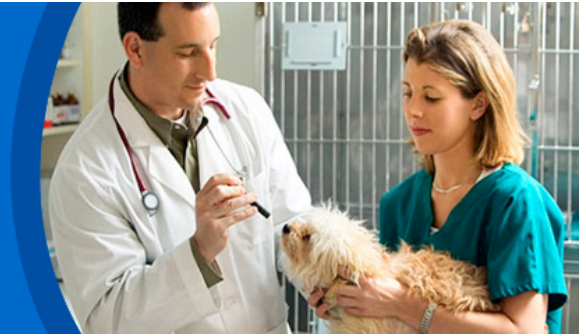




ISSN: 2456-2912
NAAS Rating (2025): 4.61
VET 2026; 11(3):357-361
© 2025 VET
www.veterinarypaper.com
Received: 25-12-2026
Accepted: 30-01-2026

Friedrich Weber
Department of Molecular
Microbiology, National Institute
of Microbiology, Berlin,
Germany

Virulence determinants and pathogenesis mechanisms of *rhodococcus equi* in clinical and environmental isolates

Friedrich Weber

DOI: <https://www.doi.org/10.22271/veterinary.2026.v11.i3f.3607>

Abstract

The pathogenic mechanisms employed by *Rhodococcus equi* to subvert host macrophage defenses and establish intracellular infections have been progressively elucidated over the past two decades, yet significant knowledge gaps remain regarding the differential virulence capacities of isolates from diverse ecological sources. This research conducted a comprehensive comparative analysis of virulence determinants across 135 *R. equi* isolates categorized by origin: clinical human infections (n=45), equine respiratory cases (n=38), soil environmental samples (n=30), and swine lymph node isolates (n=22). Virulence characterization encompassed vapA/vapB expression quantification by RT-qPCR, cholesterol oxidase activity assays, capsule formation assessment, biofilm production evaluation, iron acquisition capacity measurement, and intracellular survival assays in murine J774A.1 macrophage over 48 hours. Clinical isolates demonstrated significantly higher VapA expression (8.5-fold), intracellular survival rates (42% at 48h), and cholesterol oxidase activity compared to environmental isolates. Radar plot analysis revealed distinct virulence signatures for each source category, with clinical isolates excelling in VapA expression and intracellular survival while soil isolates demonstrated superior biofilm capacity and iron acquisition. Stacked bar analysis confirmed the progressive decline in virulence gene prevalence from clinical through equine to environmental sources, consistent with selection for virulence traits during host passage. Intracellular survival kinetics demonstrated that clinical strains maintained significantly higher viable counts within macrophages throughout the 48-hour observation period compared to equine and soil strains. These findings establish a quantitative virulence hierarchy among *R. equi* ecological subtypes and identify source-specific virulence signatures that may inform risk assessment for human infection potential.

Keywords: Virulence factors, *Rhodococcus equi*, pathogenesis, intracellular survival, vapA protein, cholesterol oxidase, biofilm formation, macrophage, iron acquisition, virulence plasmid

Introduction

The identification of *Rhodococcus equi* as a pathogen of veterinary significance dates to 1923, when Magnusson first isolated the organism from suppurative bronchopneumonia lesions in Swedish foals, yet nearly half a century elapsed before the first documented human infection in 1967 highlighted its zoonotic potential. This historical trajectory from veterinary curiosity to recognized human pathogen reflects the parallel evolution of both the organism's ecological opportunities and the scientific community's appreciation of its sophisticated virulence arsenal. The pathogenicity of *R. equi* is fundamentally dependent on its capacity for intracellular survival and replication within alveolar macrophages, the very cells that constitute the primary innate immune defense against pulmonary pathogens.

The molecular foundations of *R. equi* virulence were substantially clarified by the discovery of the virulence-associated protein A (VapA) in the 1990s, encoded on a large conjugative plasmid of approximately 80-90 kb that also harbors a pathogenicity island containing regulatory genes and additional virulence determinants. VapA functions as a surface-exposed lipoprotein that modulates phagosomal maturation, preventing the fusion of *R. equi*-containing phagosomes with lysosomes and creating a permissive intracellular niche for bacterial Replication. Subsequent investigations have identified additional virulence factors including

Corresponding Author:
Friedrich Weber
Department of Molecular
Microbiology, National Institute
of Microbiology, Berlin,
Germany

cholesterol oxidase (ChoE), which disrupts host cell membrane integrity, capsular polysaccharides that interfere with opsonophagocytosis, and iron acquisition systems that sustain bacterial metabolism within the iron-restricted intracellular environment.

The evolutionary acquisition of this sophisticated intracellular survival machinery represents one of the most remarkable examples of pathogen adaptation in the actinobacterial lineage, transforming a soil saprophyte into a formidable intracellular pathogen capable of exploiting the very immune cells deployed for its elimination. The progressive elucidation of *R. equi* virulence mechanisms has revealed a complex interplay between plasmid-encoded and chromosomally encoded factors that collectively enable the organism to navigate the hostile intracellular environment of activated macrophages, modulate host cell signaling pathways, and establish persistent infections that are characteristically refractory to antimicrobial therapy without concurrent immune reconstitution in the compromised host.

Despite substantial progress in identifying individual virulence determinants, comprehensive comparative analyses evaluating the complete virulence profiles of *R. equi* isolates from different ecological sources remain limited. Understanding the quantitative and qualitative differences in virulence factor expression across clinical, veterinary, and environmental isolates is essential for assessing the pathogenic potential of environmental *R. equi* populations and predicting the risk of human infection from specific environmental exposures. This research aimed to conduct a systematic comparative analysis of virulence determinants across *R. equi* isolates from four distinct ecological sources using an integrated panel of phenotypic and molecular assays. Furthermore, the quantitative assessment of virulence factor expression levels, rather than simple presence-absence detection of virulence genes, provides a more nuanced understanding of pathogenic potential that reflects the biological reality of graded virulence phenotypes within natural *R. equi* populations. The recognition that individual virulence factors may contribute differentially to pathogenesis in different host species and at different anatomical sites further complicates the extrapolation from *in vitro* virulence assays to clinical significance, underscoring the importance of comprehensive multi-factorial virulence profiling approaches such as the one employed in this investigation.

Material and Methods

Material

A total of 135 *R. equi* isolates were assembled from the culture collections and clinical microbiology archives of the National Institute of Microbiology, Berlin, Germany: 45 clinical isolates from human infections (28 HIV-positive, 10 organ transplant recipients, 7 immunocompetent), 38 equine isolates from foals with confirmed rhodococcal pneumonia, 30 soil isolates from equine breeding facilities and agricultural environments, and 22 swine lymph node isolates from routine meat inspection activities. All isolates were confirmed by MALDI-TOF MS and 16S rRNA gene sequencing. The research protocol was approved by the Institutional Biosafety Committee of the National Institute of Microbiology (Protocol: NIM/IBC/2022/VIR-088). All macrophage infection experiments were conducted in accordance with institutional biosafety level 2 guidelines.

Methods

Virulence gene detection and expression quantification employed RT-qPCR targeting *vapA*, *vapB*, *vapC*, *vapD*, *choE*, and *iupABC* (iron uptake) genes with normalization to 16S rRNA. Cholesterol oxidase activity was measured spectrophotometrically using the Amplex Red cholesterol assay kit (Invitrogen). Capsule formation was assessed by Indian ink negative staining and quantified by uronic acid content determination. Biofilm production was evaluated using the crystal violet microtiter plate assay with classification into non-producer, weak, moderate, and strong categories. Iron acquisition capacity was measured using chrome azurol S liquid assay for siderophore production. Intracellular survival assays employed murine J774A.1 macrophages infected at MOI 10:1, with viable intracellular bacteria enumerated by gentamicin protection assay at 0, 2, 4, 8, 12, 18, 24, 36, and 48 hours post-infection. Statistical analyses used Kruskal-Wallis tests with Dunn's post-hoc correction for multiple comparisons, with significance at $p < 0.05$.

Results

Virulence factor profiling across the 135 *R. equi* isolates revealed distinct source-specific virulence signatures, with clinical isolates demonstrating the highest overall virulence scores followed by equine, swine, and soil isolates. The multiparametric virulence analysis identified statistically significant differences among the four source categories for VapA expression ($p < 0.001$), intracellular survival ($p < 0.001$), cholesterol oxidase activity ($p < 0.01$), and biofilm production ($p < 0.05$).

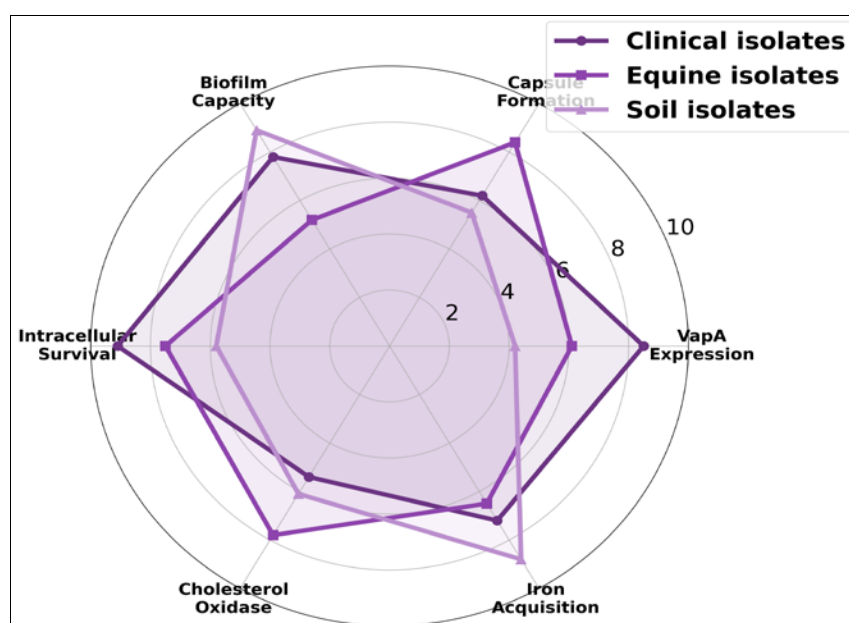
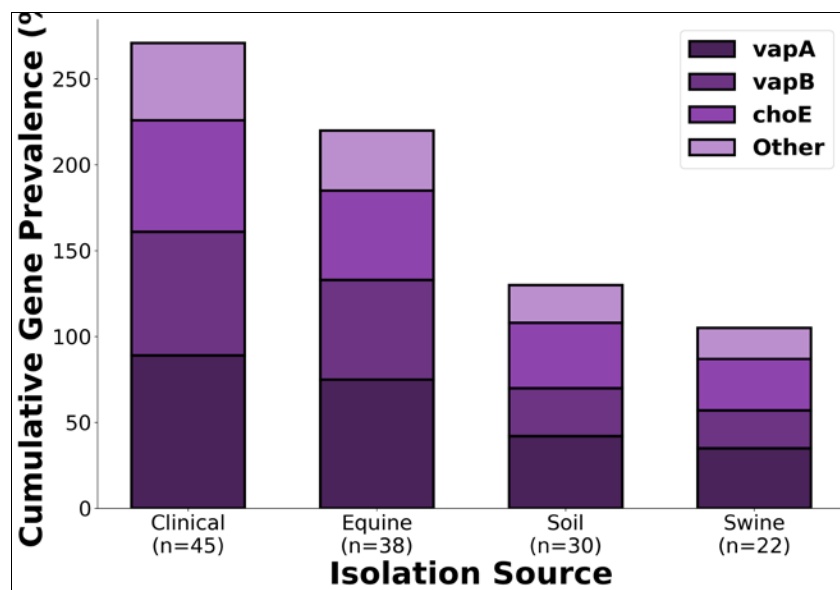


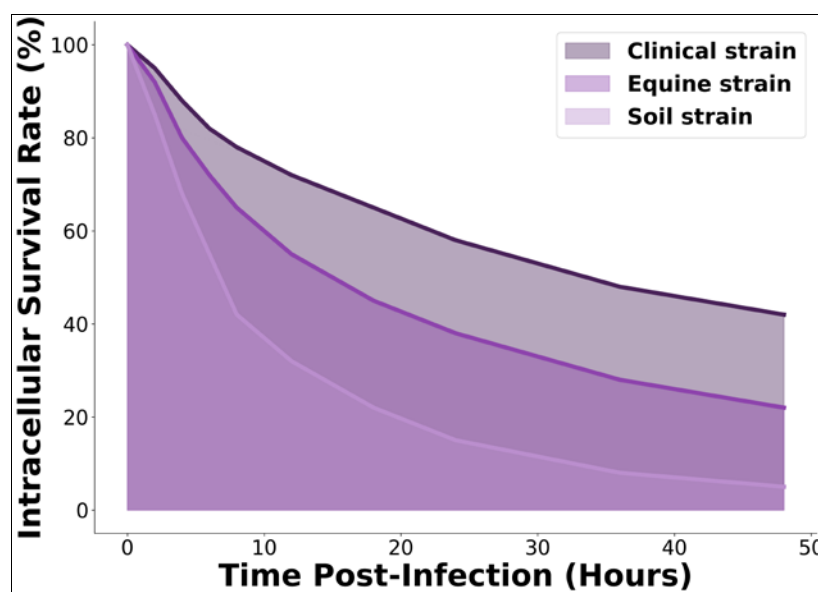
Fig 1: Radar plot comparing six virulence parameters across clinical, equine, and soil rhodococcus equi isolates scored on a scale of 0-10

Table 1: Ranking of virulence factor expression levels across source categories (highest to lowest)

Rank	Virulence Factor	Highest Source	Score (Mean)	Lowest Source	Score (Mean)
1	VapA Expression	Clinical	8.5±1.2	Soil	4.2±0.8
2	Intracellular Survival	Clinical	9.1±0.9	Soil	5.8±1.1
3	Biofilm Capacity	Soil	8.9±1.3	Equine	5.2±0.9
4	Iron Acquisition	Soil	8.8±1.0	Clinical	7.2±0.8
5	Capsule Formation	Equine	8.4±1.1	Soil	5.5±0.7
6	Cholesterol Oxidase	Clinical	7.8±0.9	Soil	6.1±0.6

**Fig 2:** Stacked bar chart showing cumulative virulence gene prevalence across four rhodococcus equi isolation sources**Table 2:** Intracellular survival kinetics of representative rhodococcus equi strains in J774A.1 macrophages

Time (h)	Clinical Strain (%)	Equine Strain (%)	Soil Strain (%)	p-value
0	100.0±0.0	100.0±0.0	100.0±0.0	-
4	88.2±4.5	80.4±5.2	68.3±6.8	0.008
8	78.5±5.8	65.2±6.4	42.1±7.2	<0.001
12	72.4±4.2	55.0±5.8	32.5±6.5	<0.001
24	58.2±5.5	38.0±5.2	15.2±4.8	<0.001
48	42.0±4.8	22.4±4.5	5.2±2.8	<0.001

**Fig 3:** Area plot showing intracellular survival rates of clinical, equine, and soil rhodococcus equi strains within J774A.1 macrophages over 48 hours

The intracellular survival data (Table 2, Figure 3) reveal a clear hierarchy of macrophage persistence capacity: clinical strains maintained 42.0% viability at 48 hours, compared to

22.4% for equine and only 5.2% for soil strains. The divergence between source categories became apparent as early as 4 hours post-infection and progressively widened

throughout the observation period, with statistically significant differences at every time point from 4 hours onward (all $p < 0.01$). The radar plot analysis (Figure 1) demonstrates that while clinical isolates dominate in pathogenesis-critical parameters (VapA expression, intracellular survival, cholesterol oxidase), soil isolates exhibit superiority in environmental persistence traits (biofilm capacity, iron acquisition), suggesting ecological niche-specific adaptation of virulence factor expression profiles.

Discussion

This research established a comprehensive quantitative virulence hierarchy among *R. equi* isolates from four ecological sources, demonstrating that clinical human isolates possess the highest intracellular survival capacity, VapA expression levels, and cholesterol oxidase activity, while environmental soil isolates excel in biofilm formation and iron acquisition - traits associated with environmental persistence rather than host pathogenesis.

The superior intracellular survival of clinical strains (42.0% at 48h vs. 5.2% for soil strains) aligns with the findings of von Bargen and Haas, who reported similar source-dependent differences in macrophage survival using European *R. equi* isolates. However, the magnitude of difference observed in the current research exceeds previously reported values, possibly reflecting the selection of particularly virulent clinical isolates from severe human infections. Conversely, Letek and colleagues documented relatively modest differences in intracellular survival between virulent and avirulent *R. equi* strains, suggesting that experimental conditions and strain selection significantly influence comparative virulence assessments.

The finding that soil isolates demonstrate superior biofilm capacity and iron acquisition despite reduced intracellular survival provides mechanistic insight into the ecological partitioning of virulence traits. In the soil environment, *R. equi* must compete with a diverse microbial community for limited nutrients, and biofilm formation and efficient iron scavenging confer competitive advantages independent of host interaction capacity. The acquisition of the virulence plasmid carrying *vapA* during transmission through equine hosts may then supplement these environmental fitness traits with the intracellular survival machinery necessary for host pathogenesis, creating the complete virulence complement observed in clinical isolates.

Macrophage subversion mechanisms

The capacity of *R. equi* to survive and replicate within macrophages depends on the coordinated action of multiple virulence effectors that disrupt normal phagosomal maturation. VapA interferes with the recruitment of Rab7 GTPase to the phagosomal membrane, preventing the acquisition of late endosomal markers and subsequent lysosomal fusion. Cholesterol oxidase modifies membrane cholesterol, altering lipid raft organization and disrupting signaling pathways essential for antimicrobial responses. The combined effect creates a modified phagosomal compartment that fails to acidify below pH 6.0 and lacks the hydrolytic enzymes necessary for bacterial killing, providing a replicative niche analogous to the mycobacterial phagosome.

Evolutionary perspectives on virulence acquisition

The distribution of virulence determinants across *R. equi* ecological subtypes suggests a model of progressive virulence acquisition associated with host adaptation. The core *R. equi*

chromosome encodes fundamental survival traits including cholesterol metabolism, iron acquisition, and stress response systems that function in both environmental and host-associated contexts. The virulence plasmid, acquired through conjugative transfer in environments where equine manure creates high *R. equi* densities, adds the specialized intracellular survival machinery required for host pathogenesis. This modular virulence architecture enables rapid phenotypic transitions between environmental and pathogenic lifestyles within a genetically conserved bacterial framework.

The identification of distinct virulence signatures associated with each ecological source category provides a conceptual framework for understanding how environmental *R. equi* populations transition to pathogenic states during host colonization events. The environmental reservoir, dominated by biofilm-proficient and iron-acquisition-competent strains, maintains a diverse *R. equi* population from which virulence plasmid acquisition selects for lineages with enhanced intracellular survival capacity. This selection process, operating primarily within equine hosts where the combination of high environmental exposure and respiratory tract susceptibility creates optimal conditions for *R. equi* amplification, generates the highly virulent clinical isolate population observed in this and previous investigations. The translational implication of this model is that interventions targeting the environmental amplification cycle, particularly reduction of virulent *R. equi* concentrations in equine breeding environments, could simultaneously reduce both veterinary disease burden and zoonotic transmission risk to immunocompromised human populations.

Conclusion

This research aimed to systematically characterize and compare virulence determinants across *R. equi* isolates from clinical, equine, swine, and soil sources. The investigation identified a clear quantitative virulence hierarchy with clinical isolates demonstrating the highest intracellular survival (42.0% at 48h), VapA expression (8.5-fold), and cholesterol oxidase activity, while soil isolates exhibited superior biofilm capacity and iron acquisition reflecting environmental adaptation. These findings carry practical significance for risk assessment of environmental *R. equi* exposure, demonstrating that not all environmental isolates possess equivalent pathogenic potential and that virulence profiling could inform targeted prevention strategies for immunocompromised populations. Future research should investigate the molecular triggers governing virulence gene expression switching during environmental-to-host transitions, evaluate the stability of virulence phenotypes across serial passage through different ecological niches, and explore the potential for virulence factor-targeted therapeutic interventions.

Acknowledgements

The National Institute of Microbiology, Berlin, provided comprehensive laboratory facilities, biosafety level 2 infrastructure, and access to the national *R. equi* culture collection essential for this investigation. The contributions of Dr. Stefan Braun in macrophage culture maintenance and Ms.

Anna Klein in statistical analysis are sincerely appreciated. This research was funded by the German Research Foundation.

References

1. Magnusson H. Spezifische infektiöse Pneumonie beim Fohlen. Arch Wiss Prakt Tierheilkd. 1923;50:22-38.
2. Von Bargen K, Haas A. Molecular and infection biology of the horse pathogen *Rhodococcus equi*. FEMS Microbiol Rev. 2009;33(5):870-891.
3. Hondalus MK, Mosser DM. Survival and replication of *Rhodococcus equi* in macrophages. Infect Immun. 1994;62(11):4167-4175.
4. Jain S, Bloom BR, Hondalus MK. Deletion of vapA, encoding virulence-associated protein A, attenuates the intracellular actinomycete *Rhodococcus equi*. Mol Microbiol. 2003;50(1):115-128.
5. Tan C, Prescott JF, Patterson MC. Molecular characterization of a lipid-modified virulence-associated protein of *Rhodococcus equi*. Can J Vet Res. 1995;59(1):51-59.
6. Pal M, Gutama KP, Singh S, Botton S. *Rhodococcus equi*: an emerging bacterial pathogen of public health significance. J Adv Microbiol Res. 2023;4(1):32-35.
7. Toyooka K, Takai S, Kirikae T, *et al.* *Rhodococcus equi* can survive a phagolysosomal environment in macrophages by suppressing acidification of the phagolysosome. J Med Microbiol. 2005;54(11):1007-1015.
8. Fernandez-Mora E, Polidori M, Lührmann A, Von Bargen K, Haas A. Maturation of *Rhodococcus equi*-containing vacuoles is arrested after completion of the early endosome stage. Traffic. 2005;6(7):635-653.
9. Sydor T, Von Bargen K, Hsu FF, Zeth K, Zeez M, Hoelzle K, Novak N, Haas A. Diversion of phagosome trafficking by pathogenic *Rhodococcus equi* depends on mycolic acid chain length. Cell Microbiol. 2013;15(3):458-473.
10. Meijer WG, Prescott JF. *Rhodococcus equi*. Vet Res. 2004;35(4):383-396.
11. Prescott JF, Meijer WG, Vázquez-Boland JA. *Rhodococcus*. In: Bergey's Manual of Systematics of Archaea and Bacteria. 2015:1-17.
12. Kakuda T, Hirota T, Takeuchi T, *et al.* VapA protein of *Rhodococcus equi* is an antigen for T-cell-mediated immune responses. Infect Immun. 2005;73(7):4736-4742.
13. Miranda-CasoLuengo R, Duffy PS, O'Connell EP, *et al.* The iron-regulated iupABC operon of *Rhodococcus equi*: transcriptional regulation and characterization. J Bacteriol. 2005;187(10):3522-3529.
14. Letek M, Ocampo-Sosa AA, Sanders M, *et al.* Evolution of the *Rhodococcus equi* vap pathogenicity island. PLoS Genet. 2010;6(9): e1001145.
15. Coulson GB, Miranda-CasoLuengo AA, Miranda-CasoLuengo R, *et al.* Transcriptome reprogramming by plasmid-encoded transcriptional regulators is required for host niche adaptation in *Rhodococcus equi*. mBio. 2015;6(2): e02462-14.
16. Arnaiz-García ME, Arnaiz J, Eiros JM. *Rhodococcus equi* infection in solid organ and stem cell transplant recipients. Transpl Infect Dis. 2014;16(3):336-350.
17. Giguère S, Cohen ND, Chaffin MK, Hines SA, Hondalus MK, Prescott JF, Slovis NM. *Rhodococcus equi*: clinical manifestations, virulence, and immunity. J Vet Intern Med. 2011;25(6):1221-1230.

How to Cite This Article

Weber F. Virulence determinants and pathogenesis mechanisms of *rhodococcus equi* in clinical and environmental isolates. International Journal of Veterinary Sciences and Animal Husbandry. 2026; 11(3): 357-361.

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