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Optimizing diagnostic sites for FMDV carrier identification: A systematic evaluation of tissue tropism in goats

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Abstract

The carrier state of Foot-and-mouth disease virus in goats remains a critical gap in disease control, as the specific anatomical sites of viral persistence have not been systematically characterized. This study evaluated 11 distinct tissue types across a cohort of 60 goats in an endemic region to identify the primary anatomical reservoirs and determine whether caprine tissue tropism aligns with bovine or ovine models of persistence. Using real-time reverse transcriptase Polymerase Chain Reaction and histopathological analysis, the research revealed a non-uniform distribution of the viral RNA. The highest positivity rates were found in the dorsal nasopharynx (up to 25.00%) and the soft palate (19.23%). In contrast, the palatine tonsils traditionally viewed as the primary reservoir in small ruminants like sheep yielded a minimal positivity rate of only 1.92%. Pareto analysis confirmed that four primary sites (the dorsal nasopharynx and soft palate) accounted for 84.62% of all positive detections, adhering to the "80/20 rule" for diagnostic optimization. Histopathological examination of these reservoirs showed subepithelial lymphoid activity, follicular hyperplasia, and epithelial mosaic and moth eating appearances, which are indicative of low-level viral maintenance. These findings demonstrate that Foot-and-mouth disease virus tissue tropism in goats mirrors the bovine nasopharyngeal model rather than the ovine tonsillar model. This research provides a data-driven foundation for refining species-specific diagnostic protocols, suggesting that relying on tonsillar sampling would fail to identify over 98% of caprine carriers. By prioritizing the dorsal nasopharynx and soft palate, surveillance programs can significantly improve the detection of subclinical carriers and mitigate the silent circulation of the virus.

Keywords: Foot-and-mouth disease virus, goats, viral persistence, Nasopharynx, soft palate, Caprine carriers

Introduction

Foot-and-mouth disease virus is recognized for its ability to establish a persistent infection in ruminants, a condition defined by the presence of infectious virus in the oropharyngeal region for 28 days or more post-infection (Jena, 2022) ^[14]. While hematobiochemical alterations during the acute phase of FMDV infection have been documented in goats, showing significant decreases in hemoglobin, total erythrocytes, packed cell volume, and total protein, there remains a paucity of literature regarding the anatomical sites of viral persistence in the carrier state. The mechanisms of viral persistence have been extensively documented in cattle, research regarding the anatomic localization and pathogenesis of the carrier state in goats remains limited (Jena, 2022; Sarry *et al.*, 2023) ^[14, 22]. Addressing this knowledge gap is essential, as persistent infection occurs across small ruminants regardless of vaccination history or initial clinical presentation (Pacheco *et al.*, 2015) ^[20]. Unlike cattle, where viral persistence is primarily localized to the nasopharyngeal mucosa, the specific tissue tropism in caprine species have not been systematically characterized (Conrady *et al.*, 2024; Stenfelt & Arzt, 2020) ^[7, 8, 24]. This study addresses this discrepancy by molecular and morphological findings to determine which tissues, serve as primary reservoirs for viral persistence in goats (Henning *et al.*, 2025; Stenfelt *et al.*, 2019) ^[13, 28]. Furthermore, this investigation aims to elucidate whether goat tissue tropism aligns more closely with the nasopharyngeal reservoirs observed in bovines or the tonsillar crypts identified in ovine models (Conrady *et al.*, 2024) ^[7, 8].

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Materials and Methods

Study Area and Experimental Animals

The research was conducted in a region endemic to Foot-and-mouth disease virus, allowing for the evaluation of goats under natural infection conditions. The study utilized a cohort of 60 goats. To investigate the subclinical carrier state, the animals were monitored to confirm they met the formal definition of an FMDV carrier: the persistence of the viral genome in the oropharyngeal region for 28 days or more following the initial infection). This phase is distinct from the active phase of infection, during which goats typically exhibit significant systemic alterations in hematobiochemical profiles, such as decreased hemoglobin and total protein.

Tissue Collection and Processing

Systemic post-mortem examinations were conducted to harvest 11 distinct anatomical tissues. To preserve the integrity of the viral RNA, samples from the nasopharynx, soft palate, and lymphoreticular tissues were collected using sterile techniques. Tissues were collected in sterile tubes containing transport media, such as phosphate-buffered glycerol with antibiotics, to stabilize the virus prior to laboratory analysis. Tissue specimens intended for morphological analysis were fixed in a 10% neutral buffered formalin solution (Bancroft and Gamble, 2008) [3]. The following eleven tissue types were evaluated: Middle tongue, Ventral soft palate, Dorsal soft palate, Dorsal nasopharynx 1(Cranial), Dorsal nasopharynx 2(Caudal), Heart, Retropharyngeal lymph node, Palatine tonsil, Prescapular lymph node, Prefemoral lymph node, Mandibular lymph node

Molecular Protocols and Instrumentation

The primary diagnostic tool for identifying the viral reservoirs was real-time reverse transcriptase PCR (rRT-PCR), which provides the sensitivity required to detect low-level viral persistence in carrier tissues (Arzt *et al.*, 2017; Pacheco *et al.*, 2015) [24, 20, 25]. Total RNA was extracted from the 11 tissue types using standardized lysis and purification protocols. The rRT-PCR assays targeted the highly conserved 3D polymerase region of the FMDV genome (Stenfeldt *et al.*, 2015) [25].

Histopathological Analysis

To characterize the micro-anatomical localization of the virus and any associated cellular changes, fixed tissues were processed for histopathology. Tissues were dehydrated through graded alcohols, cleared, and embedded in paraffin blocks. Sections were cut at a thickness of 5 micrometer and stained with Hematoxylin and Eosin to allow for the assessment of epithelial changes and lymphoid activity within the sampled niches.

Software and Statistical Analysis

Data acquired from the real-time PCR system were analyzed to determine the percentage of positivity across the 11 tissue types, facilitating the identification of tissue tropism. To identify and prioritize the primary anatomical reservoirs of FMDV, a Pareto analysis was performed. This statistical technique was used to distinguish the 'vital few' tissues that contribute the most to overall viral detection from the 'trivial many' that offer low diagnostic yield.

Results and Discussion

The combined findings of the study were discussed with their significance in the context of established foot-and-mouth disease virus pathogenesis models and diagnostic surveillance.

Tissue-Specific Distribution and Viral Load

Molecular analysis using rRT-PCR demonstrated that the highest rates of viral detection occurred in the nasopharyngeal region. The Dorsal Nasopharynx 1 exhibited the highest positivity rate at 25.00% (13/52), followed by the Dorsal Nasopharynx 2 at 23.08% (12/52). Significant viral persistence was also localized to the Dorsal Soft Palate (19.23%) and the Ventral Soft Palate (17.31%). In stark contrast, tissues that are traditionally prioritized in small ruminant surveillance, such as the Palatine Tonsils, yielded a positivity rate of only 1.92% (1/52). Peripheral sites, including the mandibular lymph nodes (1.92%), heart (3.85%), and tongue (7.69%), showed minimal involvement in long-term viral maintenance.

The mean Ct values for tissue clustered between 24 and 38, which is characteristic of the low-level replication observed in carrier animals. It serves as a molecular hallmark of the Foot-and-mouth disease virus carrier state. The detection of Ct values in the 24-38 range provides evidence of low-level genomic maintenance rather than the robust replication seen in clinical cases. This research indicates that Ct values in FMDV carriers are "generally very high, "which is "indicative of low levels of FMDV RNA" within the pharyngeal tissues. This specific range reflects several critical biological and diagnostic realities of subclinical infection. This localization is particularly noteworthy because, while these subclinical carriers often exhibit normalized hematobiochemical profiles, recovering from the acute-phase reductions in hemoglobin and total protein, they continue to harbour the virus in these specific mucosal sites.

Anatomical Discrepancy

A primary contribution of this study is the resolution of the anatomical discrepancy between bovine and ovine persistence models as they relate to goats. Historically, goats have been grouped with sheep under the small ruminant diagnostic umbrella, which identifies the epithelial crypts of the oropharyngeal tonsils as the primary viral reservoir (Conrady *et al.*, 2024; Stenfeldt *et al.*, 2019) [7, 8, 28]. However, our results proved that caprine tissue tropism significantly diverged from the ovine tonsillar model. If veterinary practitioners were to rely solely on tonsillar sampling, they would have failed to identify over 98% of the carrier animals in this study (Conrady *et al.*, 2024) [7, 8]. Instead, the findings revealed that goats mirror the bovine model of persistence. In cattle, FMDV is primarily restricted to the nasopharyngeal mucosa, specifically the dorsal nasopharynx and the superior surface of the soft palate (Pacheco *et al.*, 2015; Stenfeldt *et al.*, 2015) [20, 25]. By identifying the dorsal nasopharynx and soft palate as the definitive reservoirs in goats, this study suggests that the biological mechanisms of persistence in the caprine species are more closely aligned with those of large ruminants than with sheep (Arzt *et al.*, 2017) [24].

Pareto Analysis and Diagnostic Optimization

A Pareto analysis was applied to the tissue distribution data to prioritize diagnostic targets, revealing that four primary

sites DNP1, DNP2, DSP, and VSP accounted for 84.62% of all positive detections (Table I). This distribution adheres to the "80/20 rule," where the "vital few" tissues provide the majority of the diagnostic yield. As shown in Graph 1, the steep initial slope of the cumulative line indicates that viral persistence is highly concentrated in these anatomical niches, while the flattening of the curve toward peripheral tissues (lymph nodes and heart) suggests they contribute negligible value to identifying the carrier state.

This concentration of viral RNA confirms that goats mirror the bovine model of nasopharyngeal tropism (Arzt *et al.*, 2017; Pacheco *et al.*, 2015) [20, 25] rather than the ovine model, which focuses on the oropharyngeal tonsils (Conrady *et al.*, 2024; Stenfeldt *et al.*, 2019) [7, 8, 28]. By prioritizing the "vital few" sites identified by the Pareto graph, surveillance protocols in endemic regions can be optimized for maximum sensitivity and efficiency (Conrady *et al.*, 2024; Mardones *et al.*, 2010) [7, 8]. This targeted approach is essential for identifying subclinical carriers and preventing the silent circulation of endemic serotypes (Jena, 2022; Khan *et al.*, 2024) [14].

Histopathological alterations

Histopathological examination of the primary reservoirs, the dorsal nasopharynx and soft palate provided morphological evidence for this tissue tropism. Consistent subepithelial lymphoid activity was observed, characterized by well-defined lymphoid aggregations with follicular hyperplasia and a dense population of lymphocytes and macrophages

(Fig.1 to Fig.8). In the DNP, the epithelium exhibited a mosaic appearance, where infected cells clustered together in small groups surrounded by healthy keratinocytes. This non-uniformity in the basal layer suggests the virus specifically targets large, polygonal cells, likely basal keratinocytes or dendritic-like cells (Pacheco *et al.*, 2015; Stenfeldt *et al.*, 2015) [20, 25].

In the soft palate tissues, acantholytic-like changes were noted, marked by the loss of intercellular attachments within the stratified squamous layer. The infiltration of mononuclear cells from underlying follicles into the epithelium created a moth-eaten" appearance at the basal boundary. Conversely, the tongue showed hydropic or ballooning degeneration of prickple cells, while the myocardium maintained a regular arrangement of fibers with distinct striations, consistent with the subclinical nature of the persistent phase (Parthiban *et al.*, 2015).

Table I: Positivity percentage of Foot and Mouth Disease Virus in tissue samples organized in descending order.

Tissue Type	Code	Samples Positive	Positivity (%)
Dorsal Nasopharynx 1	DNP1	13	25
Dorsal Nasopharynx 2	DNP2	12	23.08
Dorsal Soft Palate	DSP	10	19.23
Ventral Soft Palate	VSP	9	17.31
Tongue	TONG	4	7.69
Heart	HRT	2	3.85
Mandibular Lymph Node	MLN	1	1.92
Palatine Tonsil	PT	1	1.92

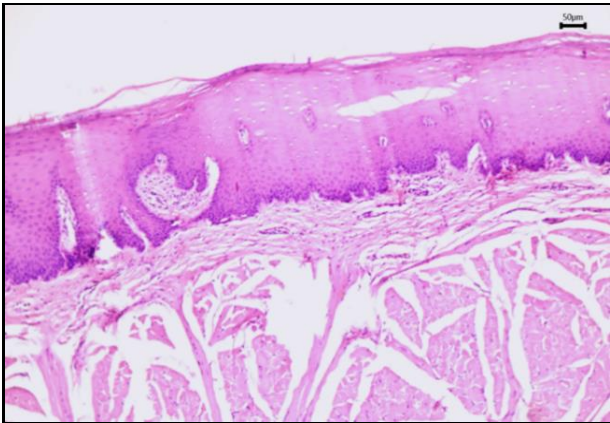


Fig 1: Tongue (H&E, 100X) Hydropic or ballooning degeneration of the prickple cells in the stratum spinosum, with acantholysis

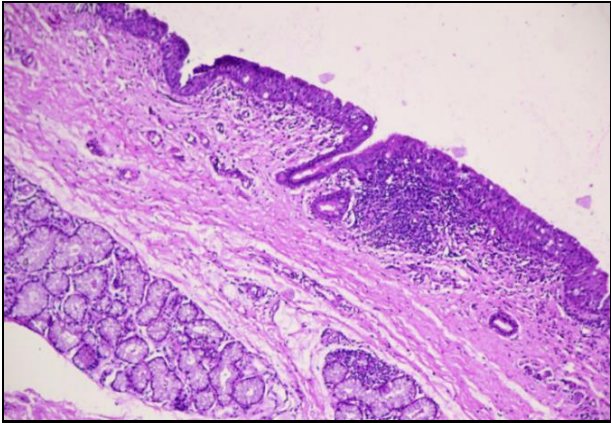


Fig 2: DNP (H&E, 100X) Well-defined, subepithelial lymphoid aggregation, exhibiting a "mosaic" appearance of the epithelium

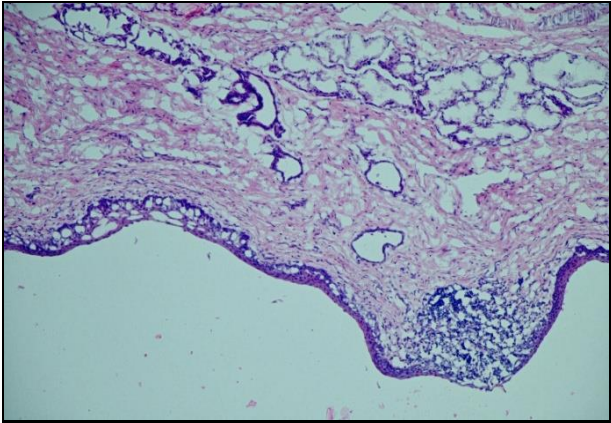


Fig 3: DNP (H&E, 100X) Well-defined, subepithelial aggregation of mononuclear cells (lymphocytes and macrophages)

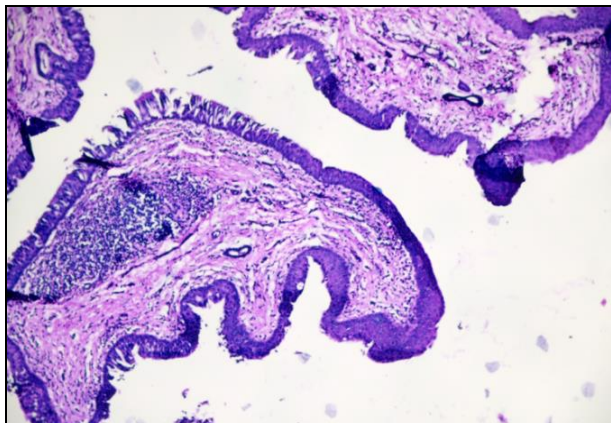


Fig 4: DSP (H&E, 100X) Well-defined, subepithelial lymphoid aggregation

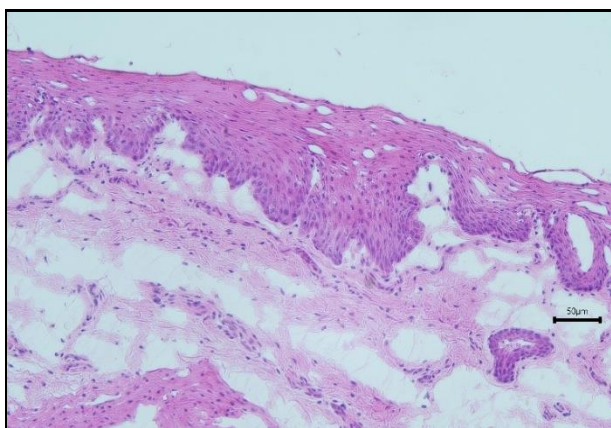


Fig 5: DSP (H&E, 100X) Acantholytic-like changes, with loss of intercellular attachments disrupting the uniform integrity of the stratified squamous layer, creating a "moth-eaten" appearance in the basal layer with non-uniform epithelium.

Conclusion

In conclusion, these results demonstrated that the dorsal nasopharynx and soft palate are the primary anatomical reservoirs for FMDV in goats. By proving that goats follow the bovine nasopharyngeal model rather than the ovine tonsillar model, this study provides a robust, data-driven foundation for refining species-specific diagnostic protocols and improving the efficacy of FMDV control programs in small ruminant populations.

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