; however, the serial dilution technique is more precise. Use of empirical antibiotics is substantial as they affect the most likely bacteria causing infection. In most cases, amoxicillin, amoxicillin-clavulanic acid, and trimethoprim-sulfonamides are considered the first empirical antimicrobial choices for UTI treatment in pets. The increasing resistance to antimicrobial drugs leaves scarce therapeutic choices for effective treatment. The emergence of MDR elevates the need to explore non-pharmacologic treatment options such as ozone therapy, probiotics, bacteriophage, herbs, and nutrient supplements. The increased incidence of MDR bacteria represents a potential cause of antibiotic failure in companion animals and constitutes a serious threat to global public health due to the presence of potentially zoonotic microbial reservoirs in pet animals.

RECOMMENDATION

Urinary tract infections are a common issue in both dogs and cats, and it's essential to understand how they're approached. Here's a breakdown of key recommendations:

*Veterinary Consultation: If you suspect your pet has a UTI, seek veterinary care immediately.

*Accurate Diagnosis: Ensure your veterinarian performs a urinalysis and, ideally, a urine culture and sensitivity test.

*Follow Veterinary Instructions: Adhere strictly to your veterinarian's treatment plan, including completing the full course of antibiotics.

*Preventative Measures: Provide plenty of fresh water and maintain a clean litter box for cats.

*Advise vet. clinicians and vet. Lab specialists about the importance of conducting bacterial culture and AST before starting UTI treatment to prevent the emergence of MDR bacteria.

*Since pets could become the reservoirs of MDR bacteria that may be transmitted to humans, veterinarians should inform pets' owners about the potential zoonotic transmission risk of these pathogens.

*Continuous monitoring of the AMR patterns of clinically important bacterial urinary pathogens is warranted to identify emerging MDR strains.

*Monitoring the antibiotic resistance profiles in pet infections is important not only for the public health implications but also to collect data useful for the treatment of diseases in pets.

*To obtain information useful for global plans aimed at fighting AMR, surveillance and rapid identification of AMR infections in pets have been highlighted within the framework of the One Health approach, which recognizes that the health of people is closely connected to the health of animals and the shared environment.

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Investigating the Effects of *Solanum nigrum Linn*. against *Spodoptera frugiperda* in *Nicotiana tabacum*

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ABSTRACT

Control, management, and eradication of *Spodoptera frugiperda* or Fall armyworm (FAW) are challenging without the extensive use of inorganic pesticides. However, extensive application of inorganic pesticides threatens human health, animals, and the environment. Hence, natural, organic, and eco-friendly substances are needed to control the proliferation of FAW. The main objective of this study was to determine the effects of *Solanum nigrum Linn*., or Black nightshade or *Am-amsi* leaf and fruit aqueous extract, on FAW found in *Nicotiana tabacum,* or Burley tobacco plants. A field experimental method using a completely randomized design was employed. Data gathered were analyzed through descriptive statistics such as mean and ANOVA. Phytochemical analysis revealed that black nightshade contains flavonoids, saponins, steroids, and terpenoids. Secondly, FAWs were irritable upon application of *Am-amsi* aqueous extract and became immobile after 12 hours of application. Moreover, there was an increasing mortality of FAW in the four treatments after 24 to 72 hours of application. In addition, there was a significant difference between T_1 (25% concentration) and T_4 (100% concentration) after 36 hours; there was a significant difference between T_2 (50% concentration) and T_4 after 60 hours; and there was a significant difference between T_1 and T_4 after 72 hours. Finally, post hoc analysis showed that T_3 (75% concentration) was comparable to T_4 when compared to a synthetic insecticide. It has been demonstrated that T_4 of the aqueous extract was the most effective against FAW, while T_3 was comparable to T_4 concentration. The phytochemical components of Black nightshade contributed to the irritability, immobility, and mortality of FAW. These findings suggest that Black nightshade is a potential natural and organic larvicide against FAW. Through the use of leaf and fruit extract of *Am-amsi*, tobacco farmers can control FAW which is a safer, more cost-effective, and more eco-friendly pesticide.

KEYWORDS: Burley tobacco, organic pesticides, phytochemical analysis, *Solanum nigrum Linn, Spodoptera frugiperda.*

Introduction

Tobacco farming is a profitable agricultural crop because it serves as one of the sources of income for the farmers in Isabela. Tobacco farming provided employment for 43,960 tobacco farmers in the Philippines with 18.17% tobacco farmers originating from Isabela (NTA, 2023a). Tobacco farming contributes employment opportunities in the agriculture sector. The agriculture sector in the Philippines provides a total employment of 23.87% or 10.36 million in 2020-2022. These data imply that almost one-fourth of the employed

population are engaged in agriculture. This population considers farming as a primary source of income among farmers (Baclig, 2022).

There are several varieties of tobacco plants, but only three varieties are commonly grown by tobacco farmers in Isabela. These include *Nicotiana rustica,* or Native tobacco; *Nicotiana tabacum* or Burley; and *Nicotiana tabacum Linn.* or Broadleaf (NTA, 2023b). In Quirino, Isabela, Burley is usually grown by most tobacco farmers. The following are the photos of tobacco species grown in Isabela.

Fig. 1. Photos of *Nicotiana rustica*, *Nicotiana tabacum*, and *Nicotiana tabacum Linn*., respectively (NTA, 2023)

However, cultivating tobacco plants requires intensive care and management. Many pests grow along with the growing tobacco plants. The existence and rapid population growth of pests such as *Spodoptera frugiperda* or Fall armyworm (FAW) is one of the major problems among tobacco farmers in the country (DA, 2020). Matova *et al*. (2020) mentioned that control, management, and eradication of FAW was challenging. This kind of pests greatly affect the growth and quality of tobacco plants. The widespread occurrence of FAW occurs during the vegetative and flowering stage of the tobacco plants. For this reason, tobacco farmers employ scouting where they manually pick the larvae out of the tobacco plants. This requires a great manpower, time, and cost. Conveniently, the majority of the tobacco farmers sprayed inorganic pesticides in tobacco plants to manage the proliferation of FAW. Accordingly, Lu (2022) revealed that farmers utilized pesticides on an average 2.31 days per week and were exposed for 3.46 months per cropping season. Tobacco farmers spray inorganic pesticides more than twice a week on the onset of vegetative stage of tobacco up to flowering stage. Consequently, FAW larvae may have developed pesticide resistance due to intensive use of pesticides. This may often lead to the disruption of tobacco growth resulting in decreased tobacco production and income. Additionally, Huyen *et al*. (2020) reported that overapplication of inorganic pesticides on crops threatens human's health and the environment.

To lessen the risks of inorganic pesticides, we can maximize the potentials of medicinal plants found in the backyards. *Solanum nigrum* or Black nightshade can be a potential natural and organic pesticide in controlling the population of FAW, which are cost-effective, harm-free, and eco-friendly.

Black Nightshade is an erect, branched and smooth herb and one meter or less in height. Stems are green and leaves are oblate to oblong and have pointed ends. Its flowers are white and fruits or berries are smooth, round and green when unripe and turn dark purple when ripens and seeds are yellow. Figure 2 shows illustrations of the *Am-amsi plant*.

In the Philippines, it is called *Am-amsi* in Ilocano, *Lubi-lubi* in Tagalog, and *Amti* in Ifugao. For Ilocanos, the young leaves *of Am-amsi* are edible and usually cooked as a fermented fish sauce soup-based dish, steamed or as a salad. They can grow anywhere.

The *Solanum nigrum Linn.* belongs to Kingdom Plantae, Phylum Magnoliophyta, Order Solanales, Solanaceae family, and Genus Solanum. It is widely spread in tropical regions and subtropical regions. It has beautiful and whitish flowers.

Fig. 2: Photos of whole *Am-amsi* plant and gathered whole *Am-amsi* plant taken by the researcher, respectively

It has 39 species and 14 varieties in China. In Southeast Asia, it is a natural and common edible medicinal herb. It is also widely distributed in the temperate regions of Europe, Asia, and America. Traditionally, it has been used by people to treat cancers, acute nephritis, urethritis, leucorrhea, sore throat, toothache, dermatitis, eczema, carbuncles, and furuncles (Chen *et al*., 2022). Additionally, Khan, Qais, and Ahmad (2019) described it as a herbaceous plant with small, green, rounded berry fruits that turns purple to black when ripe and are used to treat various diseases, including cancer and tumors.

There are various benefits of black nightshade, which include sources of food and medicine due to its various nutritional and medicinal characteristics. Mandal *et al.* (2023) reported that it served as a food supply in Indian cultures. Moreover, it was used to treat infectious diseases such as cancer, acute nephritis, leucorrhea, sore throats, toothaches, dermatitis and eczema. It was also reported that it has anti-analgesic and anti-microbial activity. Additionally, Jain *et al*. (2011) found out to have antiproliferative activity in preventing the spread and growth of tumor cells in the liver, colon, and breast, as well as antiseizure, antioxidant, anti-inflammatory, and antifungal activity.

Various studies revealed that it contains natural phenolic and flavonoid compounds (Campisi *et al*., 2019; Alam *et al*., 2022; Callano, 2021; Thejaswini *et al*., 2023). These two substances promoted antioxidant activity in preventing and managing neurodegenerative diseases and reduced liver enzymes and oxidative stress. In addition, Bibon (2021) reported that it contains solasodine. Solasodine has antibacterial activity against *Escherichia coli,* which causes diarrhea and vomiting once it enters the human body.

Apart from the nutritional and medicinal value of Black nightshade, it possesses pesticidal activity on various test insects. Spochacz *et al*. (2020) experimented with the extract of *S. nigrum* and found out that it could increase the toxicity of fenitrothion against *Tenebrio molitor* larvae. Moreover, Rahat *et al*. (2021) evaluated the insecticidal activity of *S. nigrum* on 2nd instar larvae and adults of *Drosophila melanogaster.* When the treatment was applied and ingested by the test subject, the larvae showed mortality. Malformation of adults' wings was observed after treating the different concentrations.

In another study, Rahman (2022) evaluated the efficiency of alcoholic and alkaloid extracts of leaves and fruits of *S. nigrum* against immature larvae of blue fly with varying concentrations. Alcoholic extracts have the highest effect on killing the eggs of blue flies with 89.11%, while alkaloid extracts have 88.83% mortality rates. Alcoholic extracts were more effective than alkaloids in killing the larvae of blue flies. Anti-larvicidal activity of *S. nigrum* was also explored by Mandal *et al*. (2023). 100 ml extracts of each plant part were added with ethyl acetate solvent. The larval food with concentrations was fed to the larvae. It was observed that the larvae were immobile and eventually died.

Based on the foregoing studies, the larvicidal activity of *S. nigrum* is a potential alternative and organic pesticide in controlling pests found in agricultural crops. Maximizing the use of this plant is beneficial for human health, environmental protection, and minimized use of synthetic insecticides.

FAW has the following taxonomic classification: Kingdom Animalia, Phylum Eukaryota, Class Insecta, Order Lepidoptera, Family Noctuidae, Genus *Spodoptera*, and Species *Spodoptera* frugiperda. It is a lepidopteran and polyphagous pest, which has caused major damage to maize, rice, sorghum, sugarcane, and wheat. It usually feeds on different parts of the crop, such as leaves, stems, and even fruits (Rwomushana, 2019). The first three larval stages feed on the leaves, while the older instars feed on the whorl, tassel, and ear. Pupae are obtect, whitish-green turning brown and darkened nearing adult eclosion (Navasero & Navasero, 2020). Moreover, Navik *et al*. (2021) reported that the most destructive and damaging stage of FAW is in its growing larval stage. They directly feed on stems and young shoots and leaves, which leads to slow growth and development and even death of the crops. Figure 3 presents the life cycle of FAW.

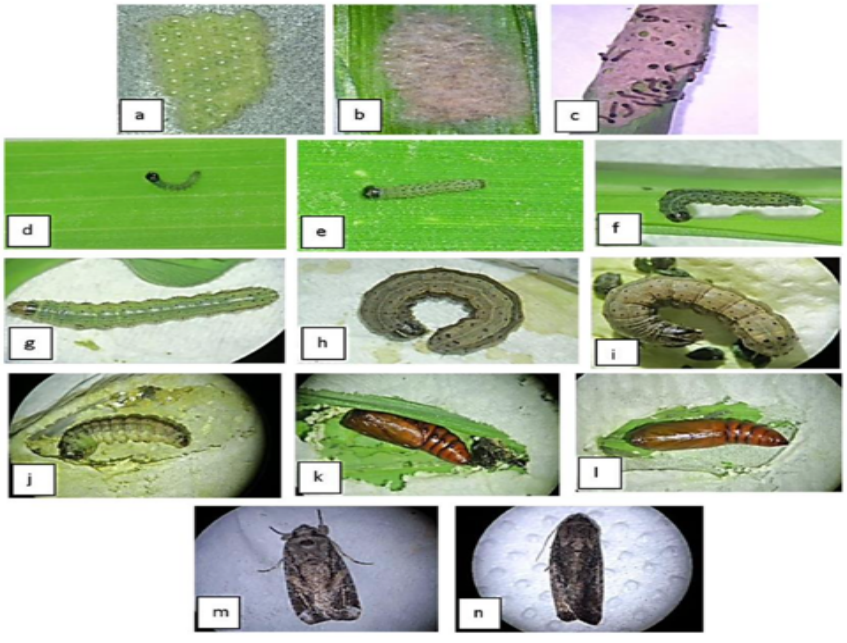

Fig. 3: The life cycle of FAW (Navasero & Navasero, 2020)

FAW is native in America and Europe, but its damage was first reported in Africa in 2016 (Rwomushana, 2019; Hruska, 2019). Additionally, Montezano *et al*. (2018) identified it as the most important noctuid pest, which became an invasive pest in Africa, while Sisay *et al*. (2018) reported it as the major pest in maize in North and South America. In 2019, Mian (2022)

stated that it is the most destructive species for several agricultural crops in Pakistan. In India, it was reported that FAW damaged maize fields with fields with 44-100% field infestation (Navik *et al*., 2021).

In the Philippines, it was first reported in Piat, Cagayan Valley, and nine more provinces in Luzon. The widespread attack of FAW drew attention for appropriate and sustainable methods of control (Navasero *et al*., 2019). Similarly, DA (2020) reported its proliferation in Mindanao in 2019.

Because of the proliferation of FAW, farmers had decreased their yield, but it led to the discovery of its sustainable management. In America, they control FAW through planting genetically modified maize, while in Africa and Asia practiced intercropping, handpicking, application of wood ash, soils, and tobacco extracts (Hruska, 2019). On the other hand, Montecalvo *et al*. (2022) experimented with the use of wettable powders such as bentonite, kaolin clay, sodium carbonate, and talcum powder. It was found out that the larvae can hardly move, growth discontinued, and reduced feeding. Kaolin clay was the most effective in controlling FAW. However, the use of wettable powders may harm farmers when ingested. It is still safe to use organic pesticides. Furthermore, Phambala *et al*. (2020) recommended farmers utilize pesticidal plants, which are effective, sustainable, and cost-beneficial in managing FAW in Africa.

Despite the introduction of sustainable management of FAW, many farmers utilized commercial pesticides for convenience. Mian (2022) reported that farmers sought management strategies to eradicate the proliferation of FAW through the use of various insecticides. In addition, Flanders, Ball and Cobb (2019) shared that the best time to apply pesticide is early morning or late afternoon, when FAW is active and usually grazes in the tobacco buds. However, frequent and intensive use of pesticides results in pest resistance (Phambala *et al*. 2020). Moreover, controlling and managing FAW through the use of pesticides is risky and harmful because it is detrimental to the farmers' health and environment (Mian *et al*., 2022).

The results of the study would offer benefits to the farmers, pharmaceutical industries, national government agencies (NGAs) such as the Department of Agriculture (DA) and the Department of Environment and Natural Resources (DENR), public, and future researchers. The results of the study would be utilized by the NGAs and pharmaceutical industries for the development of eco-friendly pesticides that will help the management of FAW and promote the use of eco-friendly, cost-efficient, and sustainable pesticides. The community would be provided with insights and perspectives with the cultivation of *Am-amsi* as a food source and the use of bio-insecticides to lessen the use of synthetic pesticides. Finally, farmers would effectively control and manage the rapid population growth of FAW that is both economical and environmental.

This study generally aimed to investigate the potential use of *Am-amsi* as a larvicide against FAW. Specifically, it aimed to answer the following questions:

1. What are the phytochemical compositions present in the fruit and leaf extracts of *Solanum nigrum Linn*?
2. What are the effects of the varying concentrations of fruit and leaf extracts of *Solanum nigrum Linn.* on *Spodoptera frugiperda* found in Burley tobacco plants?
3. Is there a significant difference in the varying concentrations of fruit and leaf extracts of *Solanum nigrum Linn.* against *Spodoptera frugiperda*?

Materials and methods

Gathering of *Am-amsi* and Phytochemical Screening of *Am-amsi* Aqueous Extracts

The proper identification of *Am-amsi* was done through the assistance of agriculturists from the Municipal Agriculture Office of Quirino, Isabela, Philippines, in the absence of the botanist in the area. The plant samples were collected in Manaoag, Quirino, Isabela, Philippines. They were washed with tap water to remove the dirt and were washed twice using distilled water to further remove the impurities. After washing and draining, the leaves and fruits were weighed, chopped into smaller pieces, and placed in the sterilized blender to attain a more refined particle. It was then filtered using sterilized cheesecloth and filter paper. The fresh extracts were placed in a beaker and measured with respective volume of 25% concentration or 15g /ml of the aqueous extract for Treatment 1 (T_1), 50% concentration or 30g/ml for Treatment 2 (T_2), 75% concentration or 45g/ml for Treatment 3 (T_3), and 100% concentration or 60 g/ml for Treatment 4 (T_4). Samples of *Am-amsi* aqueous extract were tested for the possible presence of phytochemical constituents such as flavonoids, saponins, phenols, steroids, terpenoids, anthocyanins, quinones, and tannins. The researchers sought the assistance of the Central Analytical Laboratory of Cagayan State University-Andrews Campus, Philippines for the confirmatory test of phytoconstituents due to the unavailability of laboratory equipment and chemicals in the school where the primary researcher is teaching. The different methods employed in determining the presence of phytochemical constituents were presented in Table 1.

Collection of FAW Larvae

FAW egg masses were observed in the tobacco field and were put under observations until they became larvae. The FAW larvae of the same stage, 3rd- 4th instar larvae, were collected and placed on the experimental plants. The proper identification of the test insects was conducted through the assistance of agriculturists from the Municipal Agriculture Office of Quirino, Isabela, Philippines, in the absence of the entomologist in the study site.

Land Preparation and Experimental Lay-out

The experimental area was harrowed two weeks before transplanting Burley tobacco, followed by the second harrowing five days before planting. The area was divided into five blocks representing the treatments with one meter distance from each block. Each block was subdivided into three plots 0.50 meter apart, representing replications.

The Burley plants were 60 days old when the second harvesting of leaves was taken. In this stage, the attack of FAW was rampant. The experimental Burley plants were not sprayed with commercial insecticides along with its neighboring Burley plants to ensure that the test insects were not affected and contaminated.

Data Gathering Procedure

To understand the relationship between two or more variables, the field experimental method was employed. A total of 450 FAW larvae were used and grouped into three consisting of three replicates with three plants per replication with 10 FAW larvae each. There were four treatments using the *Am-amsi* aqueous extracts with a volume of 60ml as the baseline: T_1 with 25% concentration or 15 ml of aqueous extract and 45 ml of distilled water, T_2 with 50% concentration or 30ml of aqueous extract and 30 ml of distilled water, T_3 with 75% concentration or 45 ml of aqueous extract and 15 ml of distilled water, and T_4 with 100% concentration or 60ml of pure aqueous extract. In addition, the fifth treatment, or T_0, for the commercial larvicide serves as the positive control.

There were three replicates per treatment. The different concentrations were sprayed with 5ml to the FAW early in the morning and were observed for 12 hours, 24 hours, 36 hours, 48 hours, 60 hours, and 72 hours. A completely randomized design (CRD) was used. The Least Significant Difference (LSD) Test was used to test the treatment mean difference. The data were gathered in terms of the number of mortalities at a given time interval. Observed responses such as immobility, irritability, and non-feeding were also recorded. In describing the data, mean and standard deviation were used. One-way ANOVA was used to test if there was a significant difference in the larvicidal activity of *Am-amsi* leaf and fruit aqueous extract. The statistical tool used was Statistical Package for the Social Science (SPSS) software.

Results and discussion

Phytochemical Constituents present in *Am-amsi*

Based on the results obtained from the phytochemical screening through the assistance of the Central Analytical Laboratory of Cagayan State University, Philippines, it showed that *Am-amsi* fruit and leaf aqueous extracts were proven to contain secondary metabolites of flavonoids, saponins, steroids, and terpenoids. However, anthocyanin, phenols, quinones, and tannins were absent. Table 1 shows the various phytochemical constituents present in the aqueous extract of *Am-amsi*.

Table 1: Phytochemical Constituents Present in Am-amsi Fruit and Leaf Aqueous Extract

Parameter	Method Used	Result
Anthocyanin	Alkaline Reagent Test	-ve
Flavonoids	Shinoda Test	+ve
Phenols	Ferric Chloride Test	-ve
Quinones	Munoz *et al*. (2021)	-ve
Saponins	Froth Test	+ve
Steroids	Liebermann-Burchard Test	+ve
Tannins	Braymer's Test	-ve
Terpenoids	Salkowski Test	+ve

Results of this study confirmed the study conducted by Jain *et al*. (2011), Callano (2021), and Thejaswini *et al*. (2023) that *Am-amsi* contained flavonoids. This phytochemical constituent has antioxidant anti-inflammatory, anticancer and antiviral property, which could be a potential larvicide against FAW.

Meanwhile, saponins, steroids, and terpenoids found in plants are also potential larvicides against FAW. Marrelli *et al*. (2016) reported that saponins have pharmacological properties such as anti-inflammatory, antifungal, and cytotoxic. Steroids present in plants have anti-cancer, anti-inflammatory, and anti-viral properties (Yerlikaya *et al*., 2023). Finally, Boncan *et al*. (2020) discovered that terpenoids have toxic and repellent effects on insects.

The wide array of potentials of *Am-amsi* plants due to their different pharmacological properties provides various benefits, such as medicine and natural pesticides, due to the presence of the aforementioned phytochemical constituents.

The Effects of *Am-amsi* Aqueous Extract on FAW

After 12 hours of exposure of FAW to the different treatments, only T_2 and T_4 had an effect on FAW in terms of mortality, while T_1 and T_3 had no FAW mortality. However, upon application, FAW had noticeable responses such as irritability and immobility. After 24 hours, 36 hours, 48 hours, 60 hours, and 72 hours of exposure, it was noticeable that there was an increased mean mortality of FAW on all the treatments. The FAW began to be immobile and paralyzed as time went by. They did not migrate from where they were placed before the application of the treatment. There was a significant mortality within the given population after 72 hours. Table 2 presents the increasing mortality rate of FAW in the different time intervals.

Table 2: Mortality Rate of FAW in the Different Time Intervals

Time	Treatment	Mean Mortality Rate of FAW
After 12 hrs	T_1	0.00
	T_2	1.10
	T_3	0.00
	T_4	2.23
After 24 hrs	T_1	1.10
	T_2	3.33
	T_3	2.33
	T_4	5.57
After 36 hrs	T_1	1.10
	T_2	4.43
	T_3	5.57
	T_4	7.77
After 48 hrs	T_1	4.43
	T_2	5.57
	T_3	8.90
	T_4	10.00
After 60 hrs	T_1	7.77
	T_2	8.90
	T_3	11.10
	T_4	14.43
After 72 hrs	T_1	10.00
	T_2	12.23
	T_3	15.57
	T_4	17.77

In relation to the mortality, FAW were considered dead due to the total immobility (Mandal *et al*. 2023), had dried out body, and noticeable black large spots on their head and body (Navasero and Navasero, 2020). This confirmed that the black large spots on FAW's head and body was an indication of death.

Significant Difference of the Varying Concentration of *Am-amsi* Aqueous Extract

Analysis of variance revealed that there was no significant difference among all the treatments after 12 hours, 24 hours, and 48 hours. Additionally, there is a significant difference in at least two treatments after 36 hours, 60 hours, and 72 hours. Table 3 shows mean mortality rate of FAW, standard deviation, ANOVA Test Results. In relation to this, the Post Hoc tests after 36 hours, 60 hours, and 72 hours are shown in Table 4, 5, and 6, respectively. On the other hand, T_4 had the highest mortality rate after 72 hours of exposure.

Table 3: Means, Standard Deviation and ANOVA Test Results of the Four Treatments

Time	Treatment	Mean	Standard Deviation	ANOVA Result	Interpretation
After 12 hrs	T_1	0.00	0.00	F (3, 8) = 1.83 p = 0.22	No significant difference in at least two treatments
	T_2	1.10	0.58		
	T_3	0.00	0.00		
	T_4	2.23	0.58		
After 24 hrs	T_1	1.10	0.58	F (3, 8) = 1.94 p = 0.20	No significant difference in at least two treatments
	T_2	3.33	1.00		
	T_3	2.33	0.58		
	T_4	5.57	0.58		
After 36 hrs	T_1	1.10	0.58	F (3, 8) = 6.25 p = 0.02*	Has significant difference in at least two treatments
	T_2	4.43	0.58		
	T_3	5.57	0.58		
	T_4	7.77	0.58		
After 48 hrs	T_1	4.43	0.58	F (3, 8) = 3.78 p = 0.06	Has significant difference in at least two treatments
	T_2	5.57	0.58		
	T_3	8.90	0.58		
	T_4	10.00	1.00		
After 60 hrs	T_1	7.77	0.58	F (3, 8) = 7 p = 0.01*	Has significant difference in at least two treatments
	T_2	8.90	0.58		
	T_3	11.10	0.58		
	T_4	14.43	0.58		
After 72 hrs	T_1	10.00	1.00	F (3, 8) = 6.44 p = 0.02*	Has significant difference in at least two treatments
	T_2	12.23	0.58		
	T_3	15.57	0.58		
	T_4	17.77	0.58		

** There is a significant difference.*

Table 4 shows the Post Hoc Test after 36 hours of exposure of FAW to *Am-amsi* aqueous extract. It shows that only T_1 and T_4 had significant differences. The difference was

attributed to the concentration of the applied extract because T_1 contained lesser concentration with 25% while T_4 had the highest concentration with 100%. The higher concentration of *Am-amsi* aqueous extract contributed to the higher mortality rate of FAW due to its higher concentration of phytochemical constituents.

Table 4: Post Hoc Test after 36 hours

Treatment	Other Treatments	Mean Difference	p – value	Interpretation
T_1	T_2	-3.33	0.23	Not significant
	T_3	-4.47	0.09	Not significant
	T_4	-6.67	0.01*	Significant
T_2	T_1	3.33	0.23	Not significant
	T_3	-1.13	0.89	Not significant
	T_4	-3.33	0.23	Not significant
T_3	T_1	4.47	0.09	Not significant
	T_2	1.13	0.89	Not significant
	T_4	-2.20	0.53	Not significant
T_4	T_1	6.67	0.01*	Significant
	T_2	3.33	0.23	Not significant
	T_3	2.20	0.53	Not significant

** There is a significant difference.*

The Post Hoc Test after 60 hours is presented in Table 5. It shows that T_1 and T_4 had significant differences. Additionally, T_2 and T_4 also showed significant differences. These data

Table 5: Post Hoc Test after 60 hours

Treatment	Other Treatments	Mean Difference	p – value	Interpretation
T_1	T_2	-1.13	0.89	Not significant
	T_3	-3.33	0.23	Not significant
	T_4	-6.67	0.01*	Significant
T_2	T_1	1.13	0.89	Not significant
	T_3	-2.20	0.53	Not significant
	T_4	-5.53	0.03*	Significant
T_3	T_1	3.33	0.23	Not significant
	T_2	2.20	0.53	Not significant
	T_4	-3.33	0.23	Not significant
T_4	T_1	6.67	0.01*	Significant
	T_2	5.53	0.03*	Significant
	T_3	3.33	0.23	Not significant

** There is a significant difference.*

imply that T_1, T_2, and T_3 had no significant difference on the potential of controlling FAW after 60 hours of exposure to *Am-amsi* aqueous extract.

Table 6 presents the Post Hoc Test after 72 hours. It shows that T_1 and T_4 had significant differences after 72 hours of FAW exposure to the *Am-amsi* aqueous extract. These data suggest that T_4 was more effective than T_1 and T_2 at some points. On the other hand, T_3 was comparable to T_4 because they did not have a significant difference in any time tested. Hence, T_3 and T_4 were compared to T_0 to determine if the *Am-amsi* aqueous extract was comparable to the synthetic insecticide.

Table 6: Post Hoc Test after72 hours

Treatment	Other Treatment	Mean Difference	p – value	Interpretation
T_1	T_2	-2.23	0.67	Not significant
	T_3	-5.57	0.08	Not significant
	T_4	-7.77	0.02*	Significant
T_2	T_1	2.623	0.67	Not significant
	T_3	-3.33	0.37	Not significant
	T_4	-5.53	0.08	Not significant
T_3	T_1	5.57	0.08	Not significant
	T_2	3.33	0.37	Not significant
	T_4	-2.20	0.67	Not significant
T_4	T_1	7.77	0.02*	Significant
	T_2	5.53	0.08	Not significant
	T_3	2.20	0.67	Not significant

Table 7 presents the Means, SD, and ANOVA Test Results of T_0, T_3, and T_4. Based on the table, it showed that there was a significant difference between T_0, T_3, and T_4 in the different time intervals. Post Hoc Test in the different time intervals showed that T_0 had significant differences with T_3 and T_4. This means that there is a difference in the effect of T_0 with T_3, and T_4 to the FAW. Meanwhile, T_3, and T_4 did not show significant differences. This implies that these two treatments had the same effect on the FAW in the different time intervals of exposure to the *Am-amsi* aqueous extract. Additionally, T_4 was the most effective in killing FAW in comparison with T_1, and T_2.

Rahat *et al*. (2021) reported that *Am-amsi* is toxic and has great potential as an insecticidal agent. Accordingly, the potential of *Am-amsi* as a natural pesticide is attributed to the presence of phytochemical constituents such as flavonoids, saponins, steroids, and terpenoids. The phytochemical constituents present in the *Am-amsi* potentially contributed to the gradual mortality of the FAW. Terpenoids repelled FAW, as evidenced by their frequent migration from one area to another of the tobacco plant during the time interval of observation. Moreover, it could be assumed that saponin contributed to the feeding inability, paralysis, and immobility of the FAW and eventually led to death. The toxicity content of *Am-amsi* contributed to the total immobility, dried-out body, and noticeable black color on the FAW head and body.

Although synthetic insecticide was not comparable to the T_3 and T_4 of *Am-amsi* aqueous extract, intensive use of synthetic insecticides on *Nicotiana tabacum* poses hazards to humans

as well as to animals and the environment because of the harmful chemical components (Huyen *et al*. 2020).

Table 7: Means, Standard Deviation and ANOVA Test Results of the Three Treatments

Time	Treatment	Mean	Standard Deviation	ANOVA Result	Interpretation
After 12 hours	T_3	0.00	0.00	F (2, 6) = 19.50 p= 0.002	Has a significant difference in at least two treatments
	T_4	2.23	0.58		
	T_0	7.77	0.58		
After 24 hours	T_3	2.23	0.58	F (2, 6) = 39 p = 0.00	Has a significant difference in at least two treatments
	T_4	5.57	0.58		
	T_0	15.57	0.58		
After 36 hours	T_3	5.57	0.58	F (2, 6) = 66.33 p = 0.00	Has a significant difference in at least two treatments
	T_4	7.77	0.58		
	T_0	22.23	0.58		
After 48 hours	T_3	8.90	0.58	F (2, 6) = 85.75 p = 0.00	Has a significant difference in at least two treatments
	T_4	10.00	1.00		
	T_0	30.00	0.00		
After 60 hours	T_3	11.10	0.58	F (2, 6) = 123.50 p = 0.00	Has a significant difference in at least two treatments.
	T_4	14.43	0.58		
	T_0	30.00	0.00		
After 72 hours	T_3	15.57	0.58	F (2, 6) = 73.50 p = 0.00	Has a significant difference in at least two treatments
	T_4	17.77	0.58		
	T_0	30.00	0.00		

Therefore, farmers can use *Am-amsi* aqueous extract against FAW as a substitute to synthetic insecticide. This natural pesticide is safer to use, more eco-friendly, and more cost-effective than synthetic insecticides. In using this, it eventually helps in the decreased utilization of synthetic insecticide in controlling FAW (Spochacz *et al*. 2020), decreasing pesticide emissions, and protecting farmers' health, animals, and the environment.

Conclusions and recomendations

Based on the foregoing findings, it has been demonstrated that *Am-amsi* aqueous fresh extract has a larvicidal activity against FAW. T_3 and T_4 were effective larvicidal treatments against FAW. Thus, *Am-amsi* aqueous extract could be an alternative, organic, and effective larvicide that can control FAW in *Nicotiana tabacum* or Burley.

The results of this study can help farmers to effectively and sustainably control and manage the rapid population growth of FAW through an environment-friendly method. It can also benefit farmers by reducing the hazards posed by synthetic pesticides. Additionally, it can be utilized by pharmaceutical industries in the production and manufacture of cheaper, safer, and more eco-friendly larvicidal products.

It is recommended that tobacco farmers should cultivate *Am-amsi* to have accessibility of organic pesticides to preserve and perpetuate indigenous bio-larvicidal plants. Utilization of organic pesticides such as *Am-amsi* aqueous extract can lessen synthetic pesticides that are detrimental to humans, animals, and the environment. Through this way, it can cut the cost of pesticide inputs while promoting the health and wellness of the environment and curbing greenhouse gas emissions from pesticides.

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GENETIC EVALUATION OF YIELD-RELATED AGRONOMIC TRAITS FROM HALF-SIB FAMILIES OF MAIZE (*ZEA MAYS* L.)

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Abstract

This study was conducted to evaluate half-sib families (HSF) for yield and agronomic traits in maize (*Zea mays* L.). One hundred and ninety-six half-sib families were used in this study derived from the maize variety Azam. The experiment was laid out in 14×14 partial lattice square designs with two replications. Results indicated that the phenotypic coefficient of variance was higher than the genotypic coefficient of variance for all traits except for fresh ear weight, which reflects the environmental influence on the expression of the trait. High to moderate heritability was observed for days to tasseling, days to mid silking, days to anthesis, anthesis silking-interval, kernel rows per cob, cob length, and grain yield. Highly significant and positive correlations were observed between grain yield and cob length (0.99), kernel rows per cob (0.88), grain moisture (1.00), days to tasseling (0.94), silking (0.99), and anthesis (0.96). The negative and non-significant correlation was observed between grain yield and fresh ear weight (-0.06). Maximum grain yields of 10710 kg ha^{-1} were recorded for HSF-180, while a minimum 2046 kg ha^{-1} was obtained by HSF-31. These results suggest that these half-sib families could be used as a source of maize germplasm for developing maize genotypes with superior attributes.

Keywords: Half-sib Families, Replication, Yield Attributes, Zea mays L.

Introduction

Global production of all cereal crops is not adequate to nourish the whole population, although the crops yield is increasing day-by-day (STAT, 2012). According to the FAO data,

in 2010 the coarse crop-producing countries that contribute greater than 20% of total production are the United States of America and China (STAT, 2012). Maize (*Zea mays* L.) is the world's primary coarse grain that plays a vital role as the source of bioenergy, animal feed, and human food (Zhou et al., 2012). It is the world's foremost cereal crop a production of 695 million tons and a per-unit area yield of 4815 kg, ha^{-1} and a vast quantity of production is concentrated in the United States of America. The top five maize producing countries are the USA, China, Brazil, Mexico and Argentina have drastically increased the maize production since 1961 (STAT, 2012). It is the prime crop of Sub-Saharan Africa, accounting 51% of consumed calories although the yield level is low and vehemently variable across years at less than 2 t/ha. On the other hand, in Asia, the yield level is much higher. China and Indonesia accounting an average yield of 5.2 and 4.2 t/ha (STAT, 2012).

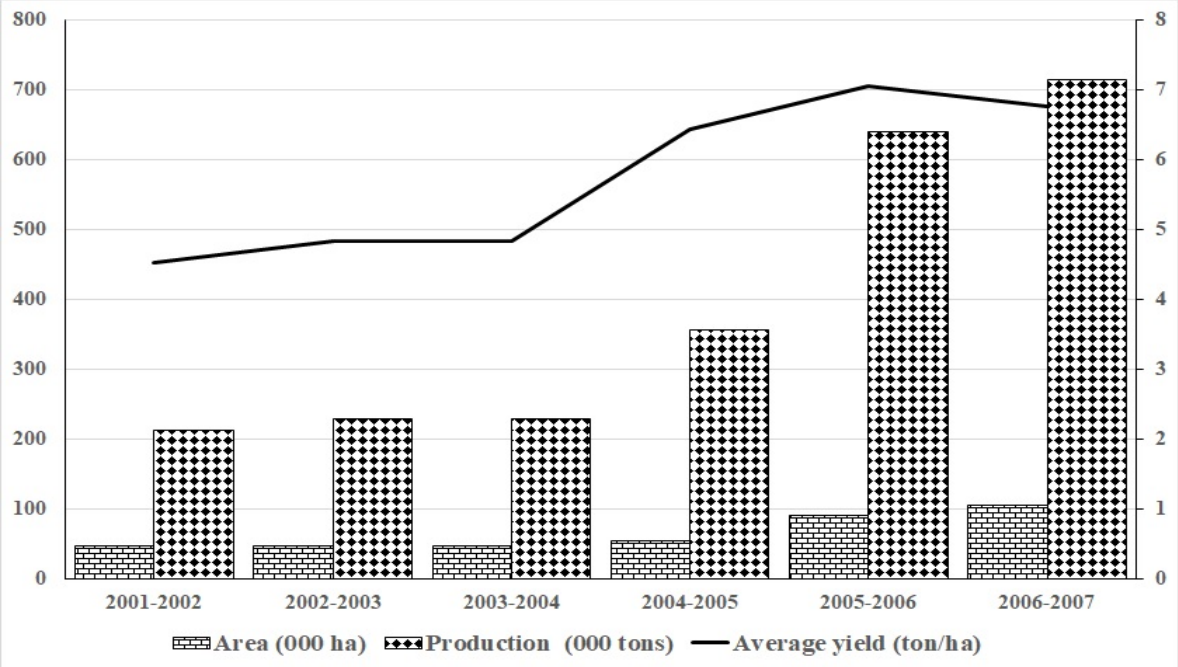

Fig. 1 Area (000 ha), Production (000 tons), and average yield (ton/ha) of spring maize in Pakistan

Pakistan contributes a production of 3.3 million tons per year with an estimated planted area of 1.016 million hectares and an average yield of 2864 $kgha^{-1}$ (Tariq and Iqbal, 2010). In Pakistan, production per year increases with a decrease in the cultivated areas (STAT, 2012). The production of maize has increased drastically over the decades from 0.38 to 3.037 million tons during 1947-2007 (Tariq and Iqbal, 2010). The area, production, and average yield of spring maize from 2001 to 2007 increased drastically (Figure 1). 99 % of the total production of maize comes from the Khyber Pakhtunkhwa and Punjab provinces of Pakistan. Khyber Pakhtunkhwa accounts for 31 % of the total maize production and 51 % of the total area (Tariq and Iqbal, 2010). In Khyber Pakhtunkhwa, maize is normally grown to produce grain and also as fodder, due to which its demand increased drastically. In combination with the Green Revolution, improved agronomic practices enhanced yields up to 40% (Evenson and Gollin, 2003). Maize improvement mainly includes evaluation, selection, and recombination of genetically distinct inbred lines or families (Pixley et al., 2006).

A number of recurrent selection methods have been used by the breeders, like mass selection, recurrent selection, half-sib selection, and full-sib family selection, for developing high-yielding maize varieties and increasing yield per unit area (Keeratinijakal and Lamkey, 1993). Recurrent selection methods comprising half-sib family selection and S_1 progeny selection are particularly of prime interest as these not only improve the breeding population for the required attributes but also sustain the genetic variability in the population (Hallauer, 2012). Coors (1988) observed a 1.5% reduction in grain moisture and a gain of 3.5% in grain yield per cycle in response to four cycles of combined half-sib and S_1 family selection in maize. Tanner and Smith (1987) recommended that half-sib family selection was highly efficient in reducing inbreeding depression in maize populations. Marquez-Sanchez (2003) evaluated different selection methods for maize and based on their results recommended that HS-selection is the best method.

The present research was designed with the objectives to evaluate half-sib families developed from maize variety Azam and to identify superior half-sib families for yield and agronomic traits that can be used in future maize breeding programs for developing maize genotypes with desirable attributes.

Materials and methods

Experimental material of the study comprised 196 half-sib family lines–having one parent in common-derived from maize variety Azam, which were provided by the Cereal Crops Research Institute (CCRI), Pirsabak, Nowshera, Pakistan. A partial lattice square design, having two replications, was used. Plant-to-plant and row-to-row distance was 25 cm and 75 cm, respectively. The land was prepared by giving three plowings followed by planking. Standard cultural practices, including irrigation, fertilizer application, and hoeing, were carried out over the growing season. Agronomic practices were carried out at the proper time. Data on yield and morphological parameters were recorded at the proper time for each character.

Silking data were recorded on a plant basis as the number of days from silking till 50% of plants in the plot showed silks, while days to anthesis were worked out by visual observation when 50% of the plants in the plot started pollen shedding. From the date of sowing, days were counted. Anthesis silking interval (ASI) was computed on a plot basis as the difference between silking and anthesis. Plant height using a graduated meter rod in cm was computed from the ground level to the topmost node of the plant, and an average of five randomly selected plants per row was taken, whereas ear height was also computed in cm as an average of five randomly selected plants per row from ground level to the node bearing the uppermost ear. Ear length was computed as an average of three per row from the tip to the base of the ear with the scale in cm, whereas kernel rows per ear were counted on the three randomly selected ears after harvesting. The moisture content of the grains was taken using a grain moisture tester after shelling the middle rows from three randomly selected ears per row at harvesting time. A hundred kernels were counted randomly from the grain lot of each row and weighed with the help of an electronic balance. Total grain yield per hectare was calculated from the data of fresh ear weight using formula:

$$\text{Grain Yield (kg ha}^{-1}) = \frac{(\text{F.wt (kg)} \times 100\text{-M.C}) \times 0.8 \times 10{,}000}{(100\text{-}15) \times \text{harvesting area}}$$

Whereas MC = moisture content (%) in grains at harvest, 0.8 = Shelling co-efficient, 15% = moisture content required in grain at storage

The data of agronomic traits were analyzed using PROC MIXED procedure to determine the relationship among the traits; phenotypic correlation coefficients were computed among all the traits. Variance components were estimated to know the environmental and genetic effects on different characters. Phenotypic (δ^2_P), genotypic (δ^2_G) and error (δ^2_E) variances were computed from mean squares of analysis of variance by using the formula suggested by Hallauer et al. (2010). The standard errors of estimates of genotypic and error variance components were computed using the methods of Hallauer et al. (2010). The genetic advance (GA) was calculated as devised by Johnson (1955). The following formulas were used are;

Error Variance, $\delta^2_E = MS_E$, where MS_E = mean square of error

Genotypic variance, $\delta^2_G = (MS_G - MS_E)/r$, where MS_G = mean square of genotype, MS_E = mean square of error, and r = number of replications

Phenotypic variance, $\delta^2_P = \delta^2_E + \delta^2_G$, where δ^2_E = error variance and δ^2_G = genotypic variance

Genotypic coefficient of variation, $GCV = \left(\frac{\sqrt{\delta^2_G}}{X}\right) \times 100$, where δ^2_G = genotypic variance and X = mean of the trait

Phenotypic coefficient of variation, $PCV = \left(\frac{\sqrt{\delta^2_P}}{X}\right) \times 100$, where δ^2_P = phenotypic variance and X = mean of the trait

Heritability $h^2 = \delta^2_G / \delta^2_P$, where δ^2_G = genotypic variance and δ^2_P = phenotypic variance

Genetic Advance $GA = k \sqrt{\delta^2_P} . h^2$, where $\sqrt{\delta^2_P}$ = Square root of Phenotypic Variance, h^2 = Heritability, and K = Constant, 2.063 at 5% selection intensity

Results and discussion

This research was conducted at (34° 0' 29" N / 71° 34' 22" E), Peshawar, Pakistan which, under Koppen's climate classification, features a semi-arid climate with very hot summers and mild winters.

Days to 50% tasseling

The analysis of variance was taken for days to 50% tasselling and observed highly significant differences at P<0.01 for half-sib families. For days to tasselling a minimum mean value of 54 was observed for HSF-13, HSF-63, HSF-163, and HSF-189, while the maximum mean value of 60 was observed for HSF-114. The grand mean of 56.14 was observed for all 196 entries (Tab. 1). These results are in agreement with those observed by Hidayat et al. (2006), who also reported a highly significant difference for days to 50% tasselling when evaluating the performance of local and exotic inbred lines of maize under agro-ecological conditions of

Peshawar. The genotypic coefficient of variance was at the very low side as it was 0.03 % (Tab. 2).

Days to 50% Silking

Analysis of variance for days to 50% showed a significant difference among the half-sib families at P<0.05. For days to 50% silking minimum mean value of 57 was observed for HSF-13, HSF-116, HSF-66, and HSF-183, while the maximum mean value of 64.5 was observed in HSF-92, with a grand mean of 60.10 computed for all 196 entries (Tab. 1). The results of Hidayat et al. (2006) are also in comparison to our results. Because he also reported a highly significant difference for days to 50% silking when evaluating the performance of local and exotic inbred lines of maize under agro-ecological conditions of Peshawar. Similar results were also observed while working on "recurrent selection for grain yield in two Spanish maize synthetic populations". The genotypic coefficient of variance computed was 0.03 (Tab. 2).

Days to 50% Anthesis

Analysis of variance regarding days to 50% anthesis showed no significant difference among the half-sib families. The minimum value for days to 50% anthesis was observed as 54.5 days for HSF-13, while the maximum of 61.5 days was observed for HSF-114, having a grand mean of 57.52 days among 196 half-sib families (Tab. 1, Tab. 6). The coefficient of variance was very low, as it was recorded as 0.02 % (Tab. 2).

Anthesis Silking Interval (ASI)

ANOVA regarding anthesis silking interval showed a significant difference (P<0.05) with the genotypic coefficient of variance as 0.52 % (Tab. 2). ASI calculated ranged from 0.5 days for HSF-102, HSF-191, HSF-194, and HSF-16 (protogynous), while a maximum of 6.5 days was calculated for HSF-24 (protandrous), having a grand mean of 2.59 days among half-sib families (Tab. 1, Tab. 6). Rahman et al. (2010) observed similar results for anthesis-silking interval in test cross-evaluation of maize synthetic "BSSS" lines.

Plant Height

Plant height requires special attention from plant breeders as it plays an important role in plant lodging. Plants having an optimum height and central or near-to- central placement of cobs are more resistant to lodging and therefore play a vital role in improving grain yield. Highly significant differences (P<0.01) were recorded for plant height among the half-sib families. The genotypic coefficient of variance 0.09 was recorded, which was very low (Tab. 2). The average plant height ranged from 130 cm for HSF-120 and HSF-159 to 186 cm for HSF-56, with a grand mean of 154.1 cm (Tab. 1). Stromberg and Compton (1989) reported significant differences regarding plant height after 10 cycles of the full-sib recurrent section in Nebraska Krung open-pollinated maize.

Ear Height

ANOVA for ear height revealed highly significant differences (P<0.01) among 196 half-sib families. The genotypic coefficient of variance was 0.13 % (Tab. 2). Minimum mean ear height of 54cm was observed for HSF-156, while the maximum ear height 91 was recorded for HSF-56, with a grand mean of 71.98 for all 196 half-sib families (Tab. 1). Stromberg and Compton (1989) reported significant differences regarding ear height after 10 cycles of full-sib recurrent selection in an open-pollinated maize population.

Table 1: General Statistics for the yield related agronomic traits.

	DT	DS	DA	ASI	PH (cm)	EH (cm)	CR	FEW	GMC	KR	CL	100-KW (g)	GY (kg ha^{-1})
Grand mean	56.14	60.10	57.52	2.59	154.11	71.98	8.67	0.98	23.44	13.35	14.81	32.27	4367.54
Maximum	60.00	64.50	61.50	6.50	186.0	91.00	14.50	2.15	30.75	16.84	19.17	41.00	10710.50
Minimum	54.00	57.00	54.50	0.50	130.0	54.00	3.00	0.30	14.95	11.00	9.34	20.00	2046.50
Standard Deviation	1.16	1.61	1.27	1.22	11.76	7.85	2.04	0.35	2.87	0.98	1.74	3.39	1180.95
Standard Error	0.08	0.12	0.09	0.09	0.84	0.56	0.15	0.02	0.21	0.07	0.12	0.24	84.35

Days To Tasseling (DT), Days to Silking (DS), Days to Anthesis (DA), Anthesis Silking Interval (ASI), Plant Height (PH), Ear Height (EH), Cobs Per Row (CR), Fresh Ear Weight (FEW), Grain Moisture Content (GMC), Kernal Rows Per Cob (KR), Cob Length (CL), 100-Kernal Weight (100-KW), Grain Yield (GY)

Table 2: Components of variance for the agronomic traits in Half-Sib Families of maize.

Parameters	(δ^2_G)	(δ^2_P)	(δ^2_E)	GCV%	PCV%
Days to Tasseling	2.36	2.71	0.35	0.03	0.03
Days to Silking	3.43	5.20	1.77	0.03	0.09
Days to Anthesis	1.84	3.22	1.38	0.02	0.06
Anthesis Silking Interval (ASI)	1.84	2.96	1.13	0.52	1.14
No of Plants/Row	6.79	11.07	4.28	0.24	1.01
Plant Height	183.87	276.73	92.86	0.09	1.80
Ear Height	81.35	123.14	41.79	0.13	1.71
No of Cobs/Row	7.45	8.34	0.89	0.31	0.96
Fresh Ear Weight/Row	0.23	0.25	0.02	0.49	0.25
Grain Moisture/Row	9.17	16.50	7.33	0.13	0.70
Kernel Rows/Cobs	1.30	1.94	0.63	0.09	0.10
Cob Length	5.72	6.06	0.34	0.16	0.41
100 Kernel Weight	21.13	22.99	1.86	0.14	0.71
Grain Yield	25.12	25.98	1.45	1.21	0.99

δ^2_G = Genotypic Variance, δ^2_P = Phenotypic Variance, δ^2_E = Error Variance, GCV % = Genotypic Co-efficient of Variance, PCV % = Phenotypic Co-efficient of Variance.

Tab. 3 Mean squares values for agronomic characteristics, of half-sib families from maize variety Azam.

Parameter	Reps	Treatments	Blocks
Tasseling	5.635	2.709**	0.635
Silking	41.145	5.198**	9.470
Anthesis	120.125	3.222$^{N.S}$	4.795
ASI	20.207	2.964**	3.948
Plant height(cm)	10175.734	276.727**	649.210
Ear height(cm)	913.873	123.140**	296.890
Kernel rows	0.130	1.935**	1.735
Cob length (cm)	15.844	6.059**	0.607
100-kernal wt.	0.125	22.992**	3.257
Grain yield (Kgha^{-1})	4671532.778	2789345.672**	2945605.393

N.S = non-significant
** = Significant at 1% level of significance

Kernels rows per cob

Analysis of variance for kernel rows cob revealed highly significant differences among half-sib families, with the genotypic coefficient of variance noted as 0.09 % (Tab. 2). Minimum average kernel rows per cob were observed as 11 for HSF-157, while maximum average values were 16.84 for HSF-83, with a grand mean of 13.35 among 196 half-sib families (Tab. 1). These results are similar to those reported by Gnanasekaran *et al.* (2008), who observed highly significant differences among the genotypes for kernel rows per cob.

Cob Length

Data recorded for cob length also showed highly significant differences among the half-sib families. The genotypic coefficient of variance was 0.16 % (Tab. 2). The cob length of half-sib families ranged from 9.34 cm to 19.17 cm for HSF-15 and HSF-41, respectively, having a grand mean of 14.81 (Tab. 1). The results are in contrast to those observed by Carlon and Russel (1989), who observed significant differences ($P<0.05$) for cob length in the testcross evaluation of maize synthetic 'BSSS' lines.

Weight of 100 Kernels

Analysis of variance revealed highly significant differences ($P<0.01$) among the half-sib families with a 5.98% coefficient of variation (Tab. 3). Mean values for the weight of 100 kernels ranged from 20 for HSF-140 to 41 for HSF-153. The grand mean was recorded as 32.27 (Tab. 1). These results are similar to those of Rahman et al. (2007), who also reported significant differences ($P<0.05$) for this trait while comparing original and selected maize populations for grain yield.

Tab. 4 Genetic Parameters for the agronomic traits in Half-Sib Families of maize.

Parameters	h^2_{BS}	GA	GG
Days To Tasseling	† 0.87	2.95	5.26
Days To Silking	† 0.66	3.10	5.15
Days To Anthesis	‡ 0.57	2.11	3.67
Anthesis Silking Interval (ASI)	† 0.62	2.20	84.63
No of Plants/Row	† 0.61	4.21	38.25
Plant Height	† 0.66	22.77	14.77
Ear Height	† 0.66	15.10	20.98
No of Cobs/Row	† 0.89	5.32	61.32
Fresh Ear Weight/Row	† 0.94	0.96	97.19
Grain Moisture/Row	‡ 0.56	4.65	19.85
Kernal Rows/ Cob	† 0.67	1.93	14.44
Cob Length	† 0.94	4.79	32.33
100 Kernal Weight	† 0.92	16.08	28.14
Grain Yield	† 0.85	20.23	26.25

†= High Heritability
‡= Moderate Heritability
h^2_{BS} = Heritability (broad scence), GA= Genetic Advance, GG= Genetic Gain

Variance Analysis

The variance analysis results for the investigated traits are shown in Table 2. The grain quantity characteristics and the grain yield of 196 half-sib families of maize were studied. To find the extent of yield variation components that are responsible for the yield differences, it must be kept in mind that total variability is contingent upon non-heritable and heritable components. The coefficient of variation measures the extent of variation present in the population, genetic advances and heritability, as these are prime important steps of the breeding program because this gives information required in the efficient breeding program. The genotypic variance (δ^2_G), phenotypic variance (δ^2_P), error variance (δ^2_E), genotypic coefficient of variance (GCV %), and phenotypic coefficient of variance (PCV %) expressed as a percentage for 14 parameters are presented in Table 2. The phenotypic coefficient of variance (PCV%) was higher than the genotypic coefficient of variance (GCV%) for all traits except for fresh ear weight, where the genotypic co-efficient of variance was greater, which reflect the environmental influence on the trait's expression (Tab. 2).

Heritability

Heritability (h^2) of a trait is vital to find out its response to selection. The genetic improvement for quantitative characters of plants requires reliable estimates of heritability for the plan of efficient breeding. A high heritability of 0.87 was observed for days to tasseling, and for days to mid-silking high heritability of 0.66 was observed, which specifies the low effect of the environment with a relative improvement of the trait (Tab. 4). A moderate heritability estimate of 0.57 was recognized for days to anthesis, which reflects considerable environmental effects on anthesis. For anthesis silking interval (ASI), high heritability estimates of 0.62 were observed. For a number of plants and plant height, high heritability

estimates of 0.61 and 0.66 were observed. Mahmood and Hubbard (2004) recognized a high heritability estimate of 0.99 for plant height that shows our results are the same as his results. A high heritability estimate of 0.67 was observed for kernel rows per cob. Mahmood and Hubbard (2004) also observed a high heritability estimate of 0.87 for this parameter. For cob length and 100-kernel weight, a high heritability of 0.94 and 0.92 was observed (Tab. 4), which are in agreement for 100-kernel weight, scrutinized by Sujiprihati *et al*. (2007). High heritability estimates of 0.85 were observed for the grain yield parameter, and such high heritability estimates were also observed (Mohamed and Mohamed, 2017).

Tab. 5 Correlations among yield related agronomic traits.

	DT	DS	DA	ASI	PR	PH	EH	CR	FEW	GM	KR	KW	GY
DT		0.98**	1.00**	1.00**	0.96**	0.93**	0.93**	0.97**	0.28**	0.95**	0.99**	0.98**	0.94**
DS			0.99**	0.99**	0.99**	0.98**	0.98**	1.00**	0.09 N.S	0.99**	0.94**	1.00**	0.99**
DA				1.00**	0.97**	0.95**	0.95**	0.98**	0.22**	0.97**	0.98**	0.99**	0.96**
ASI					0.97**	0.94**	0.94**	0.97**	0.24**	0.96**	0.98**	0.98**	0.95**
PR						1.00**	1.00**	1.00**	-0.01 N.S	1.00**	0.90**	1.00**	1.00**
PH							1.00**	0.99**	-0.09 N.S	1.00**	0.86**	0.99**	0.90**
EH								0.99**	-0.09 N.S	1.00**	0.86**	0.99**	0.94**
CR									0.02 N.S	1.00**	0.91**	1.00**	1.00**
FEW										-0.04 N.S	0.42**	0.07 N.S	-0.06N.S
GM											0.89**	0.99**	1.00**
KR												0.93**	0.88**
CL													0.99**

DT-Days To Tasseling, DS-Days To Silking, DA-Days To Anthesis, ASI-Anthesis Silking Interval (ASI), PR-No of Plants/Row, PH-Average Plant Height of Five Plants, EH-Average Ear Height of Five Plants, CR-No of Cobs/Row, FEW-Fresh Ear Weight/Row, GM-Grain Moisture/Row, KR-Kernal Rows/ Cob (Average of 3 Cobs), CL-Cob Length (Average Cob Length In 3 Cobs), KW-100 Kernal Weight, GY-Grain Yield

N.S = non-significant, ** = Significant at 1% level of significance

Correlation Analysis

The degree of correlation is important to factor in yield, which is a complex character among different characters. Steel and Torrie (1960) scrutinized that correlation is the intensity of the measures between the traits. The assortment of one trait affects the progress of all characters that are positively correlated. The correlation coefficients among the different traits were studied (Tab. 5), which shows a highly significant and positive correlation between grain yield with all parameters, especially with the number of plants per row and the number of cobs per row. This shows that our results are quite good and true because when the number of plants per row increases, the grain yield will be increasing; this will automatically increase the number of cobs per row, which results in the increment of grain yield. A highly significant and positive correlation was also observed between grain yield and cob length, kernel rows per cob, grain moisture, days to tasseling, silking, and anthesis. However, a non-significant and negative correlation factor was observed between grain yields and fresh ear weight. A non-significant and positive correlation factor was observed between 100-kernel weights with

fresh ear weight, and a highly significant and positive correlation was observed with no plant per row (Tab. 5).

Tab. 6 (Part-A) List of half-sib families (HSF) showing grain yield (GY kg ha^{-1}) of two replications.

HSF	GY	HSF	GY	HSF	GY	HSF	GY	HSF	GY	HSF	GY	HSF	GY
HSF-1	**3697**	HSF-15	**2293.5**	HSF-29	**3661.5**	HSF-43	**3670**	HSF-57	**4107.5**	HSF-71	**3026**	HSF-85	**3462**
HSF-2	**2870.5**	HSF-16	**3404**	HSF-30	**3065**	HSF-44	**2803**	HSF-58	**3042.5**	HSF-72	**3895.5**	HSF-86	**3055**
HSF-3	**3131**	HSF-17	**3676.5**	HSF-31	**2046.5**	HSF-45	**4043**	HSF-59	**3970**	HSF-73	**3662**	HSF-87	**4785.5**
HSF-4	**4023**	HSF-18	**3527.5**	HSF-32	**3066**	HSF-46	**3839**	HSF-60	**3785**	HSF-74	**4949**	HSF-88	**3996.5**
HSF-5	**4767**	HSF-19	**4061**	HSF-33	**3734.5**	HSF-47	**3725**	HSF-61	**4578**	HSF-75	**4719.5**	HSF-89	**4527**
HSF-6	**4174**	HSF-20	**3316**	HSF-34	**3428**	HSF-48	**6905.5**	HSF-62	**5180**	HSF-76	**3845**	HSF-90	**5198**
HSF-7	**5333**	HSF-21	**4843.5**	HSF-35	**4169.5**	HSF-49	**4148**	HSF-63	**5755.5**	HSF-77	**5215**	HSF-91	**4475.5**
HSF-8	**4942**	HSF-22	**4695**	HSF-36	**5996**	HSF-50	**4313.5**	HSF-64	**6660**	HSF-78	**5299.5**	HSF-92	**4299.5**
HSF-9	**7225.5**	HSF-23	**3484**	HSF-37	**3178.5**	HSF-51	**4460.5**	HSF-65	**5099.5**	HSF-79	**5115.5**	HSF-93	**3335**
HSF-10	**3980.5**	HSF-24	**4033**	HSF-38	**4409.5**	HSF-52	**5300.5**	HSF-66	**4989.5**	HSF-80	**4017**	HSF-94	**5557**
HSF-11	**4053**	HSF-25	**5387.5**	HSF-39	**4117.5**	HSF-53	**6613**	HSF-67	**5494**	HSF-81	**4345.5**	HSF-95	**4105.5**
HSF-12	**4436.5**	HSF-26	**4752**	HSF-40	**4474**	HSF-54	**4347**	HSF-68	**4354**	HSF-82	**3350.5**	HSF-96	**3430**
HSF-13	**3816**	HSF-27	**6555**	HSF-41	**5373.5**	HSF-55	**5936.5**	HSF-69	**4367**	HSF-83	**6940**	HSF-97	**5593**
HSF-14	**4645.5**	HSF-28	**4601**	HSF-42	**5112.5**	HSF-56	**4711**	HSF-70	**5068**	HSF-84	**8378**	HSF-98	**2437.5**

Tab. 6 (Part-B) List of half-sib families (HSF) showing grain yield (GY kg ha^{-1}) of two replications.

HSF	GY	HSF	GY	HSF	GY	HSF	GY	HSF	GY	HSF	GY	HSF	GY
HSF-99	3060.5	HSF-113	2446.5	HSF-127	6048.5	HSF-141	3447	HSF-155	3817	HSF-169	3658	HSF-183	4193.5
HSF-100	3153	HSF-114	3449.5	HSF-128	3662.5	HSF-142	3208	HSF-156	3589	HSF-170	3288	HSF-184	3490.5
HSF-101	3745	HSF-115	5169.5	HSF-129	3519	HSF-143	3846	HSF-157	4438.5	HSF-171	3915.5	HSF-185	3367.5
HSF-102	4407.5	HSF-116	3791.5	HSF-130	3894	HSF-144	3271.5	HSF-158	4043.5	HSF-172	4131	HSF-186	3551
HSF-103	4907.5	HSF-117	3175	HSF-131	3074	HSF-145	4525.5	HSF-159	3867	HSF-173	3843	HSF-187	4746.5
HSF-104	4224.5	HSF-118	4808	HSF-132	4758.5	HSF-146	4360.5	HSF-160	4442	HSF-174	4850.5	HSF-188	4552
HSF-105	4420	HSF-119	5298	HSF-133	3550.5	HSF-147	3623.5	HSF-161	5200	HSF-175	4365	HSF-189	4151
HSF-106	5409.5	HSF-120	2385	HSF-134	4551.5	HSF-148	3217	HSF-162	3631.5	HSF-176	5285.5	HSF-190	5560
HSF-107	4460	HSF-121	3013.5	HSF-135	4561	HSF-149	4634.5	HSF-163	5735.5	HSF-177	5424.5	HSF-191	4197.5
HSF-108	2427	HSF-122	3296	HSF-136	3677	HSF-150	5099.5	HSF-164	5931	HSF-178	3455	HSF-192	4141
HSF-109	5024.5	HSF-123	3657.5	HSF-137	3137	HSF-151	3850.5	HSF-165	5268	HSF-179	7376.5	HSF-193	4157.5
HSF-110	3684	HSF-124	3330	HSF-138	3226	HSF-152	4686.5	HSF-166	8257.5	HSF-180	10710	HSF-194	8283.5
HSF-111	3687	HSF-125	4326	HSF-139	5262.5	HSF-153	3661.5	HSF-167	4841.5	HSF-181	4268.5	HSF-195	5782.5
HSF-112	5700.5	HSF-126	3919	HSF-140	4804	HSF-154	3239.5	HSF-168	6138.5	HSF-182	4752	HSF-196	2729.5

Grain yield (kg ha^{-1})

Grain yield improvement is one of the major aims of every plant breeding program. Several methods of selection have been used by maize breeders to improve yield per unit area and develop high- yielding genotypes. Among them, the four major types are mass selection, selection based on half-sib progeny performance, full-sib performance, and selfed progeny selection (Okoye et al., 2018). Statistical analysis of the data regarding grain yield revealed highly significant genetic variation ($P<0.01$) among the half-sib families. The genetic coefficient of variance (CV) for grain yield was 15.62 % (Tab. 3). Grain yield of half-sib families ranged from 2046.50 kg ha^{-1} for HSF-31 to 10710.50 kg ha^{-1} for HSF-180. The grand mean calculated was 4367.54 kg ha^{-1} (Tab. 1). Our results are consistent with those of Tanner and Smith (1987), who conducted eight cycles of half-sib family ($BSK_{(S)}$) recurrent selection in the Krug yellow dent maize population, in which they obtained significant variances among test crosses for grain yield.

Conclusion

The phenotypic coefficient of variance was higher than the genotypic coefficient of variance for all traits except for fresh ear weight which reflects the environmental influence on the expression of the trait. High to moderate heritability was observed for days to tasseling, days to mid silking, days to anthesis, anthesis- silking interval, kernel rows per cob, cob length, and grain yield. Highly significant and positive correlations were observed between grain yield and cob length (0.99), kernel rows per cob (0.88), grain moisture (1.00), days to tasseling (0.94), silking (0.99), and anthesis (0.96). The negative and non-significant correlations were observed between grain yield and fresh ear weight (-0.06). Maximum grain yields of 10710 kg ha^{-1} was recorded for HSF-180 while the minimum 2046 kg ha^{-1} was obtained by HSF-31

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Authors contributions:

Nasr Ullah Khan and Muhammad Saad Ahmed conceived the idea and designed the project. Muhammad Ishfaq Khan, Rida Nisar, Muhammad Muddasir, and Muhammad Umer Mustafa conducted the experiment and collected the data. Muhammad Arshad, Rehan Naeem, Mohsin Khurshid, and Muhammad Khuram Razzaq analyzed the data. Nasr Ullah Khan, Muhammad Saad Ahmed, Muhammad Ishfaq Khan, and Abdul Majid drafted the manuscript. All authors read the manuscript before submission.

Conflict of interests:

None

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