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Dermatophytosis in Bovine, Recent Clinical analysis, Treatment, and updated zoonotic infection: A Review Article

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ABSTRACT

Dermatophytosis is a worldwide disease caused by pathogenic keratinolytic fungi known as dermatophytes inside the cutaneous layer of both cattle and human skin. are divided into three categories: *Microsporum* species, *Epidermophyton* species, and *Trichophyton* species. Infected animals develop skin lesions with a thick, grey-white crust that rises above the skin approximately 3cm in diameter or more. The back, head, and perineum are the only places where lesions are localized; otherwise, the body is covered with them, with severe cases coalescing. *Trichophyton verrucosum* has the greatest prevalence in bovines. Many have also reviewed new herbal extract that has been employed as a substitute therapy for fungus activity (fungicide or fungistatic) in several non-filamentous and filamentous fungi species typically identified in veterinary clinical practice. This review aims to understand the acquired and innate responses briefly, and discovered the recent technique in the detection of fungi, with treatment by chemical, extract herbal plant, and incidence by chief causal fungal species, and the final method that increases the risk of zoonotic infections between bovine and human.

1. Introduction

Bovine dermatophytosis poses a significant threat to the health of both humans and animals on a global scale [1]. The infection is commonly known as "ringworm"[2]. Dermatophytes are divided into three groups based on their natural habitats: *anthropophilic* species (only infect people), *zoophilic* species (obligate diseases for diverse animals), and *geophilic* species (living in the earth). Based on microscopic features, dermatophytes are anamorphic taxa that

are divided into several groupings and classed as *Trichophyton* species, *Epidermophyton* species, and *Microsporum* species [3].

Apart from generating conidia or asexual spores, fungi have the ability to sexually reproduce by producing sexual spores. There are three different forms of sexual spores: *zygospores*, *ascospores*, and *basidiospores*. Ascospores are generated within an ascus, while basidiospores are produced externally on the basidium, which is a stalk-like structure. *Zygospores* are characterized by their large size and solitary nature, with thick walls surrounding them. Fungi that do not produce sexual spores are classified as Fungi *Imperfecti*. They are called *imperfect* because they lack sexual spores, and instead, asexual spores are produced through the process of mitosis [4].

The interaction between humans and animals can lead to the transmission of infectious diseases, known as zoonoses, either directly or indirectly [5]. Individuals who work with animals in fields such as farming, veterinary medicine, and animal care, along with their families, are susceptible to contracting infections either directly from the animals or indirectly through contact with contaminated tools and equipment [6].

A dermatophyte infection is identifiable by a raised, circular crust on the skin, measuring around 3cm in diameter, with a thick, grey-white appearance [7]. In the initial stages of the infection, the skin underneath the crust remains moist, but as the disease advances, the scab starts to peel off, and only hair loss and flaky skin may be noticeable. While lesions can be found throughout the body, they are more prevalent on the back, head, and perineum, with calves being particularly susceptible. In severe cases, the lesions may appear in clusters [8].

The ringworm fungus mainly infects keratinized tissues, including the stratum corneum and hair shafts, which are vital for the organism's growth and development [9], this can cause autolysis of the hair fiber structure, hair breakage, and hair loss (alopecia) [8], the fungus has the ability to produce proteolytic and keratolytic enzymes, such as keratinase, elastase, and collagenase. These enzymes enable the fungus to utilize keratin as its sole source of nutrition after colonization, thus promoting fungal growth in the stratum corneum and leading to epidermal keratinization [10]. The lesions continue to develop if environmental conditions that favor mycelial growth, such as a warm, humid atmosphere and slightly alkaline skin pH, are present, making it easier for the fungus to penetrate the skin. The dry crusts, which are a distinctive feature of the disease, are formed due to exudation from the invaded epithelial layers, fungal hyphae, and epithelial debris. The ringworm fungus is strictly aerobic, and in most lesions, the fungus dies off in the center, leaving only the perimeter active. The centrifugal progression and the characteristic ring-shaped lesions are a result of this pattern of development [8]. In the early stages of infection, dermatophytes respond to the skin by de-repressing multiple genes that produce various proteins and enzymes, such as adhesins, lipases, DNAases, phosphatases, non-specific proteases, and keratinases. These proteins and enzymes enable the pathogen to adhere to and

infiltrate host tissue, scavenge resources, and overcome host defensive mechanisms, as the acidic pH of human skin is optimal for their function [11], When fungal spores germinate in an acidic environment, it has been observed that cuticular fatty acids exhibit various toxic and fungistatic effects [12]. The most common infective component of the dermatophyte organism is arthrospores, which are formed by the fragmentation and segmentation of fungal hyphae. These arthrospores adhere to keratin and can germinate within a few hours of contact with the skin, penetrating the cornified tissue and causing hair infections [8]. As a result, the ability of dermatophytes to infect the host depends on several factors such as the species of dermatophyte, the number of infectious spores, virulence factors, and the immune status of the host [9]. The path of dermatophyte infection is depicted in **Figure** 1.

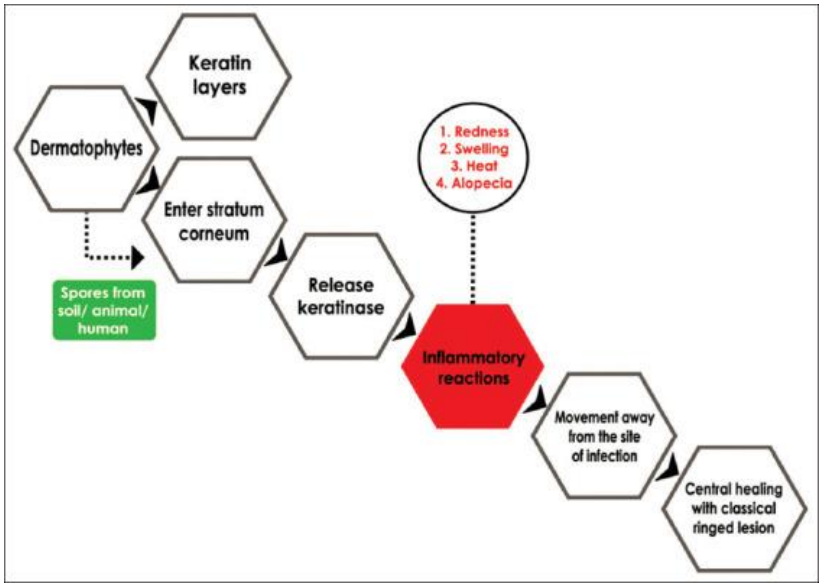

Figure 1: The graphic depicts the route of dermatophyte entrance into the host [14]

Clinical pathology

In laboratory diagnosis, the identification of spores and mycelia in skin scrapings from the edge of the lesion and in culture is necessary [13], Alternatively, to prevent bacterial contamination, the sample can be transferred to sterile black chart paper and kept dry [14]. To examine for dermatophyte infection, skin scrapings should be taken, decontaminated with 70% ethyl alcohol, placed in a 10% potassium hydroxide solution, and potentially supplemented with lactophenol cotton blue before being examined under a microscope. While this method is simple, affordable, and provides a quick and effective screening, it may take a long time to obtain results [15], The sensitivity and specificity of the diagnosis can be affected by the adequacy of the technique used for sample collection, the quality of the sample, and the expertise of the person performing the test [14]. An alternative method for diagnosis involves adding 36 percent dimethyl sulfoxide (DMSO) to KOH solution, which can speed up the removal of thick scales and increase the transparency of keratinocytes, making it

easier to visualize the fungi causing the infection [16]. To identify dermatophytes, the Chicago Sky Blue stain method is more effective than the potassium hydroxide wet mount testing [17]. The most accurate approach to identify fungus in skin, hair, and nails under a microscope is to fluorescently stain the material with an optical brightener (diaminostilbene), which binds to chitin, a component of the fungal cell wall [18]. The distinctive feature of *Trichophyton* spp. and *Microsporum* spp. are spores that appear as reflective, rounded or polyhedral shapes in chains or mosaics on epithelial scales, on the surface of hair fibers, and within hair follicles [8]. Fluorescent staining with Calcofluor white (CFW) is believed to be the most sensitive method for detecting fungi in hair, skin, and nails under a microscope. The fluorescent antibody in CFW binds specifically to the glucan and chitin layers found in the fungal cell wall, making fungal fluorescence staining a useful new diagnostic technique for identifying fungi [18]. When using fluorochromes like Calcofluor or Blankophor in combination with fluorescence microscopy, identification of fungi is faster, simpler, and more reliable than with KOH testing. These fluorochromes bind equally well to the chitin and glucan present in the fungal cell wall. Under UV light in fluorescence microscopy, fungal filaments and spores appear as blue-white [19]. Chlorazol black E (CBE) stain has a high affinity for the chitin found in the cell walls of fungi, but not in the tissues of vertebrates. This stain creates a blue-black color on the cell walls of filamentous fungi and yeast, making it easier to visualize and more sensitive under microscopy [20]. Wood's Light Fluorescence is a painless and rapid method to screen for pigmentation and viral diseases in the hair and skin. By emitting long-wave UV radiation, it detects fluorescence on skin surfaces that would not typically fluoresce under normal conditions. This technique can also identify a range of skin conditions and is commonly used to detect *Microsporum* species [21]. The lactophenol Cotton Blue mount is a helpful tool for examining fungal growth characteristics, such as the type of mycelium, the presence or absence of microconidia, and the shape and morphology of macroconidia. This mount is particularly useful for identifying various dermatophyte species under the microscope [22]. Fungal culture is a valuable tool for verifying fungal species, and Sabouraud's dextrose agar (SDA) is the most commonly used medium for this purpose. SDA modified with chloramphenicol, gentamicin, and cycloheximide is even more selective for dermatophytes [23]. Dermatophyte Test Medium (DTM) has phenol red which serves as a pH indicator, and it is better suited for screening purposes compared to SDA which is more useful for identification. The media changes color from yellow to bright red as dermatophytes break down proteins, leading to the release of ammonium ions and an alkaline environment [24]. Potatoes are an excellent inclusion in media, such as Potato Dextrose Agar (PDA), because they supply essential minerals and carbohydrates required for fungal growth. By incorporating tartaric acid in a specific proportion (10%), it reduces the pH of the medium and prevents bacterial growth [16]. The chitin

synthase 1 primer pair can be utilized for PCR and nucleic acid sequence-based amplification to promptly detect medication resistance in fungi while also aiding in rapid diagnosis [25], Real-time PCR was utilized and found to be more specific than direct microscopy and culture techniques in detecting the genomic material of dermatophytes. Compared to microscopic inspection, it was able to detect *Trichophyton verrucosum* and *T. benhamiae* more accurately [26]. Moreover, it is important to note that various methods have been employed to identify dermatophyte species, including the use of enzymes such as keratinase, phospholipase, elastase, DNase, lipase, protease, and gelatinase. Nevertheless, presently, only keratinase has been linked to the development of dermatophyte infections [27]. Lastly, when it comes to diagnosing Majocchi granuloma, histology has been employed. Hyphae can be observed in the stratum corneum through H&E staining. The use of periodic acid Schiff staining and Gomori methanamine silver stains can enhance the visibility of fungal hyphae [28] **Figure 2.**

Figure 1: Bovines infected with ringworm [29]

Treatment

The former treatment method is utilized for minor lesions, while the latter is used for extensive lesions, and may involve either localized or systemic therapy. Due to the risk of zoonotic transmission, protective clothing and gloves should be worn when handling sick animals. Currently, systemic mycoses can be treated with five different categories of antifungal medications, including polyenes (such as amphotericin B), azoles (such as fluconazole, itraconazole, posaconazole, voriconazole, and isavuconazole), echinocandins (such as caspofungin, micafungin, and anidulafungin), allylamines (such as terbinafine), and antimetabolites (such as flucytosine). Amphotericin B is the most effective polyene for treating invasive fungal infections, as it has a broad spectrum of fungicidal activity. The mechanism of action for polyenes involves binding to ergosterol in the fungal cell membrane, which leads to increased permeability and release of intracellular components, ultimately resulting in cell death [30]. Azoles and polyenes both target ergosterol to achieve a fungicidal effect. Azoles specifically

inhibit the lanosterol 14-demethylase enzyme, which blocks the production of ergosterol [30]. The enzyme 1,3-d-glucan synthase plays an important role in the weakening of fungal cell walls, which can lead to cell lysis. Echinocandins are essential in preventing this process by inhibiting the enzyme and converting uridine diphosphate glucose towards d-glucan, which strengthens the cell walls [31].

Terbinafine, an allylamine topical medication that works by blocking squalene epoxidase, is exclusively employed to treat dermatophytes [32]. The fungal cell absorbs 5-flucytosine (5-FC), a pyrimidine analogue, through cytosine permease. Once inside, it transforms into 5-fluorouracil by deamination and then integrates into the fungus's RNA, impeding the creation of proteins [33].

Sodium thiosulfate, Whitefield ointment (which contains 6% benzoic acid and 3% salicylic acid), and Iodine preparation are examples of local or topical treatments that can hinder spore transmission within specific time frames. According to research, these time frames are as follows: days + (standard deviation) for sodium thiosulfate, 7 (1.1) for Whitefield ointment, 14 (0.7) for Iodine preparation, and 20.9 (0.6) for Iodine preparation [1]. In veterinary medicine, imidazoles (such as clotrimazole, ketoconazole, and miconazole) and triazoles (such as itraconazole and fluconazole) are the preferred topical medications for treating fungal infections in animals. These antifungal drugs contain two and three nitrogen molecules respectively. Furthermore, azole antifungal drugs are known to inhibit the formation of cell membrane sterols via cytochrome P450-dependent enzymes [34]. Natamycin, which is a type of antifungal polyene antibiotic, exerts its effects on the fungal cell membrane by selectively binding to ergosterol [35], Natamycin is employed topically to cure fungal infections like mycotic keratitis in the eyes, ringworm, and yeast mastitis in the udder. When dealing with Candida mastitis in cows, the use of natamycin has demonstrated positive results (an infusion of 20 ml of a 2.5% solution or 10 ml of a 5% solution into the affected quarter of the udder once daily for three days). For efficient therapy, it is suggested to apply double whole-body sprays of natamycin (0.1%) separated by three days for cattle that are naturally infected with *T. verrucosum* [36]. Nystatin, which is a type of polyene antibiotic, disrupts the membranes of fungi by binding to sites that normally bind to ergosterol. This binding creates complexes that cause physical changes to the membrane, resulting in altered permeability. As a result, ions within the cell are able to flow out, leading to membrane disorganization [34].

In a group of 33 dairy cows with ringworm, the application of silver nitrate as a topical treatment resulted in the development of hair on the lesion after one week [37]. The unadjuvanted live vaccine for fungi comprises freeze-dried *T. verrucosum* conidia and hyphal elements of the LTF-130 strain. Ivermectin injection can effectively cure dermatophytosis in cattle through immunopotentiation, which increases the number of total white blood cells, including lymphocytes [38].

Administering the intramuscular vaccine on the neck's side serves both therapeutic and preventive purposes, and it's recommended to vaccinate calves between the ages of two and four weeks. A yearly booster is unnecessary as the immunity lasts a lifetime. If given to calves between four and six days old, a lesion measuring 1-2 cm with mild scaling and hair loss can develop at the injection site, which regenerates after a few weeks due to the vaccine strain's residual pathogenicity. Vaccination and ongoing surveillance have enabled Norway to remain free of bovine ringworm [39]. It has been observed that horses with multiple ringworm lesions respond positively to two inactivated lyophilized *T. verrucosum* vaccinations given 14 days apart [40].

Propolis, a natural extract, has been utilized as a substitute treatment to inhibit or prevent the growth of fungi (either by killing or inhibiting their growth) in several types of fungi commonly found in veterinary clinical settings, both filamentous and non-filamentous. These fungi include *Malassezia pachydermatis*, *Microsporum canis*, *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida guilliermondii*, *Candida parapsilosis*, and other species [41]. Henna leaves, specifically the compound fraxetin found in them, have demonstrated effective antifungal properties against 30 different species of dermatophytes in clinical isolates. This suggests that fraxetin could be a viable alternative treatment for dermatophytosis in both humans and animals [42]. Moreover, the ointment containing an alcoholic extract of garlic with a concentration of 20 percent showed significant therapeutic effectiveness. The skin redness decreased within two days of starting the treatment, while the scales and keratosis disappeared within six days. Furthermore, after two weeks of treatment, there was no sign of hair growth on the treated area [29] as in figure 3. Bergamot oil (*Citrus Bergamia*) has strong antifungal properties and is effective in fighting against *Trichophyton verrucosum*, both alone and in combination with salicylic acid, across a range of different dosages [43]. Topical application of clove oil three times a day for ten consecutive days can completely cure ringworm lesions within two weeks, owing to its potent antifungal properties. Meanwhile, in cases of emergency infection, culture filtrate vaccine can be administered as a subcutaneous injection suspension, with a dose of 5ml followed by a second dose after ten days, which has been found to be an effective therapy [44].

In comparison to both Ketoconazole (an antifungal standard) and *L. coronopifolia* extract, green synthesized Ag NPs exhibited notable antifungal properties against the tested isolates from cows, with a p-value of less than 0.01. Ag NPs showed remarkably significant effects against fungi [45], Moreover, it was demonstrated that zinc oxide (ZnO) nanoparticles exhibited anti-fungal properties against various fungi including Trichophyton mentagrophyte, Candida albicans, Microsporum canis, and Aspergillus fumigatus in cattle. The highest inhibition of fungal growth was observed at a concentration of 40 mg/mL of ZnO nanoparticles [46].

Figure 3: (A) showd a bovine infected with ringworm (B) bovine treated with alcoholic extract of garlic 20% showed appearance of hair completely after 14 days from the beginning of the treatment [29].

Incidence

The major fungal species responsible for the condition were *M. gypseum, M. nanum, M. canis, T. terrestre, T. equinum, T. verrucosum, and T. mentagrophytes*. Dermatophytes are found worldwide in various species and clinical presentations (64). The rate of infection was 73 percent, with the most significant incidence of infection occurring in animals aged less than one year, with a rate of 63 percent. Males had a higher rate of infection than females, with 54.8 percent and 45.2 percent, respectively. The winter months, particularly January, had the highest incidence of infection, with a rate of 27.4 percent. *Trichophyton Mentagrophytes*, which has three species (T. verrucosum, T. rubrum), had a higher rate of isolation for T. verrucosum at 68.5 percent compared to the other species (65), Additionally, according to another observation, female cattle had the greatest incidence rate (91.7%) and male cattle had the lowest percentage (88.9%%) [66]. Out of the 508 animals analyzed, *Trichophyton verrucosum* caused ringworm in 126 of them, which was 24.8 percent. The incidence of ringworm was higher in young calves below the age of one year, at 54.76 percent. Moreover, it was more frequent in males than in females, with rates of 67.46 percent and 32.53 percent, respectively (66). The occurrence of Trichophyton verrucosum among cattle is subject to fluctuations based on factors such as location, age, and time of year [67]; Bovine dermatophytosis, specifically *Trichophyton verrucosum*, was found to have a higher frequency rate of occurrence (54.2%) compared to *Trichophyton mentagrophytes* (45.8%). The incidence of the disease was significantly linked to various factors such as age, management practices, breed, and season, with a p-value of less than 0.05 [68].

Zoonotic infection

Trichophyton verrucosum is a type of fungus that is commonly found in animals and is the main cause of dermatophytosis in cattle. Recently, there has been a steady increase in the number of *T. verrucosum* infections in humans, which is linked to the presence of farms that rear cattle [6].

In recent times, there has been increased attention on the quality of indoor air in stables, cowsheds, and henhouses due to the potential risk of zoonotic diseases. The fungal load in cowsheds is known to be significant, particularly when there is insufficient attention paid to regulating the micro-climate within with respect to heating, ventilation, and illumination. Additionally, airborne zoophilic dermatophytes, such as *T. verrucosum*, have become more prevalent, which further increases the risk of zoonotic diseases. The examination of fungal pollutants showed an average of 0.084 dermatophyte propagules (CFU) per cubic meter of air in spring and 0.0239 CFU/m3 in summer, with 64.6% of colonies containing these propagules. *Trichophyton verrucosum* was found to be the dominant species on all five farms examined [69]. Moreover, the two factors that increase the severity of the disease are present in Trichophyton (T.) verrucosum, a highly infectious fungus that causes bovine ringworm and can be transmitted to humans. These factors, Sub3 and 6, are exclusively produced by spores and can be identified in the epidermis, dermis, and hair parts of the cow's skin [70]. Furthermore, when assessing the microbial composition of indoor air quality in cattle farms, researchers identified 172 and 210 genera of bacteria/archaea and 89 and 43 genera of fungi in the dust and air, respectively. Some of these genera could potentially pose a threat to the health of humans and animals [71]. Moreover, there is a considerable risk of zoonotic transmission when humans experience poorly healing skin conditions as a result of being infected with Trichophyton verrucosum, a zoophilic dermatophyte associated with bovine dermatophytosis [72]. In addition, fungi can cause surface infections in animals by accidentally penetrating and colonizing on damaged skin, and they can be transmitted to humans through contact. The fact that opportunistic fungi are increasingly responsible for causing dermatomycosis makes it important for veterinarians to be cautious when identifying these molds in wound samples [73]. Moreover, *T. erinacei* is a type of dermatophyte that can be transmitted to humans through contact with the abdominal skin lesions that resemble dermatophytosis, typically appearing around two weeks after the onset of the disease in animals [74].

Conclusion

To summarize, this review confirms a relationship between persistent fungal infections in bovines and humans, emphasizing the need to address co-infection with other pathogens. The study also investigates the link between *Trichophyton verrucosum* infection and the amount of dermatophyte spores in the air or direct contact with farm animals. This is a critical issue to be addressed in the treatment of ringworm, and a vaccine can aid in early diagnosis and prompt elimination of the fungus. To decrease horizontal transmission and reduce severe cases of *Trichophyton verrucosum*, it is strongly recommended to implement control programs for the fungus in farm animals and provide treatment for bovines with ringworm.

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Conflict of Interest

The author certifies that this work was carried out without financial assistance and that he or she does not believe there is a conflict of interest.

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القوباء الحلقية في الأبقار ، التحليل السريري الحديث ، العلاج ، واحدث الاصابات المشتركة : دراسة مراجعة

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الملخص

الخلاصة

القوباء الحلقية هي حالة عالمية تسببها الفطريات الكيراتينية المرضية والمعروفة باسم الفطريات الجلدية داخل الطبقة الجلدية لجلد الأبقار والبشر. تصنف إلى أنواع الشَّعْرَوِيَّة والبَشْرَوِيَّة والبويغاء. الحيوانات المصابة تظهر بآفات جلدية بقشرة سميكة رمادية بيضاء ترتفع فوق الجلد بقطر 3 سم أو أكثر. الظهر والرأس والعجان هي الأماكن الوحيدة التي تتوضع فيها الآفات ؛ وبخلاف ذلك يكون الجسم مغطى بها وتندمج مع بعضها في الحالات الشديدة. تنتشر الشعروية الثؤلوليَّة في الأبقار بشكل كبير. كما تمت مراجعة العديد من المستخلصات العشبية الجديدة التي تم استخدامها كعلاج بديل لنشاط الفطريات (ايقاف نمو الفطريات أو قتل الفطريات) في العديد من أنواع الفطريات غير الخيطية والخيطية التي تم تحديدها عادةً في الممارسة السريرية البيطرية. تهدف هذه المراجعة إلى فهم الاستجابات المكتسبة والفطرية باختصار ، واكتشاف التقنية الحديثة في الكشف عن الفطريات ، مع العلاج الكيميائي ، والمستخلصات النباتات العشبية ، والإصابة بالأنواع الفطرية المسببة الرئيسية ، والطرق النهائية التي تزيد من خطر العدوى الحيوانية المنشأ بين الابقار والبشر..