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RESEARCH ARTICLE

A 40-Year Retrospective Analysis of *Mannheimia haemolytica*, *Pasteurella multocida* and *Bibersteinia trehalosi* Cases from South Africa: Insights into Distribution and Serotype Diversity

Itumeleng Matle[1*], Skhumbuzo Mbizeni[1], Emmanuel Moloko Seakamela[2], Davies Mubika Pfukenyi[3] and Mohamed Mahmoud Sirdar[4,5]

[1]Department of Agriculture and Animal Health, University of South Africa, Pretoria, South Africa; [2]Bacteriology Division, Agricultural Research Council: Onderstepoort Veterinary Research, Onderstepoort, Pretoria, South Africa; [3]Department of Veterinary Sciences, Faculty of Animal and Veterinary Sciences, Botswana University of Agriculture and Natural Resources; [4]International Society for Infectious Diseases (ISID), Boston, United States of America; [5]Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa
*Corresponding author: ematlei@unisa.ac.za

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ABSTRACT

Respiratory diseases caused by *Mannheimia haemolytica*, *Pasteurella multocida* and *Bibersteinia trehalosi* cause substantial economic losses in livestock. This study investigated the long-term epidemiological trends of *Pasteurellaceae* based on diagnostic laboratory reports submitted to the Agricultural Research Council–Onderstepoort Veterinary Research diagnostic laboratory in South Africa between 1969 and 2018. The study aimed to characterize the temporal distribution of *Pasteurellaceae* species from 1969 to 2018 and describe long-term patterns in species diversity and dominance. A total of 2,317 bacterial laboratory reports were retrospectively analyzed. *M. haemolytica* was the predominant species (57.0%), followed by *P. multocida* (27.1%) and *B. trehalosi* (13.3%). Most laboratory reports originated from sheep and cattle, which together accounted for more than two-thirds of submissions. Host-specific analyses demonstrated marked differences in bacterial distribution, with *M. haemolytica* predominating in ruminants, whereas *P. multocida* was more frequently recorded among pigs, companion animals and laboratory animals. Although temporal variation was statistically significant, year of isolation was not independently associated with bacterial species after multivariable analysis. Serotyping revealed considerable diversity, with *M. haemolytica* serotypes A1, A2, A6, A7 and A9 predominating, while serogroup A was the most common among typed *P. multocida*. This study provides one of the longest retrospective assessments of *Pasteurellaceae* in South Africa, demonstrating persistent host-associated patterns, geographic variation and considerable recorded serotype diversity over four decades. These findings support continued host-focused surveillance, improved serotyping and molecular characterization, and demonstrate the value of historical diagnostic records as a baseline for future surveillance.

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INTRODUCTION

The family *Pasteurellaceae* comprises a phylogenetically diverse group of Gram-negative bacteria that commonly colonize mucosal surfaces of vertebrate hosts, particularly the respiratory tract (Harper *et al*., 2006; Crosby and Woolums, 2022). Although many members may occur as commensals, disruption of host-microbe equilibrium by stress, environmental factors or co-infections can contribute to disease (Harper *et al*., 2006; Brooks *et al*., 2020). Among the clinically important members are *Mannheimia haemolytica, Pasteurella multocida,* and *Bibersteinia trehalosi,* which are associated with respiratory and systemic diseases in livestock and poultry and contribute to substantial economic losses through mortality, reduced productivity, treatment costs, and carcass condemnation (Griffin *et al*., 2010; Boulianne, *et al*., 2020; Jilo *et al*., 2020; Abera and Mossie, 2023).

Their epidemiological importance is further influenced by considerable antigenic and serotypic diversity, which varies among host species and disease syndromes.

P. multocida is classically categorized into five capsular serogroups (A, B, D, E and F) based on capsular polysaccharides and further differentiated into 16 somatic (lipopolysaccharide) serotypes according to the Heddleston scheme (Heddleston, 1962; Brogden and Packer, 1979). These capsular groups are strongly associated with distinct disease syndromes across host species, including fowl cholera (serogroups A and F), hemorrhagic septicemia (serogroups B and E), pneumonic pasteurellosis (serogroup A) and atrophic rhinitis (serogroup D), reflecting functional variation in pathogenic potential (Chung *et al*., 2001; Wilkie *et al*., 2012).

Historically, *P. haemolytica* was divided into two major biotypes based on carbohydrate fermentation patterns: biotype A, which fermented arabinose, and biotype T, which fermented trehalose (Angen *et al*., 2002). The *P. haemolytica* complex was further differentiated into 17 capsular serotypes using the indirect hemagglutination test. These included 13 A serotypes: A1, A2, A5, A6, A7, A8, A9, A11, A12, A13, A14, A16 and A17 and four T serotypes: 3, 4, 10 and 15 (Getnet *et al*., 2022). Subsequent phenotypic, serological and molecular studies demonstrated that the former *P. haemolytica* complex represented taxonomically distinct organisms. As a result, biotype A strains were reclassified under the genus *Mannheimia*, with the principal species renamed *M. haemolytica*, while biotype T strains were reassigned to *B. trehalosi*. Further taxonomic refinement also showed that serotype A11 was sufficiently distinct from *M. haemolytica* and is now recognized as *M. glucosida* (Mohamed and Abdelsalam, 2008; Sarah, 2011). Consequently, *M. haemolytica* is currently considered to comprise 12 recognized capsular serotypes: A1, A2, A5, A6, A7, A8, A9, A12, A13, A14, A16 and A17.

In sub-Saharan Africa, particularly in South Africa, there remains a critical lack of longitudinal data describing the distribution, temporal patterns, and serotype diversity of *Pasteurellaceae*. This gap limits the ability to detect epidemiological shifts, the emergence of dominant or novel strains, and changes in pathogen populations that may be driven by evolving livestock production systems, antimicrobial usage patterns, vaccination practices, and environmental pressures. Such information is essential for strengthening epidemiological understanding, informing evidence-based disease control strategies, and guiding future research aimed at improving diagnostic approaches and characterizing strains relevant to vaccine development. Retrospective analyses of archived diagnostic cases and laboratory reports provide a unique and underutilized opportunity to reconstruct historical epidemiological trends, identify persistent or emerging serotypes and generate baseline data against which future surveillance efforts can be benchmarked.

In this context, the present study directly addresses these knowledge gaps by leveraging the extensive repository of laboratory cases maintained by the Agricultural Research Council – Onderstepoort Veterinary Research (ARC-OVR), spanning four decades of diagnostic submissions. By systematically characterizing the temporal distribution of *Pasteurellaceae* species from ≤1969 to 2018, this study aims to generate robust epidemiological insights into long-term patterns of pathogen diversity and dominance. These findings are expected to improve understanding of the historical distribution and serotype diversity of these pathogens in South Africa and provide a baseline for future diagnostic, surveillance and molecular investigations.

MATERIALS AND METHODS

Data source: This study used laboratory records from the ARC-OVR diagnostic information system. A total of 2,317 *Pasteurellaceae* laboratory reports from routine veterinary diagnostic submissions received from all nine provinces of South Africa and selected international sources between 1969 and 2018 were analyzed. Variables included host and bacterial species, year of isolation, month of submission, season, province of origin, locality, serotype, sample type and, where available, geographic coordinates. To align with current taxonomy, former *P. haemolytica* biotype T serotypes 3, 4, 10 and 15 were reclassified as *B. trehalosi*, while isolates recorded as serotype A11 were classified as *M. glucosida*. These classifications were applied retrospectively across the entire dataset.

Laboratory analysis: Samples were cultured and isolates identified using standard bacteriological methods (Carter, 1984; Sharma and Adlakha, 1996; Quinn *et al*., 1996; Quinn *et al*., 2002). Serotyping was performed by indirect hemagglutination according to established protocols (Biberstein *et al*., 1960; Biberstein and Thompson, 1966). Agglutination >50% at a serum dilution of ≥1:20 was considered positive (Frank and Wessman, 1978). Isolates were tested against all *M. haemolytica* serotypes (1, 2, 5, 6, 7, 8, 9, 11, 12, 13, 14, 16 and 17), *B. trehalosi* serotypes (3, 4, 10 and 15) and *P. multocida* serogroups A-F. Isolates showing no agglutination were classified as untypeable. Positive and negative control sera supplied by ARC-OVR were included throughout testing.

Data analysis: Data were cleaned and standardized, while records were retained irrespective of completeness to preserve the historical dataset. Categories such as "Not mentioned" and "Not typed" were retained where applicable. Descriptive analyses summarize bacterial species by host, geographic origin, year, season, sample type and serotype. Seasons were classified as summer (December-February), autumn (March-May), winter (June-August) and spring (September-November). Geographic origin was reported by provinces for domestic submissions and by country for international submissions. Frequencies and proportions were calculated using Microsoft Excel and IBM SPSS Statistics version 31 (IBM Corp., Armonk, NY, USA) and maps were produced using ArcMap 10.8 (Esri, Redlands, CA, USA).

Comparative analyses focused on *M. haemolytica* and *P. multocida* (n = 1,832). Host species were analyzed using the original laboratory categories and grouped into ruminants, avian, companion/equine, porcine, laboratory animals (rabbit and mice), and other hosts. The year of isolation was grouped into six periods (≤1969, 1970-1979, 1980-1989, 1990-1999, 2000-2009 and 2010-2018). Associations between bacterial species and explanatory

variables were evaluated using Pearson's χ^2 test, with sparse categories combined where necessary. Effect size was measured using Cramér's V.

A multivariable binary logistic regression model was fitted to determine whether host group, province and year of isolation were independently associated with bacterial species. *M. haemolytica* was coded as 1 and *P. multocida* as 0. Laboratory animals and Gauteng were used as the reference categories for host groups and province, respectively, while year of isolation was modelled continuously. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were estimated. Model fit was assessed using the Hosmer-Lemeshow goodness-of-fit test and discrimination using the area under the receiver operating characteristic curve. Statistical significance was defined as $P<0.05$.

Serotype distributions were summarized descriptively by bacterial species and host group. Geographic patterns were visualized using available coordinates and interpreted as the distribution of diagnostic submissions rather than estimates of population-level disease occurrence.

Ethical statement: Farmer's and owner's identities were kept anonymous. Diagnostic laboratories are authorized to publish data for epidemiological purposes for the benefit of the country and the world.

RESULTS

Descriptive epidemiology of laboratory records: A total of 2,317 laboratory reports were included in the descriptive analyses, representing submissions collected between ≤1969 to 2018 from a wide range of host species, geographic locations, sample types, and serotypes (Table 1). Most reports were identified as *M. haemolytica* (1320/2317; 57.0%), followed by *P. multocida* (627/2317; 27.1%) and *B. trehalosi* (309/2317; 13.3%). Less frequently identified organisms included *M. glucosida* (36/2317; 1.6%), while all other *Pasteurella* species collectively accounted for fewer than 1% of reports.

The distribution of reports across host species was markedly skewed toward ruminant hosts. Ovine submissions accounted for the largest proportion of reports (959/2317; 41.4%), followed by bovine (610/2317; 26.3%), porcine (161/2317; 6.9%), and chicken (150/2317; 6.5%) submissions. Other host species were less frequently represented, each accounting for less than 5% of the total reports, including rabbits (98/2,317; 4.2%), avian species (70/2,317; 3.0%), caprine (61/2,317; 2.6%), canine (54/2,317; 2.3%) and feline (51/2,317; 2.2%) hosts. Overall, the predominance of ovine and bovine submissions indicates that ruminant production systems were principal source of reports in this dataset.

Geographic distribution was uneven, with a clear concentration of submissions originating from Gauteng and Eastern Cape provinces. Gauteng accounted for nearly half of all submissions (1099/2317; 47.4%), followed by Eastern Cape (509/2317; 22.0%) and Free State (135/2317; 5.8%). International submissions represented 5.5% (127/2317) of the dataset, while smaller contributions (<5% each) were received from the remaining provinces (Table 1). The temporal distribution of reports spanned the period from ≤1969 –2018, reflecting the long-term archival and surveillance value of the dataset. Seasonal distribution of submissions was broadly uniform across the four South African seasons, with similar proportions recorded in spring (September-November; 591/2317; 25.5%), winter (June-August; 590/2317; 25.5%), autumn (March-May; 573/2317; 24.7%), and summer (December-February; 563/2317; 24.3%) (Table 1).

Table 1: Descriptive epidemiology of bacterial laboratory reports recorded between ≤1969 and 2018 (*n*=2317) [**]

Variable	Category	*n* (%)
Bacterial species	*Mannheimia haemolytica*	1320 (57.0)
	Pasteurella multocida	627 (27.1)
	Bibersteinia trehalosi	309 (13.3)
	M. glucosida	36 (1.6)
	Other *Pasteurella* spp.	25 (1.1)
Host species	Ovine	959 (41.4)
	Bovine	610 (26.3)
	Porcine	161 (6.9)
	Chicken	150 (6.5)
	Rabbit	98 (4.2)
	Avian	70 (3.0)
	Caprine	61 (2.6)
	Canine	54 (2.3)
	Feline	51 (2.2)
	Other hosts	103 (4.4)
Province of origin	Gauteng	1099 (47.4)
	Eastern Cape	509 (22.0)
	Free State	135 (5.8)
	International	127 (5.5)
	Western Cape	94 (4.1)
	North-West	82 (3.5)
	Mpumalanga	78 (3.4)
	Northern Cape	66 (2.8)
	Limpopo	65 (2.8)
	KwaZulu-Natal	62 (2.7)
Season of submission	Spring (September-November)	591 (25.5)
	Winter (June-August)	590 (25.5)
	Autumn (March-May)	573 (24.7)
	Summer (December-February)	563 (24.3)
Sample type	Not mentioned	1019 (44.0)
	Lung	992 (42.8)
	Lung and liver	60 (2.6)
	Liver	42 (1.8)
	Milk	35 (1.5)
	Brain	33 (1.4)
	Abscess	24 (1.0)
	Organs	18 (0.8)
	Nasal swab culture	17 (0.7)
	Other sample types	77 (3.3)

[**]Values are presented as frequencies and proportions. Percentages are calculated using the total number of laboratory reports (*n* = 2317). "Other *Pasteurella* spp." includes infrequently identified *Pasteurella* taxa each contributing <0.5% of the dataset. "Other hosts" includes host categories individually contributing <2% of total submissions.

Sample type distribution was heterogeneous, although respiratory-derived samples predominated. Lung tissue represented the most frequently recorded sample type (992/2317; 42.8%), followed closely by records in which sample type was not specified (1019/2317; 44.0%). Less frequent sample types (< 3% for each) included lung and liver (60/2317; 2.6%), liver (42/2317; 1.8%), milk (35/2317; 1.5%), brain (33/2317; 1.4%), abscesses (24/2317; 1.0%), and organs (18/2317; 0.8%) (Table 1).

Serotype diversity was broad; however, a substantial proportion of laboratory reports were recorded as untyped, indicating incomplete serological characterization across the dataset. Among typed reports, several serotypes were recurrently represented, suggesting persistent circulation of

specific serotype groups within host populations. The coexistence of typed and untyped reports likely reflects historical variation in diagnostic laboratory practices, including changes in serotyping capacity, availability and coverage over time.

Epidemiological patterns and predictors of *M. haemolytica* and *P. multocida*: In the preceding section, the overall laboratory report collection was described to provide a broad epidemiological overview of bacterial reports across host species, geographic regions, sample types and time. From this section onward, the analysis focuses specifically on *M. haemolytica* and *P. multocida*, which represented the two most epidemiologically significant bacterial species in the dataset and together accounted for most reports submitted for analysis. A total of 1,832 reports were included in the species-specific analysis, of which 1,239 (67.6%) were *M. haemolytica* and 593 (32.4%) were *P. multocida* (Table 2).

Table 2: Distribution of *M. haemolytica* and *P. multocida* by host species

Host species	Total (*n*)	*M. haemolytica n* (%)	*P. multocida n* (%)
Ovine	779	658 (84.5)	121 (15.5)
Bovine	444	293 (66.0)	151 (34.0)
Porcine	141	42 (29.8)	99 (70.2)
Chicken	102	80 (78.4)	22 (21.6)
Laboratory animals*	97	22 (22.7)	75 (77.3)
Canine	51	15 (29.4)	36 (70.6)
Caprine	46	42 (91.3)	4 (8.7)
Feline	46	5 (10.9)	41 (89.1)
Avian	43	38 (88.4)	5 (11.6)
Vaccine	33	18 (54.5)	15 (45.5)
Buffalo	16	3 (18.8)	13 (81.2)
Ostrich	13	10 (76.9)	3 (23.1)
Wildlife	8	4 (50.0)	4 (50.0)
Equine	8	6 (75.0)	2 (25.0)
Unknown	3	2 (66.7)	1 (33.3)
Dolphin sow	1	1 (100)	0 (0.0)
Total	1832	1239 (67.6)	(32.4)

*Laboratory animals include rabbits and mice. Geographic distribution of the diagnostic submissions differed between the two bacterial species (χ^2 (8) =124.38, P<0.001; Cramér's V=0.261) (Table 3). Gauteng contributed 45.5% (564/1,239) of *M. haemolytica* and 69.1% (410/593) of *P. multocida* records, while Eastern Cape contributed 27.7% (343/1,239) and 9.1% (54/593), respectively. These findings describe geographic patterns among diagnostic submissions and should not be interpreted as estimates of provincial disease prevalence.

Table 3: Distribution of *M. haemolytica* and *P. multocida* by province

Province	Total (*n*)	*M. haemolytica* n (%)	*P. multocida* n (%)
Gauteng	974	564(45.5)	410(69.1)
Eastern Cape	397	343(27.7)	54(9.1)
Free State	115	93(7.5)	22(3.7)
Western Cape	78	54(4.4)	24(4.0)
North-West	75	54(4.4)	21(3.5)
Mpumalanga	67	50(4.0)	17(2.9)
Limpopo	59	41(3.3)	18(3.0)
KwaZulu-Natal	42	29(2.3)	13(2.2)
Northern Cape	25	11(0.9)	14(2.4)
Total	1832	1239(100.0)	593(100.0)

χ^2(8) = 124.38, P<0.001; Cramér's V=0.261. Percentage calculated using all *M. haemolytica* (n=1239). Percentage calculated using all *P. multocida* (n=593).

The distribution of bacterial species reports differed markedly among host categories. After hosts were combined into biologically meaningful groups, the association between host group and bacterial species was significant (χ^2(5) = 336.48, P<0.001; Cramér's V = 0.428). *M. haemolytica* predominated among ruminant (996/1,285; 77.5%) and avian submissions (128/158; 81.0%), whereas *P. multocida* predominated among companion/equine (80/106; 75.5%), porcine (99/141; 70.2%) and laboratory-animal submissions (75/97; 77.3%) (Fig. 1). Host category therefore showed the strongest association with bacterial species among the factors examined.

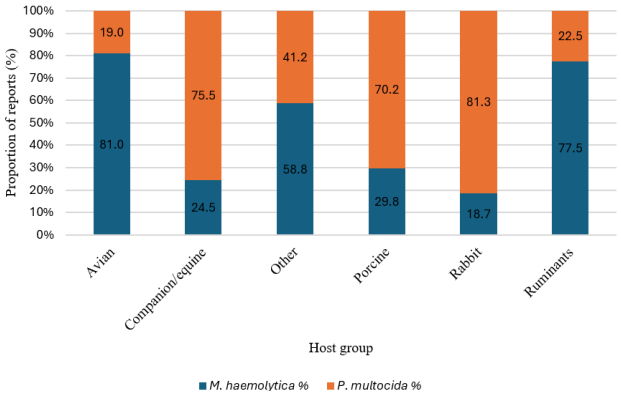


Fig. 1: Proportional distribution of *M. haemolytica* and *P. multocida* across grouped host categories.

Bacterial species distribution varied across grouped periods of isolation (χ^2(5) = 37.55, P<0.001; Cramér's V = 0.143) (Table 4). The largest contribution of *M. haemolytica* occurred during 1990-1999 (441/1,239; 35.6%), whereas *P. multocida* was more evenly distributed across the study periods. The relatively small Cramér's V indicated that temporal differences were weaker than host-associated and geographic differences.

Table 4: Temporal distribution of *M. haemolytica* and *P. multocida*

Year group	Total (n)	*M. haemolytica* n(%)	*P. multocida* n(%)
≤1969	96	62(5.0)	34(5.7)
1970–1979	181	112(9.0)	69(11.6)
1980–1989	364	233(18.8)	131(22.1)
1990–1999	570	441(35.6)	129(21.8)
2000–2009	282	178(14.4)	104(17.5)
2010–2018	339	213(17.2)	126(21.2)
Total	1832	1239(100.0)	593(100.0)

χ^2(5) = 37.55, P<0.001; Cramér's V = 0.143. Percentage calculated using all *M. haemolytica* reports (n=1239). Percentage calculated using all *P. multocida* reports (n=593).

Serotyping demonstrated considerable diversity within both bacterial species (Table 5). Among *M. haemolytica* records, 542/1,239 (43.7%) were untyped; the most frequently recorded typed serotypes were A1 (10.3%), A2 (8.8%), A6 (7.3%), A9 (5.6%) and A7 (4.7%). Among *P. multocida* records, 320/593 (54.0%) were untyped; serogroup A was the most frequently identified typed category (26.8%), followed by D (8.8%), B (7.1%) and E (1.2%). The high proportion of untyped records limits interpretation of the complete serotype distribution.

A multivariable binary logistic regression model was used to identify factors independently associated with bacterial species (Table 6). Host group remained the strongest associated factor after adjustment for province and year of isolation. Compared with laboratory animals, ruminant submissions had 12.29-fold higher adjusted odds of *M. haemolytica* rather than *P. multocida* (95% CI 7.34-20.56; P<0.001), while avian submissions had 18.46-fold higher odds (95% CI 9.65-35.29; P<0.001). Porcine submissions also had higher odds (OR 1.99; 95% CI 1.07-

3.69; P=0.030), whereas the association for companion/equine submissions was not significant (OR 1.25; 95% CI 0.63-2.45; P=0.525).

Table 5: Serotype distribution of *M. haemolytica* and *P. multocida*

Serotype	*M. haemolytica n*(%)	*P. multocida n*(%)
Not Typed	542(43.7)	320(54.0)
A1	128(10.3)	-
A2	109(8.8)	-
A6	91(7.3)	-
A9	70(5.6)	-
A7	58(4.7)	-
A	-	159(26.8)
D	-	52(8.8)
B	-	42(7.1)
E	-	7(1.2)

Table 6: Multivariable logistic regression model for factors associated with *M. haemolytica* relative to *P. multocida*

Predictor	Adjusted OR	95% CI	P-value
Host group (overall)			<0.001
Laboratory animals	Reference	-	-
Ruminants	12.29	7.34-20.56	<0.001
Avian	18.46	9.65-35.29	<0.001
Companion/equine	1.25	0.63-2.45	0.525
Porcine	1.99	1.07-3.69	0.030
Other hosts	6.70	3.03-14.83	<0.001
Province (overall)			<0.001
Gauteng	Reference	-	-
Eastern Cape	3.57	2.54-5.02	<0.001
Free State	3.04	1.74-5.31	<0.001
Mpumalanga	2.05	1.08-3.90	0.028
Western Cape	1.57	0.81-3.08	0.184
Northwest	0.93	0.54-1.62	0.804
Limpopo	0.95	0.51-1.76	0.870
KwaZulu-Natal	1.03	0.50-2.12	0.927
Northern Cape	0.86	0.49-1.52	0.600
Year of isolation	0.995	0.984-1.005	0.306

Outcome: *M. haemolytica* = 1 and *P. multocida* = 0. OR, adjusted odds ratio; CI, confidence interval. Reference categories were laboratory animals for host group and Gauteng for province.

Province remained associated with bacterial species after adjustment, with higher odds of *M. haemolytica* for Eastern Cape (OR 3.57; 95% CI 2.54-5.02; P<0.001), Free State (OR 3.04; 95% CI 1.74-5.31; P<0.001) and Mpumalanga (OR 2.05; 95% CI 1.08-3.90; P=0.028) compared with Gauteng. Years of isolation were not independently associated with bacterial species (OR 0.995 per year; 95% CI 0.984-1.005; P=0.306).

Serotype and geographic patterns: Descriptive examination of serotypes by grouped host category showed that the most frequently recorded *M. haemolytica* serotypes occurred predominantly among ruminant submissions, reflecting the large contribution of ruminants to the diagnostic dataset (Supplementary Table S1). *P. multocida* serogroup A occurred across several host groups, including ruminant, porcine, companion/equine and laboratory-animal submissions (Supplementary Table S2). Given the substantial proportion of untyped records, these patterns were interpreted descriptively.

Geographic mapping illustrated differences in the locations from which diagnostic submissions originated (Fig. 2; Fig. 3). The maps are presented to describe the geographic distribution of recorded submissions and serotypes and are not intended to represent disease prevalence, spatial risk or transmission patterns.

Supplementary Table S1: Serotype distribution across grouped host categories among *M. haemolytica*

Host group	Not Typed	A1	A2	A6	A7	A9	Other serotypes	Total
Ruminants	401	121	103	87	54	66	164	996
Avian	51	2	1	2	1	3	68	128
Companion/equine	8	1	1	0	1	0	15	26
Porcine	21	1	2	1	0	1	17	43
Laboratory animals	12	1	1	0	0	0	8	22
Other	49	2	1	1	2	0	20	24
Total	542	128	109	91	58	70	292	1239

Supplementary Table S2: Serotype distribution across grouped host categories among *P. multocida*

Host group	Not Typed	A	B	D	E	Other serotypes	Total
Ruminants	147	88	21	24	3	6	289
Avian	17	8	1	3	0	1	30
Companion/equine	41	28	4	6	0	1	80
Porcine	45	34	7	11	0	2	99
Laboratory animals	48	30	4	2	1	0	85
Other	22	11	5	6	3	3	10
Total	320	159	42	52	7	13	593

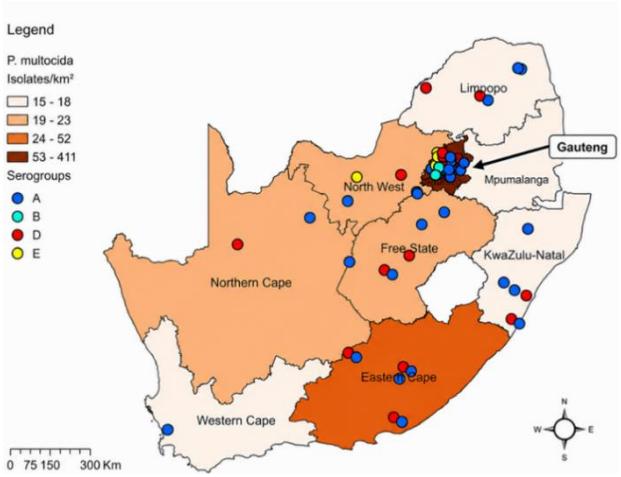


Fig. 2: Geographic distribution of *P. multocida* serogroups among diagnostic submissions in South Africa.

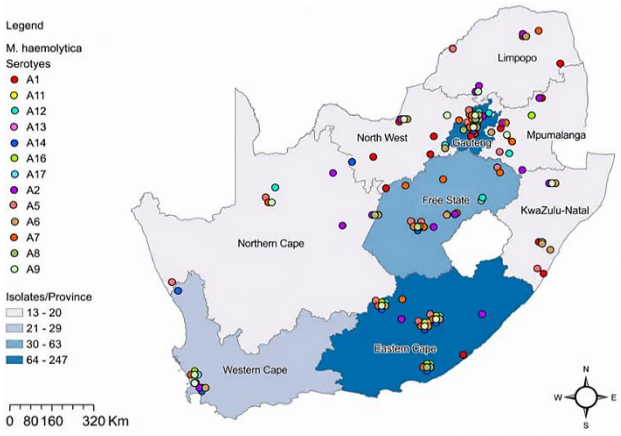


Fig. 3: Geographic distribution of *M. haemolytica* serotypes among diagnostic submissions in South Africa.

DISCUSSION

This study is among the most comprehensive long-term, laboratory-based epidemiological assessments of *Pasteurellaceae* reports conducted in South Africa. The analyses of historic diagnostic cases submitted to the ARC–OVR demonstrate the enduring value of historical

veterinary laboratory records for understanding long-term pathogen distribution, host associations, geographical variation, and serotype diversity. Increasingly, retrospective diagnostic datasets are recognized as valuable resources for syndromic surveillance, outbreak detection, and the reconstruction of long-term disease trends (Dórea *et al*., 2013; McCluskey, 2021; Shapiro *et al*., 2025). Their interpretation requires consideration of inherent limitations such as submission bias, changing diagnostic practices, laboratory methodologies, and differential access to veterinary services.

The predominance of *M. haemolytica* throughout the study confirms its longstanding importance as the principal *Pasteurellaceae* pathogen affecting domestic ruminants in South Africa (Schneider *et al*., 1979; Van Amstel *et al*., 1987; Odendaal and Henton, 1995). Representing 57.0% of all laboratory reports, *M. haemolytica* remained consistently more common than *P. multocida* and *B. trehalosi*, reflecting its recognized role in pneumonic pasteurellosis and the bovine respiratory disease complex (Rice *et al*., 2007; Confer, 2009). The continued predominance of *M. haemolytica* over four decades suggests remarkable epidemiological stability despite substantial changes in livestock production, veterinary practices and diagnostic technologies. Although *P. multocida* and *B. trehalosi* were recovered less frequently, their continued detection indicates that respiratory disease in South African livestock involves multiple *Pasteurellaceae* members rather than a single dominant pathogen. *P. multocida* remains an important opportunistic pathogen with a broad host range, whereas *B. trehalosi* continues to play an important role in respiratory and systemic disease in sheep (Harper *et al*., 2006; Wilson and Ho, 2013; Guo *et al*., 2024). These findings emphasize the importance of considering the entire Pasteurellaceae complex during diagnostic investigations of respiratory disease.

One of the clearest findings was the strong association between host category and bacterial species within the diagnostic submission dataset. *M. haemolytica* was predominantly reported by ruminant and avian hosts, whereas *P. multocida* was more frequently recorded among porcine, companion/equine and laboratory-animal submissions. These patterns are consistent with the recognized host associations of these organisms (Rice *et al*., 2007; Abbas *et al*., 2023; Tawor *et al*., 2024). The multivariable analysis showed that host category remained strongly associated with bacterial species after adjustment for province and year; however, the retrospective submission-based design does not permit inference regarding population-level risk or causality.

The predominance of ovine and bovine submissions also reflects the epidemiology of respiratory disease in South African livestock production systems. Pasteurellaceae commonly colonize the upper respiratory tract of healthy animals but become opportunistic pathogens following transportation, overcrowding, nutritional stress, adverse climatic conditions or concurrent viral infections (Confer, 2009; Griffin *et al*., 2010). Consequently, respiratory disease remains one of the leading causes of production losses in cattle and sheep worldwide (Ackermann *et al*., 2010; Taylor *et al*., 2010; O'Donoghue *et al*., 2025). The large number of submissions from these species therefore likely reflects both the economic importance of ruminant production and the historical role of ARC–OVR as the national reference laboratory for bacterial diseases of livestock.

Geographic variation was observed among diagnostic submissions, with Gauteng contributing the largest number of records. This pattern is likely influenced by the location of ARC-OVR within Gauteng, referral pathways and differential access to veterinary diagnostic services. Province remained associated with bacterial species after adjustment for host and year, but this finding should be interpreted cautiously because the dataset lacks population denominators and is affected by submission and referral bias. The geographic results therefore describe the origins of submitted diagnostic records rather than provincial prevalence or disease risk.

Although bacterial species distribution differed across grouped time periods, year of isolation was not independently associated with bacterial species after adjustment for host category and province. This indicates that the unadjusted temporal pattern was largely accounted for by differences in the measured host and geographic composition of submissions. The retrospective dataset does not allow determination of whether remaining temporal variation resulted from changes in animal populations, diagnostic practices, referral patterns or other unmeasured factors.

The predominance of lung tissue among submitted specimens further supports the central role of *Pasteurellaceae* in respiratory disease. Lung samples represented the largest proportion of all submissions, consistent with the recognized involvement of *M. haemolytica*, *P. multocida*, and *B. trehalosi* in pneumonia and respiratory disease complexes affecting domestic ruminants (Dabo *et al*., 2007; Rice *et al*., 2007). Together with the predominance of ruminant hosts, these findings indicate that a substantial proportion of the reports may have originated from clinically significant respiratory disease cases rather than incidental colonization. Nevertheless, the large proportion of records lacking sample-type information illustrates a challenge associated with historical laboratory databases and highlights the importance of improving metadata quality within veterinary surveillance systems.

Serotyping demonstrated considerable diversity among both bacterial species. The frequent recording of *M. haemolytica* serotypes A1, A2 and A6 is consistent with previous reports of their occurrence in bovine and ovine respiratory disease (Rice *et al*., 2007; Confer, 2009; Mason *et al*., 2022; Sahay *et al*., 2024; Wang *et al*., 2025), while *P. multocida* serogroup A was the most frequently identified typed category. However, 43.7% of *M. haemolytica* and 54.0% of *P. multocida* records were untyped, substantially limiting interpretation of complete serotype patterns. The findings therefore primarily provide a historical description of recorded serotypes and highlight the value of more complete contemporary molecular characterization.

A principal strength of this study is the long temporal span and size of the historical diagnostic dataset, which provides a baseline description of Pasteurellaceae submissions over four decades. Nevertheless, the retrospective design has important limitations, including

referral and submission bias, incomplete metadata, changes in diagnostic methods over time, the high proportion of untyped records and the absence of molecular confirmation for historical records. The dataset is therefore not representative of the national livestock population, and the findings should be interpreted as patterns among diagnostic submissions rather than estimates of national prevalence, incidence or causal epidemiological relationships. Future systematic surveillance incorporating standardized metadata and contemporary molecular typing would allow these historical patterns to be evaluated more rigorously.

Conclusion: This 40-year retrospective study provides a historical description of *M. haemolytica, P. multocida* and *B. trehalosi* recorded through the ARC-OVR diagnostic laboratory. Host category showed the strongest association with bacterial species within the submitted records, while additional geographic variation was observed and year of isolation was not independently associated with bacterial species after adjustment. These findings should be interpreted within the diagnostic submission-based nature of the dataset and do not represent national disease prevalence. The observed serotype diversity, together with the high proportion of untyped records, highlights the value of continued laboratory surveillance and improved molecular characterization. The dataset provides a historical baseline against which future systematic and representative surveillance can be compared.

Authors Contribution: IM, SM, EMS conceptualized, conceived, and designed the study; IM, EMS executed the laboratory analysis; MMS, DMP analyzed the data; All authors interpreted the data, critically revised the manuscript for important intellectual content and approved the final version.

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