Prevalence and risk factors for *Mycoplasma* spp. positivity in cat blood donor units from Portugal, Spain and Belgium in 2022: retrospective study on 7573 blood donations from 4121 healthy donor cats

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Haemotropic mycoplasmas are epi-erythrocytic parasitic bacteria that can cause haemolytic anaemia. Prevalence for haemoplasma infection varies geographically among *Mycoplasma* species. Male adult outdoor non-pedigree cats are at increased risk for infection. Some studies identified an association between haemoplasmas and retroviruses. As *Mycoplasma* spp. can be transmitted via blood transfusion, routine quantitative polymerase chain reaction (qPCR) screening of donor cats is recommended. This retrospective study assessed prevalence and risk factors for *Mycoplasma* spp. positivity in cat donor units from Portugal, Spain, and Belgium. A private blood bank database of cat donations performed in 2022 was reviewed. Studied risk factors for *Mycoplasma* spp. positivity included age, sex, pedigree, blood type, geographic area, season, and retroviral co-infection (FeLV and FIV). A multiple generalised estimation equation model was used to account for repeated blood donations on a same cat. A total of 7573 blood donations from 4121 privately-owned mixed breed donor cats from Portugal (n=4034, 97.9%), Spain (n=70, 1.7%), and Belgium (n=17, 0.4%) were studied. Most cats donated blood once (n=1996, 48.4%); the remainder donated twice (n=1099, 26.7%), three (n=725, 17.6%) or four (n=301, 7.3%) times. Two-hundred and twelve Portuguese cats tested positive at least once for *Mycoplasma* spp. leading to an estimated prevalence of 5.3% (95% CI: 4.6-5.9). The prevalence did not significantly differ between Portuguese regions (P=0.28). Two cats in Spain had positive *Mycoplasma* spp. qPCR, whilst all Belgian cats were negative. The small sample sizes in these countries prevented robust prevalence estimation. Among positive Portuguese cats, 30 cats donated blood >1 time in 2022: 26 cats were negative first then subsequently tested positive, 3 cats were positive on two occasions, and 1 cat was initially positive and subsequently tested negative. Blood units collected from male cats were at higher risk for *Mycoplasma* spp. positivity (OR 1.9, P<0.001). Increased risk was also observed for blood units that tested positive for FeLV either by serology and/or qPCR (OR 2.9, P=0.0018) and for blood donations performed in winter (OR 2.5, P<0.0001). None of the other studied risk factors was associated with *Mycoplasma* spp. positivity. European cat blood donors displayed a low prevalence of *Mycoplasma* spp. with an increased risk in cats affected with FeLV and male cats. The seasonality for *Mycoplasma* spp. positivity, with an increased risk in winter, remains to be elucidated. Positive *Mycoplasma* spp. qPCR results identified in previously negative donors emphasizes the importance of testing on every donation instead of annually.

Ethical comment: Screening for mycoplasma spp. and other infectious agents was performed on blood issued from blood donation as part as routine procedures performed by the animal blood bank; no unnecessary procedures were done to blood donors. Every procedure at the blood bank required a signed informed owner consent provided in attachment. This study was conducted according to European legislation (86/609/EU).