Praca oryginalna *Original paper*

Mycoplasma bovis seroprevalence and risk factors in Polish cattle

PIOTR ŻMUDA[1], MAGDALENA NOWAK[2], WIKTORIA TCHÓRZ[2], DANIEL KLICH[3], EWELINA KWIECIEŃ[4], WOJCIECH WÓJCIK[5], KRZYSZTOF ANUSZ[2], ANNA DIDKOWSKA[2]

[1]University Centre of Veterinary Medicine UJUR, Al. Mickiewicza 24/28, 30-059 Kraków, Poland
[2]Department of Food Hygiene and Public Health Protection, Institute of Veterinary Medicine, Warsaw University of Life Sciences (SGGW), Nowoursynowska 166, 02-787 Warsaw, Poland
[3]Department of Animal Genetics and Conservation, Warsaw University of Life Sciences (SGGW), Ciszewskiego 8, 02-786 Warsaw, Poland
[4]Department of Preclinical Sciences, Institute of Veterinary Medicine, Warsaw University of Life Sciences (SGGW), Ciszewskiego 8, 02-786 Warsaw, Poland
[5]Veterinary Clinic "Animed", Juliana Tuwima 8, 38-300 Gorlice, Poland; "Dziki Projekt", Krzywa 25, 38-307 Sękowa, Poland

Received 02.08.2024 *Accepted 17.08.2024*

Żmuda P., Nowak M., Tchórz W., Klich D., Kwiecień E., Wójcik W., Anusz K., Didkowska A.

***Mycoplasma bovis* seroprevalence and risk factors in Polish cattle**

Summary

This study aimed to assess the occurrence of *M. bovis* antibodies and their risk factors in cattle in Poland. Serum samples were collected from 154 cattle from 13 Voivodeships during routine veterinary procedures from February to March 2024. The animals' ages ranged from 6 months to 13 years. *M. bovis* antibodies were detected in serum samples by Monoscreen Ab ELISA *Mycoplasma bovis* kits (Bio-X Diagnostics S.A., Belgium). The obtained results were subjected to statistical analysis using Sterne's exact method and logistic regression model. Antibodies against *M. bovis* were detected in sera from 40/154 animals. None of the analysed factors influenced seroprevalence. Our study revealed 26% *M. bovis* seroprevalence in cattle from different regions of Poland. Based on previous research performed in Europe, the results of this study can be classified as medium seroprevalence. Due to the lack of effective vaccines against *M. bovis*, increasing antimicrobial resistance of field isolates, *M. bovis* infections and seroprevalence in Polish cattle should continue to be monitored.

Keywords: antibodies, cattle, *Mycoplasmopsis bovis*, seroprevalence

Mycoplasma spp. are pathogens that can cause mastitis, otitis media, arthritis, pneumonia, and reproductive disorders in cattle, and is a significant concern (12, 18, 30). Among *Mycoplasma* spp., *Mycoplasma bovis*, now also called *Mycoplasmopsis bovis*, is the most common (10, 13). Mycoplasmosis is a leading cause of economic losses for cattle breeders (4). Despite developing numerous potential vaccines, their widespread commercial use remains challenging (6, 25). The poor response to treatment, nonspecific clinical and pathological signs, and associated implications for affected stock make accurate monitoring of *Mycoplasma* spp. a critical necessity (8, 24). Moreover, *M. bovis* is highly contagious (14, 27). Given the escalating problem of antimicrobial resistance, monitoring becomes even more vital to prevent the use of broad-spectrum antimicrobial drugs (11, 17).

One of the methods of diagnosis is the detection of specific antibodies against *M. bovis* in plasma, serum, or milk samples within two weeks of infection (23, 31). Antibody levels remain high for several months (3). Therefore, antibodies can be detected even in animals that are not actively shedding *M. bovis* at the time of sample collection (22). The most often used serological technique for detecting *M. bovis*-specific antibodies is the Enzyme-Link Immunosorbent Assay (ELISA) (23).

In Poland there have been two major *M. bovis* seroprevalence monitoring studies in cattle, with the last one 10 years ago (2, 26). Antibodies against *M. bovis* were also detected in Polish wildlife (5, 7, 15). Due to the increasing antibiotic-resistant problem and lack of current data, the aim of this study was to assess the *M. bovis* seroprevalence and its risk factors in cattle in Poland.

Material and methods

From February to March 2024, serum samples were collected from 154 cattle (100 females, 54 males) during

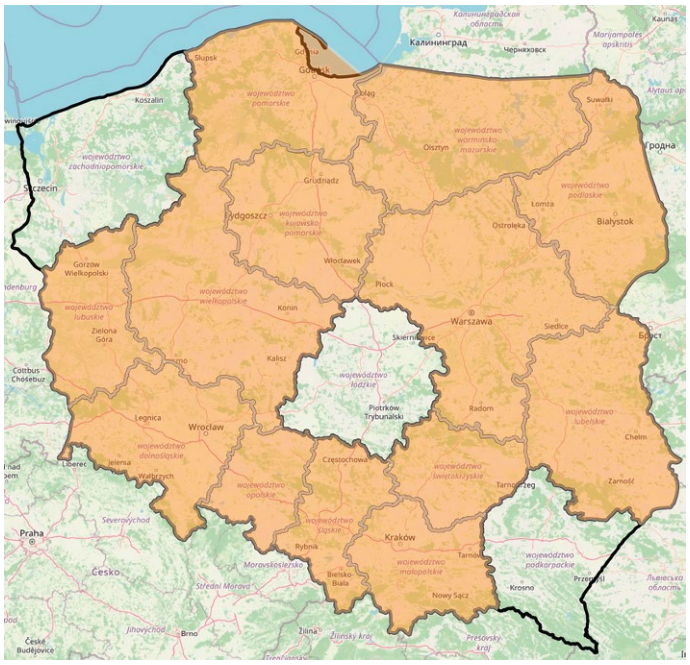

Fig. 1. Voivodeships from which the test samples come (marked in orange)

routine veterinary procedures. The age of animals ranged from 6 months to 13 years (mean age: 3.3 years). The breeds presented as follows: Polish Holstein-Friesian Black-White Cattle (n = 67), Limousine (n = 20), crossbreed with meet breeds (n = 25), Bond'e Aquitaine (n = 11), Simental (n = 11), Polish Holstein-Friesian Red-White Cattle (n = 7), Belgian blue (n = 3), Montbeilarde (n = 3), crossbreed without meet breeds (n = 3), Red Polish Cattle (n = 2), Charolaise (n = 1) and Red Angus (n = 1). Animals came from 42 counties of 13 Voivodeships (Dolnośląskie, Kujawsko-Pomorskie, Lubelskie, Lubuskie, Małopolskie, Mazowieckie, Opolskie, Podlaskie, Pomorskie, Śląskie, Świętokrzyskie, Warmińsko-Mazurskie, Wielkopolskie) (Fig. 1).

The blood was collected into sterile 6 ml tubes with a clot activator from the jugular vein. The samples were transported to the laboratory at 4°C. After centrifugation and serum separation, samples were stored at –20°C until further testing.

Before testing, serum samples were allowed to thaw at room temperature. Next, *M. bovis* antibodies were detected by Monoscreen Ab ELISA *Mycoplasma bovis* kits (Bio-X Diagnostics S.A., Belgium). The test was performed according to the manufacturer's manual. Briefly, serum samples were diluted and added to the wells sensitized by a recombinant protein from *M. bovis* expressed by *Escherichia coli*. Next, conjugate (protein G peroxidase-labeled) was added to the wells after incubation and washing steps. After a second incubation and washing, the chromogen (tetramethylbenzidine) was distributed. If specific antibodies were present in the serum sample, the conjugate remained bound, and the enzyme-catalyzed a color change from colorless into a pigmented compound. After adding stop solution, results were read using an EPOCH spectrophotometer (BioTek Instruments Inc., US) at a wavelength of 450 nm. The intensity of the resulting yellow colour was proportionate to the titter of the specific antibodies in the sample. Results were calculated in accordance with manufacturer instructions.

We calculated 95% confidence limits (95% CI) using Sterne's exact method. We analysed the potential impact of sex, age, and cow breed types on seroprevalence. For this purpose, we performed a logistic regression model, where the dependent variable was the test result, i.e., seropositive results were marked as 1, and seronegative results were marked as 0. The covariates from the model were two qualitative variables: the sex of the animals (male or female) and the breed type (meat, meat and dairy, and dairy breeds). The breeds were grouped because, for many of them, the number of samples was too small to be assessed separately. The third covariate was age expressed in years.

Results and discussion

Antibodies against *M. bovis* were detected in sera from 40/154 animals (26%; 95 CI, 19.41 to 33.41). None of the variables, sex, age, or breed type of cows, influenced seroprevalence (Tab. 1). The logistic regression model was insignificant ($Chi^2 = 0.756$). The model presented a low Nagelkerke R2 value (0.024), and despite a relatively high percentage of correctly classified cases (72.6%), all values predicted by the model were 0.

Tab. 1. The Effect of sex, age, and breed type on the presence of *Mycoplama bovis* antibodies in cattle in a regression logistic model

Source	B	SE	Wald Chi^2	P
Intercept	–1.131	0.496	5.192	0.023
Sex (F)	0.546	0.452	1.460	0.227
Sex (F)	0*			
Age	–0.066	0.095	0.490	0.484
Breed (meet)	0.243	0.514	0.223	0.636
Breed (meat and dairy)	–0.065	0.517	0.016	0.900
Breed (dairy)	0*			

Explanations: None of the variables was statistically significant. B – beta coefficient; SE – standard error; Wald Chi^2 – test of significance; P – P value; * – redundant category.

Our study revealed 26% *M. bovis* seroprevalence in cattle from different regions of Poland, which corresponds with studies conducted over eight years ago (26). This shows that it would be worth introducing new ways of dealing with *M. bovis* in cattle herds to lead to a decrease in its occurrence. Particular attention should be paid to its role in the bovine respiratory disease complex (BRDC) and the immunosuppressive effect of *M. bovis*, as it can predispose cattle to other respiratory infections (8, 16).

In this study, none of the variables was statistically significant. However, in other conducted studies, some other variables were checked, such as herd size and the number of neighbouring farms (19), corporation-type farms, and purchased cattle (21), which were significant risk factors for seropositivity to *M. bovis*.

M. bovis is widely spread worldwide in cattle (28). Seroprevalence in Europe varies from country to country, from very low seroprevalence (e.g. Finland – less than 1%; (14)), mid seroprevalence (e.g. Serbia – 9.92%; (29)) to high seroprevalence (e.g., Hungary – 82.91%, (9)), making results of this study classified as medium seroprevalence.

Even though ELISA is a cheaper and less time-consuming method than culture, it has some limitations. Seroconversion may last two to three weeks, so at the first stage of infection, ELISA may give false negative results (32). Moreover, ELISA specificity can be decreased by cross-reactivity with other closely related bacteria (20). We have received relatively low seroprevalence, taking into consideration that *M. bovis* is highly contagious; it might be because of the low sensitivity of the test used in our study (1).

Considering that seroprevalence in Poland is not decreasing, the lack of efficient vaccines against *M. bovis* and the increasing antimicrobial resistance of *M. bovis* field isolates, *M. bovis* infections and seroprevalence should be further monitored in Polish cattle. Combining serology with other diagnostic DNA-based methods, like PCRs, or new methods, like isothermal amplification technology (IAT), should also be considered.

References

1. *Andersson A. M., Aspán A., Wisselink H. J., Smid B., Ridley A., Pelkonen S., Autio T., Lauritsen K. T., Kensø J., Gaurivaud P., Tardy F.*: A European inter-laboratory trial to evaluate the performance of three serological methods for diagnosis of Mycoplasma bovis infection in cattle using latent class analysis. BMC Vet. Res. 2019, 25, 15 (1), 369.
2. *Bednarek D., Ayling R. D., Nicholas R. A., Dudek K., Szymańska-Czerwińska M.*: Serological survey to determine the occurrence of respiratory Mycoplasma infections in the Polish cattle population. Vet. Rec. 2012, 171 (2), 45.
3. *Byrne W. J., Ball H. J., Brice N., McCormack R., Baker S. E., Ayling R. D., Nicholas R. A. J.*: Application of an indirect ELISA to milk samples to identify cows with Mycoplasma bovis mastitis. Vet. Rec. 2000, 146, 368-369.
4. *Chockalingam A., Zarlenga D. S., Bannerman D. D.*: Antimicrobial activity of bovine bactericidal permeability–increasing protein–derived peptides against Gram-negative bacteria isolated from the milk of cows with clinical mastitis. Am. J. Veter. Res. 2007, 68, 1151-1159.
5. *Didkowska A., Klich D., Nowak M., Wojciechowska M., Prolejko K., Kwiecień E., Rzewuska M., Olech W., Anusz K.*: A serological survey of pathogens associated with the respiratory and digestive system in the Polish European bison (Bison bonasus) population in 2017-2022. BMC Vet. Res. 2023, 19 (1), 74.
6. *Dudek K., Bednarek D., Ayling R. D., Kycko A., Szacawa E., Karpinska T. A.*: An experimental vaccine composed of two adjuvants gives protection against Mycoplasma bovis in calves. Vaccine 2016, 34, 3051-3058.
7. *Dudek K., Bednarek D., Szacawa E., Ayling R. D., Krzysiak M. K., Marczuk J.*: A serological and molecular study on the occurrence of mycoplasmas in European bison (Bison bonasus) from two areas of Eastern Poland. Pol. J. Vet. Sci. 2015, 18 (4), 881-883.
8. *Dudek K., Nicholas R. A. J., Szacawa E., Bednarek D.*: Mycoplasma bovis infections – occurrence, diagnosis and control. Pathogens 2020, 9 (8), 640.
9. *Fodor L., Jánosi K., Makrai L., Gyuranecz M.*: Screening of Hungarian cattle herds for seropositivity to Mycoplasma bovis. Acta Vet. Hung. 2017, 65 (2), 166-172.
10. *Fox L. K.*: Mycoplasma mastitis: causes, transmission, and control. Vet. Clin. North Am. Food Anim. Pract. 2012, 28, 225-237.
11. *Gautier-Bouchardon A. V.*: Antimicrobial resistance in Mycoplasma spp. Microbiol. Spectr. 2018, 6 (4).
12. *Gelgie A. E., Desai S. E., Gelalcha B. D., KerroDego O.*: Mycoplasma bovis mastitis in dairy cattle. Front. Vet. Sci. 2024, 11, 1322267.
13. *Gioia G., Addis M. F., Santisteban C., Gross B., Nydam D. V., Sipka A. S., Virkler P. D., Watters R. D., Wieland M., Zurakowski M. J., et al.*: Mycoplasma species isolated from bovine milk collected from US dairy herds between 2016 and 2019. J. Dairy Sci. 2021, 104, 4813-4821.
14. *Haapala V., Pohjanvirta T., Vähänikkilä N., Halkilahti J., Simonen H., Pelkonen S., Soveri T., Simojoki H., Autio T.*: Semen as a source of Mycoplasma bovis mastitis in dairy herds. Vet. Microbiol. 2018, 216, 60-66.
15. *Krzysiak M. K., Dudek K., Krajewska M., Bednarek D., Szulowski K.*: Serological studies to determine the occurrence of Johne's disease and mycoplasma infection in the Northern-East Polish population of European bison (Bison bonasus). Pol. J. Vet. Sci. 2014, 17 (4), 721-723.
16. *Lachowicz-Wolak A., Klimowicz-Bodys M. D., Płoneczka-Janeczko K., Bykowy M., Siedlecka M., Cinciała J., Rypuła K.*: The prevalence, coexistence, and correlations between seven pathogens detected by a PCR method from South-Western Poland dairy cattle suffering from Bovine Respiratory Disease. Microorganisms 2022, 10 (8), 1487.
17. *Lysnyansky I., Ayling R. D.*: Mycoplasma bovis: mechanisms of resistance and trends in antimicrobial susceptibility. Front. Microbiol. 2016, 7, 595.
18. *Maunsell F. P., Woolums A. R., Francoz D., et al.*: Mycoplasma bovis infections in cattle. J. Vet. Intern. Med. 2011, 25, 772-783.
19. *McAloon C. I., McAloon C. G., Tratalos J., O'Grady L., McGrath G., Guelbenzu M., Graham D. A., O'Keeffe K., Barrett D. J., More S. J.*: Seroprevalence of *Mycoplasma bovis* in bulk milk samples in Irish dairy herds and risk factors associated with herd seropositive status. J. Dairy Sci. 2022, 105 (6), 5410-5419.
20. *Miller J. J., Levinson S. S.*: Interferences in Immunoassays; Eleftherios D., Theodore C., Eds. Immunoassay, Academic Press: Cambridge, UK 1996, p. 165-190.
21. *Murai K., Higuchi H.*: Prevalence and risk factors of Mycoplasma bovis infection in dairy farms in northern Japan. Res. Vet. Sci. 2019, 123, 29-31.
22. *Nicholas R. A., Ayling R. D.*: Mycoplasma bovis: disease, diagnosis, and control. Res. Vet. Sci. 2003, 74 (2), 105-112.
23. *Okella H., Tonooka K., Okello E.*: A systematic review of the recent techniques commonly used in the diagnosis of Mycoplasma bovis in dairy cattle. Pathogens 2023, 12 (9), 1178.
24. *Parker A. M., Sheehy P. A., Hazelton M. S., Bosward K. L., House J. K.*: A review of mycoplasma diagnostics in cattle. J. Vet. Intern. Med. 2018, 32 (3), 1241-1252.
25. *Soehnlen M. K., Aydin A., Lengerich E. J., Houser B. A., Fenton G. D., Lysczek H. R., et al.*: Blinded, controlled field trial of two commercially available Mycoplasma bovis bacterin vaccines in veal calves. Vaccine 2011, 29, 5347--5354.
26. *Szacawa E., Szymańska-Czerwińska M., Niemczuk K., Dudek K., Bednarek D., Aylling R. D.*: Comparison of serological, molecular and cultural diagnostic method for the detection of Mycoplasma bovis infections in cattle. Anim. Sci. Pap. Rep. 2016, 34 (4), 351-359.
27. *Ter Laak E., Noordergraaf J. H., Dieltjes R. P. J. W.*: Prevalence of Mycoplasmas in the respiratory tracts of pneumonic calves. J. Vet. Med. Ser. B. 1992, 39, 553-562.
28. *Vähänikkilä N., Pohjanvirta T., Haapala V., Simojoki H., Soveri T., Browning G. F., Pelkonen S., Wawegama N. K., Autio T.*: Characterisation of the course of Mycoplasma bovis infection in naturally infected dairy herds. Vet. Microbiol. 2019, 231, 107-115.
29. *Vojinovic D., Zdravkovic N., Prodanović R., Vujanac I., Nedić S., Giadinis N. D., Panuonis N., Manic M. M., Bugaraski D., Palamarevic M., Bogicevic N., Dobrosavljevic I., Spalević L., Žutić J., Prodanov-Radulović J., Bojkovski J.*: Seroprevalence of Mycoplasma bovis in grazing dairy cows from five different areas in Serbia. J. Hellenic Vet. Med. Soc. 2019, 69 (4), 1241-1246.
30. *Walz P. H., Mullaney T. P., Render J. A., Walker R. D., Mosser T., Baker J. C.*: Otitis media in preweaned Holstein dairy calves in Michigan due to Mycoplasma bovis. J. Vet. Diagn. Investig. 1997, 9, 250-254.
31. *Wawegama N., Browning G., Kanci A., Marenda M., Markham P.*: Development of a recombinant protein-based enzyme-linked immunosorbent assay for diagnosis of Mycoplasma bovis infection in cattle. Clin. Vaccine Immunol. 2013, 21.
32. *Wawegama N. K., Browning G. F., Kanci A., Marenda M. S., Markham P. F.*: Development of a recombinant protein-based enzyme-linked immunosorbent assay for diagnosis of Mycoplasma bovis infection in cattle. Clin. Vaccine Immunol. 2014, 21, 196-202.

Corresponding author: Anna Didkowska, DVM, PhD, Nowoursynowska 166, 02-787 Warsaw, Poland; e-mail: anna_didkowska@sggw.edu.pl