ISVEE17
11-15 November 2024 • Sydney, Australia
International Convention Centre Sydney
17th International Symposium on Veterinary Epidemiology and Economics 2024
Veterinary epidemiology and economics down under in leaps and bounds

CityU
香港城市大學
City University of Hong Kong

Accuracy of three diagnostic tests for diagnosis of *Mycobacterium avium* subsp. *paratuberculosis* in domestic ruminants using Bayesian latent class models

Ibrahim Elsohaby[1], Polychronis Kostoulas[2], Mahmoud Fayez[3,4], Juan Carlos Arango-Sabogal[5]

[1] Department of Infectious Diseases and Public Health, Jockey Club of Veterinary Medicine and Life Sciences, City University of Hong Kong, Hong Kong SAR, China

[2] Laboratory of Epidemiology and Artificial Intelligence, Faculty of Public and One Health, School of Health Sciences, University of Thessaly, Karditsa, Greece

[3] Department of Bacteriology, Veterinary Serum and Vaccine Research Institute, Ministry of Agriculture, Cairo, Egypt

[4] Al Ahsa Veterinary Diagnostic Lab, Ministry of Environment, Water and Agriculture, Saudi Arabia

[5] Département de pathologie et microbiologie, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada

INTRODUCTION

- Paratuberculosis, is a chronic wasting disease affecting domestic and wild ruminants globally, caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP).
- The primary route of MAP transmission is faecal-oral.
- Infected animals can shed MAP in faeces and milk for up to two years before clinical signs appear, leading to environmental contamination and persistence.
- MAP infections often go undetected; however, in late stages, symptoms such as intermittent or persistent diarrhea, weight loss, decreased milk production, and mortality may develop.
- Various diagnostic tests exist for detecting MAP, though none possess perfect accuracy as a reference standard, largely due to low sensitivity.
- Commonly used MAP diagnostic tests include direct tests (e.g., faecal smear, culture, PCR) and indirect tests (e.g., ELISA, interferon-γ).
- Assessing the accuracy of diagnostic tests traditionally requires knowledge of the true infection status. However, a definitive gold standard for MAP detection in animals is currently lacking.
- Latent class models (LCMs) offer a valuable approach to estimate sensitivity (Se) and specificity (Sp) of multiple diagnostic tests in the absence of a reference standard.

Objectives:

1. Estimate the Se and Sp of faecal smear (FS), faecal PCR, and serum ELISA for detecting MAP infections in sheep, goats, cattle, and camels in flocks/herds with a previous history of MAP infection using LCM.
2. Estimate the prevalence of MAP infection in each ruminant species in this context.

MATERIALS AND METHODS

Study design: A cross-sectional study was conducted in the Eastern province of Saudi Arabia to collect faecal and blood samples from animals over 2 years old including 240 camels, 220 sheep, 123 goats, and 66 cattle.

Diagnostic tests: three tests were performed including:

- **Faecal smears (FS):** Faecal sample was decontaminated with 0.9% hexadecylpyridinium chloride, and the supernatant was smeared, stained with Ziehl-Neelsen, and examined microscopically. A sample was considered positive if at least 10 acid-fast bacilli were observed across 100 fields.
- **Faecal *IS900*-PCR:** MAP DNA extraction was performed using QIAamp® PowerFecal® kit (QIAGEN, Courtaboeuf, France). Amplification of the *IS900* gene was carried out using an iCycler PCR machine (BIO-RAD, Hercules, CA, USA). A sample was deemed positive if the *IS900*-PCR yielded positive results.
- **Serum antibody-ELISA:** IDEXX ELISA kit (*Mycobacterium paratuberculosis* antibody test kit, IDEXX, Hoofddorp, Netherlands) was used. A sample was deemed positive if the S/P ratio was ≥55%.

Statistical analyses:

Model and assumptions: A three-tests-one-population BLCM was fitted and FS and PCR to be dependent conditionally on the true MAP health status of animals.

Prior information: Prior information about the accuracy of FS, PCR and ELISA for each ruminant species was obtained from the literature where available (**Table 1**).

Table 1. Prior information and corresponding distributions assumed in BLCM to estimate Se and Sp of ELISA, FS, and PCR in four different ruminant species.

Parameter	Prior information (% and 2.5th or 97.5th percentile) and corresponding β distribution							
	Camels		Sheep		Goats		Cattle	
Prevalence	NA	β(1, 1)	NA	β(1, 1)	NA	β(1, 1)	NA	β(1, 1)
Se-ELISA	NA	β(1, 1)	32 (68)	β(3.2, 5.7)	69 (46)	β(13.7, 6.7)	27.3 (38.3)	β(21.6, 55.9)
Sp-ELISA	95 (80)	β(56.6, 7.1)	97 (94)	β(100, 4.0)	95 (90)	β(100, 6.2)	97.4 (96.6)	β(100, 3.6)
Se-FS	NA	β(1, 1)	NA	β(1, 1)	NA	β(1, 1)	43.7 (48.7)	β(100, 128.5)
Sp-FS	NA	β(1, 1)	NA	β(1, 1)	NA	β(1, 1)	98 (95.4)	β(100, 3.0)
Se-PCR	NA	β(1, 1)	36 (58)	β(8.1, 13.6)	19 (35)	β(7.5, 29.1)	50.3 (36.4)	β(24.3, 24.0)
Sp-PCR	95 (80)	β(56.6, 7.1)	98 (94)	β(100, 3.0)	98 (93)	β(98.2, 2.9)	93.5 (87.5)	β(100, 7.8)

RESULTS

- The number of tested animals per herd/flock ranged from 10 to 41 camels, 6 to 55 sheep, 11 to 38 goats, and 6 to 11 cattle.
- Overall, FS yielded more positive results than ELISA or PCR across all species.
- At least one positive test result was observed in each herd/flock.
- Cross-tabulated results by species are shown in **Table 2**.

Table 2. Cross tabulated test results by species used in the BLCM to estimate Se and Sp of ELISA (Test 1), FS (Test 2), and PCR (Test 3)

Species	+/+/+	+/+/–	+/–/+	+/–/–	–/+/+	–/+/–	–/–/+	–/–/–	Total
Camels	17	5	0	0	9	31	0	178	240
Sheep	52	5	0	0	10	23	0	130	220
Goats	19	6	0	0	4	13	0	81	123
Cattle	12	2	0	0	4	17	0	31	66

- The highest MAP prevalence was observed in cattle (78.5%), followed by sheep (32.5%), goats (29.0%), and camels (15.0%).
- Across all species, FS showed the highest median Se among tests (**Figure 1**).
- Median Se estimates for all tests were generally lower in cattle (<50%) compared to other species.
- In sheep, Se values for all tests exceeded 70%, while in camels, Se ranged from 56.7% for ELISA to 82.3% for FS (**Figure 1**).
- Among goats, PCR had a lower Se (median = 40.3) compared to ELISA (median = 69.3) and FS (median = 79.1).
- Median Sp across tests was above 86%, with FS yielding the lowest Sp in camels (86.6%), sheep (87.0%), and goats (90.4%). In cattle, PCR had the lowest Sp (93.8%).

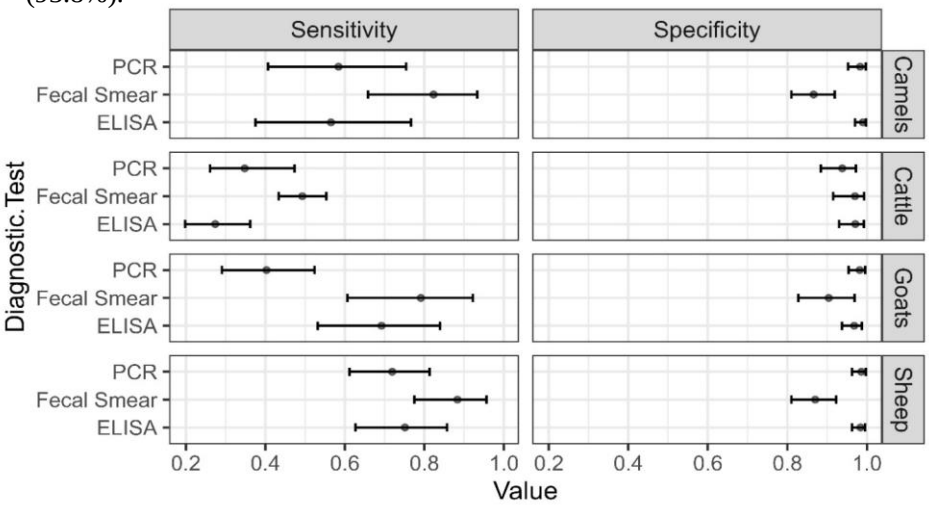

Figure 1. Median (point) and 95% Bayesian credible intervals (bars) of Se and Sp of ELISA, FS, and PCR to detect MAP infections in four different ruminant species

CONCLUSIONS

- Considering individual and population test metrics, cost, and laboratory turnaround time, ELISA appears to be a suitable option for identifying MAP-infected animals in this region and prevalence context.
- Our results underscore the importance of understanding population context and pre-test disease probability when interpreting test accuracy estimates.