

## Spread and consequences of secondary infections in cattle affected with foot-and-mouth disease in Karbala province, Iraq

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**Citation:** Hameed, M. A. K., Al-Dawmy, F., Alqatab, A. H., Zabeel, A. K., & Ibrahim, Z. K. (2025). Spread and consequences of secondary infections in cattle affected with foot-and-mouth disease in Karbala province, Iraq. *Scientific Progress & Innovations*, 28(4), 172–177. doi: 10.31210/spi2025.28.04.27

Foot-and-mouth disease (FMD) is a highly contagious viral disease caused by aphthovirus belonging to the family of *Picornaviridae*. Although the primary viral infection is known to cause the typical clinical manifestations, such as fever, salivation, and the formation of vesicles around the mouth, muzzle, teats, and interdigital spaces, the breakage of these vesicles opens the entry for opportunistic microorganisms. The latter can greatly affect the disease outcome, however, neither their spread, nor the species composition diversity among cattle herds in Iraqi have not been adequately documented. Thus, the current study was aimed at isolating and identifying the secondary microbial agents that complicate the foot-and-mouth disease lesions in cattle in Karbala province, Iraq. Following the rules of asepsis, a total of one hundred smear samples were collected from the mouth cavity (n=50) and extremities (n=50) of cows that had been clinically diagnosed with FMD on the basis of the sudden appearance of the disease classical symptoms during its regional outbreak. The microbiological investigation showed a complex polymicrobial community consisting of various opportunistic pathogens that were colonizing as well as infecting the primary viral lesions. Moreover, the isolation of several pathogens from a single lesion was a common phenomenon. Among the isolated bacterial pathogens, both Gram-positive and Gram-negative forms were well represented. *Trueperella pyogenes* turned out to be the most predominant pathogen (18 % of total isolates). *Pasteurella multocida* was found to be the second (16 % of total isolates) after *T. pyogenes*. Notably large percentages of *Staphylococcus aureus* (14 %), both methicillin-sensitive and methicillin-resistant strains, as well as *Escherichia coli* (13 %), both commensal and pathogenic strains, were found. Other bacterial pathogens that were significant enough to be mentioned individually were *Streptococcus dysgalactiae* (7 %), *Fusobacterium necrophorum* (6 %), *Pseudomonas aeruginosa* (5 %). Fungal pathogens were found less frequently. They were mostly *Candida* sp. (5 %), as well as *Cryptococcus* sp. (3 %). It can be noted from these results that bacterial and fungal complications are not only incidental but also common and significant complications that occur as a result of FMD infection. These secondary infections play a vital role complicating the disease pathogenesis. Thus, this research confirmed the urgent necessity of changing the paradigm in the treatment of FMD cases, introducing integrated and holistic treatment approaches, beyond merely supportive therapy of the viral infection.

**Keywords:** foot and mouth disease (FMD), cattle, secondary infection, microbes, bacteria, fungi.

## Поширення і наслідки вторинних інфекцій у великої рогатої худоби хворої на ящур в умовах провінції Кербела, Ірак

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Ящур – висококонтагіозне захворювання яке викликається вірусом з роду *Aphthovirus*, що належить до родини *Picornaviridae*. Нині відомо, що первинна вірусна інфекція викликає типові клінічні прояви, такі як лихоманка, слизовидлення та утворення везикул навколо ротової порожнини, дійок та міжпальцевого простору в корів. Розрив цих везикул відкриває ворота інфекції для умовно-патогенних мікроорганізмів, які можуть значно впливати на прогноз захворювання. Незважаючи на це, поширення і наслідки вторинних інфекцій серед поголів'я великої рогатої худоби хворої на ящур, в Іраку не були належним чином задокументовані. Таким чином, метою дослідження було виділити та ідентифікувати вторинні мікробні агенти, що ускладнюють перебіг ящуру у великої рогатої худоби в провінції Кербела (Ірак). Для дослідження з дотриманням правил асептики було відібрано 100 мазків із ротової порожнини (n=50) та кінцівок (n=50) корів, яким клінічно діагностували ящур на основі раптової появи класичних симптомів захворювання у період регіонального спалаху хвороби. Мікробіологічне дослідження показало складну полімікробну асоціацію, що складається з різних умовно-патогенних мікроорганізмів, які колонізували та інфікували первинні вірусні ураження. При цьому виділення одночасно кількох патогенів з одного вогнища ураження було поширеним явищем. Серед виявлених бактеріальних патогенів були добре представлени як грампозитивні, так і грамнегативні форми. *Trueperella pyogenes* виявився найпоширенішим патогеном (18 % від загальної кількості ізолятів). На другому місці після *T. pyogenes* виявилася *Pasteurella multocida* (16 % від загальної кількості ізолятів). Також, достатньо високим виявився відсоток *Staphylococcus aureus* (14 %), як метицилін-чутливих, так і метицилін-резистентних штамів, а також *Escherichia coli* (13 %) як коменсальних, так і патогенних штамів. Іншими бактеріальними патогенами, які є достатньо значущими були *Streptococcus dysgalactiae* (7 %), *Fusobacterium necrophorum* (6 %), *Pseudomonas aeruginosa* (5 %). Грибкові патогени були виявлені рідше, переважали *Candida* sp. (5 %) та *Cryptococcus* sp. (3 %). Отже, бактеріальні та грибкові ускладнення є не лише випадковими, але й поширеними та значними ускладненнями, що виникають як наслідок інфекції ящуру. Ці вторинні інфекції відіграють життєво важливу роль, ускладнюючи патогенез захворювання. Проведене дослідження підтвердило нагальну необхідність зміни парадигми в лікуванні випадків ящуру, впроваджуючи інтегровані та цілісні підходи, що виходять за рамки лише підтримуючої терапії.

**Ключові слова:** ящур, велика рогата худоба, вторинна інфекція, патогени, бактерії, гриби.

**Бібліографічний опис для цитування:** Хамед М. А. К., Аль-Даумі Ф., Алькатаф А. Х., Забіл А. К., Ібрагім З. К. Поширення і наслідки вторинних інфекцій у великої рогатої худоби хворої на ящур в умовах провінції Кербела, Ірак. *Scientific Progress & Innovations*. 2025. № 28 (4). С. 172–177.

## Introduction

*Picornaviridae* virus is a member of the genus *Aphthovirus*, the foot and mouth disease virus (FMDV). A tiny, non-enveloped, positive-sense single-stranded RNA virus, FMDV has a genome of around 8500 bases and is encased in an icosahedral capsid made up of four structural proteins. Seven immunologically distinct serotypes A, O, C, SAT1, SAT2, SAT3, and Asia1 are present, which do not cause cross-immunity. Some FMD virus serotypes have more variation than others but as a collective group they contain over 60 strains. The disease is endemic in most parts of Asia, Africa, the Middle East and South America. Serotypes A and O are widely distributed, SAT serotypes are mostly observed in Africa, and Asia1 is currently only in Asia [1, 2].

The FMDV is primarily responsible for the initial clinical sign in cattle, with vesicle formation and oral and foot epithelium erosions. However, secondary bacterial complications are primarily responsible for the major economic losses and animal welfare concerns [3].

The predisposition to secondary bacterial infection in foot-and-mouth disease is based on the total breakdown of the host's initial physical and immunological defenses. Not only are the mouth, teats, and foot epithelial linings physically affected through vesicle breakage but they also become immunologically vulnerable. The FMD virus has been reported to preferentially infect keratinocytes and epithelial cells, resulting in widespread cytolysis [4]. Moreover, the virus is now also known to infect and deplete some populations of dendritic cells and macrophages directly in lesions and local lymph nodes and thereby suppress the early innate immune response that would otherwise keep infiltrating bacteria in check [5].

The characteristics of secondary infections have moved from viewing them as simple contaminations to an understanding of the complex synergisms in the polymicrobial populations that colonize FMD lesions. The isolation of the pathogens like *Trueperella pyogenes* and *Pasteurella multocida* along with other microbes like *Fusobacterium necrophorum* and *Staphylococcus aureus* is not a coincidence. There is often a synergistic association, particularly in connection with foot abscesses. For instance, initial colonization by facultative anaerobes will reduce the oxygen tension in the region, providing it an ideal culture for such anaerobes as *Fusobacterium necrophorum* [6].

The organism, in fact, produces leukotoxins that kill host phagocytes, and *T. pyogenes* provides required growth factors through its proteolytic activity. This synergistic pathogenesis intensifies tissue necrosis and pyogenesis, leading to more severe and refractory clinical disease than would be caused by any of these pathogens alone [7].

## The aim of the study

The current study was aimed at isolating and identifying the secondary microbial agents that complicate

the foot-and-mouth disease lesions in cattle in Karbala province, Iraq

## Materials and methods

In order to survey the dominant microbial species causing these secondary infections in Karbala province, the study was undertaken. Aseptically collected one hundred smear samples of ruptured oral ulcers and foot lesions, especially from interdigital space and coronary band, of confirmed FMDV infected cattle were from various locations in Karbala province.

Standard microbiological techniques for the isolation and identification of bacterial and fungal pathogens were employed. The initial cultivation of the collected samples was done on general-purpose media (nutrient agar, blood agar, MacConkey agar) and selective media (e.g., Mannitol salt agar for staphylococci, Edwards modified medium for *Trueperella pyogenes*, Sabouraud dextrose agar with chloramphenicol for fungi).

Presumptive identification of the isolated cultures was made on the basis of their colonial characteristics and Gram staining. For fungal cultures, the identification was made on the basis of their macroscopic and microscopic characteristics.

## Results and discussion

The microbiological culture showed a polymicrobial population of complexity as presented in **Table 1**.

**Table 1**

Spread of microorganisms isolated from infected cattle

The organism	Smear samples
<i>Trueperella pyogenes</i>	18/100
<i>Staphylococcus aureus</i>	14/100
<i>Escherichia coli</i>	13/100
<i>Streptococcus dysgalactiae</i>	10/100
<i>Pasteurella multocida</i>	16/100
<i>Streptococcus agalactiae</i>	9/100
<i>Salmonella</i> sp.	7/100
<i>Candida</i> sp.	5/100
<i>Cryptococcus</i> sp.	3/100
Non growth	10/100

*Trueperella pyogenes*, *Escherichia coli*, and *Pasteurella multocida* were some of the isolates, along with other opportunistic pathogens such as *Staphylococcus aureus*, *Salmonella* sp., *Streptococcus dysgalactiae*, and other microbes such as *Aspergillus* sp. and *Candida* sp. There were ten samples with no growth due to some possible reasons like technical error during inoculation, mishandling, incubation defects, and other environmental factors.

The information provided in **Table 2** is a crucial, site-specific analysis of the secondary microbial flora associated with foot-and-mouth disease lesions, which shows characteristic ecological patterns for oral and foot infection sites. This secondary colonization is not random but is affected by anatomical, physiological, and

environmental parameters, which have important implications for disease control and outcome.

**Table 2**

Spread of microorganisms isolated from infected cattle depending on the sampling location

Isolated microorganisms	Smear samples	
	oral cavity	affected hooves
<i>Trueperella pyogenes</i>	10	8
<i>Staphylococcus aureus</i>	9	5
<i>Escherichia coli</i>	9	4
<i>Streptococcus dysgalactiae</i>	5	5
<i>Pasteurella multocida</i>	7	9
<i>Streptococcus agalactiae</i>	5	4
<i>Salmonella</i> sp.	3	4
<i>Candida</i> sp.	4	1
<i>Cryptococcus</i> sp.	2	1
Non growth	3	7

One of the first things that emerge is the fairly even representation of *Trueperella pyogenes* in oral (10 isolates) and hoof lesions (8 isolates). This opportunistic nature is well established in necrotic tissue, and its proteolytic enzymes and biofilm-forming properties make it ideally suited to the devitalized tissue of both sites. This observation is consistent with the findings of [8, 9] that reported *T. pyogenes* as the most prominent secondary invader in bovine interdigital necrobacillosis and mastitis, respectively, and emphasized its non-specific tropism for compromised epithelial barriers.

On the other hand, the distribution of *Pasteurella multocida* indicates a notable disposition for lesions involving the hooves, as shown by 9 isolates as opposed to 7 ones from the oral lesions. This may be explained by the fact that, due to the environmental contamination, injuries on the feet often become exposed to soil and manure, which often contain *P. multocida*. Moreover, the ischemic and traumatic nature of severe foot lesions may provide a suitable environment for its establishment. This is supported by the study made by [8, 9], which showed that *P. multocida* was more frequently isolated from extremities' infections than from oral lesions in cattle, attributed to the environmental factors and traumatic inoculation.

The spread of *Staphylococcus aureus* and *Escherichia coli* was higher in the oral lesions than in the hoof lesions. For *S. aureus*, it could be attributed to the fact that the organism has a natural habitat in the nasopharyngeal region and skin. These organisms could easily be transferred to the oral lesions through grooming, licking, or even the act of feeding. The oral cavity might provide a more favorable environment for the growth of *S. aureus* due to the warmth and moisture. The high prevalence of *E. coli* in the oral lesions could be attributed to the fact that the organism has a natural habitat in the perineal region. It could be transferred to the oral lesions through the act of grooming the perineal region. This could also be attributed to the fact that the organism could be present in the feed and water.

This high spread of the organisms in the oral lesions is in agreement with the study carried out by [11] that reported a higher load of staphylococci and enterobacteria in the oral lesions than in the cutaneous lesions in FMD-infected cattle.

The isolation of fungal agents (*Candida* sp. and *Cryptococcus* sp.), primarily from oral lesions, is a significant finding. It suggests that mycotic overgrowth may be precipitated by the disruption of the normal mucous flora, the necrotic environment, and possibly the immune suppressive effects of the primary FMD virus or stress. Their presence, particularly in lesions that may be treated empirically with antibacterial agents, signals the risk for complicated, mixed infections that are not responsive to antibiotic therapy alone. Recent literature, such as the report by [12] on secondary infections in bovine respiratory disease, has increasingly highlighted the under-recognized role of fungal opportunists in prolonged bovine illnesses.

The microbiological analysis showed a complex polymicrobial community in lesions of FMD infected cattle, which confirms the known pathophysiology of the virus. FMD vesicle rupture creates extremely large portals of entry in the oral cavity and feet epithelium, and secondary colonization by a broad range of opportunistic pathogens is possible [13]. The isolation of multiple bacterial species, for example, *T. pyogenes*, *E. coli*, and *P. multocida*, and fungi from the same clinical samples strongly indicates the presence of the polymicrobial infection environment and synergistic interactions between bacteria may result from this, which may worsen tissue damage, slow down the healing process, and intensify clinical symptoms such as mastitis and lameness [14]. The difficulty in the treatment of FMD complications is highlighted by the existence of a polymicrobial ecosystem, as it may be necessary to address multiple pathogenic pathways simultaneously.

The extremely high prevalence of *Trueperella pyogenes* (18 %) is consistent with its established reputation as a leading opportunistic pathogen in bovine disease. The bacterium is a facultatively anaerobic, non-motile coccus which is a common mucous membrane entry to the upper respiratory and urogenital tracts. Its pathogenicity is mainly attributed to a highly active exotoxin, pyolysin, which is a cholesterol-dependent cytolysin and induces cell lysis by forming pores in the membranes of host cells. *T. pyogenes* also possesses several other virulence factors such as fimbriae for adherence, a neuraminidase to cleave protective glycoproteins on mucosal surfaces, and collagen-binding proteins that facilitate invasion to deeper tissue. Its common occurrence among the FMD isolates from lesions defends its position as a first-order causative agent for secondary infection, where it is highly present in abundance in the necrotic cells of the burst vesicles [15].

These resulting deep, suppurative infections, particularly pododermatitis in cattle feet and mastitis in udder, are normally chronic and crippling, inducing

severe prolonged lameness and decrease in milk production, which are central causative agents for economic losses during FMD outbreaks [2].

Similarly, the significant segregation of *P. multocida* (16 %) stresses its ecological adaptability and virulence beyond its classical application in bovine respiratory disease (BRD). *P. multocida* is a short, Gram-negative, facultatively anaerobic coccobacillus. Pathogenicity is exerted through an elaborate array of virulence factors, primarily the polysaccharide capsule, whose capsular antigens A, B, D, E, and F determine its serogroup and host tropism and disease manifestation. Lipopolysaccharide (LPS) endotoxin, a potent stimulator of inflammatory response, and adhesions and iron-acquisition systems are also significant virulence factors. For FMD, systemic stress and cortisol release secondary to the viral infection can result in immune suppression, i.e., in dysfunction of neutrophils. This immune compromised state allows *P. multocida*, as a commensal in the nasopharynx, to translocate and infect localized skin that is breached by the feet and mouth. Such infections can lead to cellulitis, abscessation, and in extreme cases, bacteremia and septicemic pasteurellosis, thereby severely impairing the clinical outcome and increasing the levels of mortality in infected herds [7, 16].

Significant issues concerning animal welfare and public health are brought to light by the isolation of pathogens from FMD lesions, including *Salmonella* sp. (7 %) and *E. coli* (13 %), as well as *S. aureus* (14 %). The large open wounds caused by ruptured vesicles are easily contaminated by these organisms, which are common residents of the farm environment and the gastrointestinal system of cows. Their presence is the immediate indicator of a severe violation of the normal cutaneous and mucosal barriers, damaged by the prior infection with the virus. Bacteremia, septicemia, and endotoxic shock are disease conditions that have high prevalence rates in progressive FMD disease infections particularly in young animals, as predictors of mortality. Not only is the potential of local colonization available in this invasion, but also the mechanisms of the potential systemic spread [13, 14].

The result of such a serious polymicrobial disease is painful and immobilizing, which greatly affects the clinical outcome, and extends the convalescence period, and is a significant animal welfare problem. The incidence of the given zoonotic infections concerns public health. Close contact with infected cattle or fomites predisposes the transmission of the infection to veterinarians, farm workers and other animals. The environment of the farms is polluted massively with these pathogens [17].

One of the risks is the development of such isolates that would be resistant to popular antibiotics. *S. aureus*, *E. coli* and *Salmonella* are a big concern due to their ability to inflict infections in individuals that are difficult to treat [18]. Moreover, *E. coli* and *Salmonella* species directly endanger the food supply and may cause foodborne disease outbreaks in case of the lack of control measures. These findings therefore stress the importance

of proper biosecurity measures, the need to use personal protective gear, when communicating with infected fauna, and the importance of using antibiotic susceptibility testing in the process of conducting effective treatment.

The detection of the fungal organisms, in this case *Candida* sp. (5 %), is an important discovery which reveals the severe physiological damage of the fungi by FMD viral infection. This is in line with the theory that immune compromised states such as lymphopenia induced by the virus and systemic stress of an ailing fever may enable commensal fungi to multiply [19].

The major FMD viral infection destroys the oral mucosal, gastrointestinal tract barriers, killing the epithelial protective layer and offering a physical niche that is colonized by the fungi. Moreover, the extensive use of broad-spectrum antibiotics applied to treat secondary bacterial infections can destroy the normal microbiota and leave bacterial competitors dead, which makes the ecological vacuum allowing the development of fungi especially *Candida* species [20]. The progress from the infection to colonization has the capability of inducing clinically apparent conditions such as oral thrush that, besides impairing prehension are also capable of further affecting the nutritional status of the animal. More seriously, in systemically immune compromised host the local infection can result in invasive systemic candidiasis [21].

The disease is typically insidious and difficult to diagnose antemortem, but it has the potential to lead to disseminated microabscesses within the organs such as the kidneys, liver, and abomasum and develop a fatal outcome. The isolation of *Candida* sp. in 5 % of cases is therefore not only a laboratory isolation but also a marker for severely diseased animals at risk of a complex and nonspecific fungal infection. It demands heightened clinical suspicion and, in severe and non-reactive cases – potential antifungal therapy, specifically in instances where iatrogenic effects of earlier antibiotic therapy are suspected [22].

Finally, 10 % non-growth cultures are a common laboratory experience because of numerous reasons. Pre- or concurrent antimicrobial treatment of the animals before sampling would significantly reduce the number of viable bacteria. Additionally, the bacteria with specific nutritional requirements may not grow on standard media, and antifungal or antibacterial compounds in the smear transportation system can inhibit growth [23, 24].

Generally, a broad range of opportunistic bacterial and fungal pathogens were isolated from the lesions of foot-and-mouth disease infected cattle in this study. The most frequent isolates, including *T. pyogenes*, *P. multocida*, *S. aureus*, and *E. coli*, were repeatedly isolated, emphasizing their significant role in secondary infection. Furthermore, the fungal organisms including *Candida* sp. were also isolated. The complexity of these polymicrobial infections is emphasized. Infected herds suffer severe economic losses, prolonged morbidity, and chronic lameness as a result of these secondary complications.

## Conclusions

This research verifies the existence of secondary bacterial and fungal infections as a common and dangerous complication of foot-and-mouth disease in cattle, creating complex polymicrobial biofilms on oral and feet lesions. The isolation of important pathogens such as *Trueperella pyogenes*, *Pasteurella multocida*, *Staphylococcus aureus*, and *Escherichia coli* indicates a clear opportunistic infection pattern that contributes to the primary viral pathology. The specific information on the spread for each location indicates the presence of specific microbial communities, with staphylococcal and enteric bacteria predominating in oral lesions and a higher degree of environmental contamination with pathogens such as *P. multocida* in hoof lesions. These secondary infections directly affect the morbidity and serious clinical course of the disease, as well as the convalescence period due to delayed wound healing.

Therefore, a complex treatment approach must incorporate specific antimicrobial treatment according to local susceptibility patterns and strict site-specific wound care practices. In any case, the treatment of these secondary infections is crucial for solving animal welfare issues and decreasing the significant economic losses incurred during FMD outbreaks.

*Prospects for further research.* Future studies should aim at investigating long time observations of lesion development with and without specific intervention, molecular analysis of virulence factors of the spread isolates, and the assessment of particular antiseptic regimens in outbreak settings.

## Conflict of interest

The author (s) state that there is no conflict of interest.

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