# Molecular and serological detection of *Anaplasma phagocytophilum* in dogs in Germany (2008-2020)

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## INTRODUCTION

*Anaplasma* (*A.*) *phagocytophilum* is an obligate intracellular bacterium causing granulocytic anaplasmosis.[1,2] Ticks of the *Ixodes persulcatus* complex are the primary vectors.[3]

## AIMS OF THE STUDY

Aim of the study was to assess the percentage of positive test results for *A. phagocytophilum* in dogs in Germany and to identify possible risk factors for infection/pathogen contact.

## MATERIAL AND METHODS

Results of direct (PCR) and indirect (IFAT, ELISA) tests for *A. phagocytophilum* requested by veterinarians in Germany between 2008 and 2020 from the LABOKLIN laboratory (Bad Kissingen, Germany) were included in the retrospective study. Binomial logistic regression was performed to determine the effect of sex, age groups, and time of testing. A distribution machine learning model (random forest) was trained and build based on PCR results. The output of this model was then aggregated to the 2-digit postal codes of Germany and reclassified in a low, medium and high classification (figure 1).

**Table 1: Dogs tested for *Anaplasma phagocytophilum* by PCR from 2008 to 2020 in the laboratory LABOKLIN (*n* positive/*N* total (%))**

| Time frame | PCR | IFAT/ELISA |
|---|---|---|
| **2008-2012** | 248/5,826 (4.3) | 5,932/22,591 (26.3) |
| **2013-2016** | 354/7,633 (4.6) | 5,424/29,517 (18.4) |
| **2017-2020** | 730/13,909 (5.2) | 13,364/38,268 (34.9) |
| **Total** | 1,332/27,368 (4.9) | 24,720/90,376 (27.4) |

**Table 2: Number of positive tested dogs including direct and indirect detection methods for *Anaplasma phagocytophilum* in dogs living in Germany from 2008-2020 sorted by age (*n* positive/*N* total [%])**

| Age-group | PCR | IFAT/ELISA |
|---|---|---|
| 0 – 2 years (junior) | 206/4,934 (4.2) | 2,557/20,749 (12.3) |
| >2 – 7 years (adult) | 478/9,529 (5.0) | 8,924/31,198 (28.6) |
| >7 – 10 years (mature) | 285/6,019 (4.7) | 6,473/16,349 (39.6) |
| >10 – 13 years (senior) | 213/3,809 (5.6) | 3,704/8,757 (42.3) |
| >13 years (geriatric) | 49/762 (6.4) | 600/1,475 (40.7) |
| Total | 1,231/25,053 (4.9) | 22,258/78,528 (28.3) |
| Mann-Whitney-U-test | P < 0.001 | P < 0.001 |

PCR: Mann-Whitney-U-test, *P* < 0.001, 154666055.500
IFAT/ELISA: Mann-Whitney-U-test, *P* < 0.001, 825081293.000


**Figure 1: Likelihood of being tested PCR-positive for *Anaplasma phagocytophilum* in different German federal states based on 27,368 dogs**

**Table 3: Binominal logistic regression analysis in dogs tested for *Anaplasma phagocytophilum* by PCR and IFAT/ELISA, all with known sex, age, years as well as months of testing from 2008 to 2020**

| | B | SE | Wald | P | Odds Ratio | 95%-CI for Odds Ratio | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower bound | Upper Bound |
| **PCR-testing (*N* = 23,829 dogs)** | | | | | | | |
| **Sex (male)** | 0.166 | 0.214 | 0.605 | 0.437 | 1.181 | 0.777 | 1.795 |
| **Age (> 7 years)** | 0.177 | 0.207 | 0.728 | 0.393 | 1.193 | 0.795 | 1.791 |
| **Years** | 0.058 | 0.032 | 3.249 | 0.071 | 1.060 | 0.995 | 1.129 |
| **Season (summer)** | **1.107** | **0.208** | **28.395** | **< 0.001** | **3.026** | **2.014** | **4.548** |
| **Constant** | -120.016 | 65.282 | 3.380 | 0.066 | 0.000 | - | - |
| **Antibody-testing (IFAT/ELISA, *N* = 74,073 dogs)** | | | | | | | |
| **Sex (male)** | **0.238** | **0.017** | **203.113** | **< 0.001** | **1.269** | **1.228** | **1.312** |
| **Age (> 7 years)** | **0.872** | **0.017** | **2656.929** | **< 0.001** | **2.392** | **2.314** | **2.472** |
| **Years** | **0.054** | **0.002** | **623.234** | **< 0.001** | **1.056** | **1.051** | **1.060** |
| **Season (summer)** | **0.338** | **0.017** | **379.519** | **< 0.001** | **1.403** | **1.356** | **1.451** |
| **Constant** | -117.757 | 4.868 | 585.175 | < 0.001 | 0.000 | - | - |

B: unstandardized regression weight; SE: standard deviation to the mean
Variables entered in step 1: male, age > 7 years, year, summer
Degrees of freedom were 1 for all Wald statistics

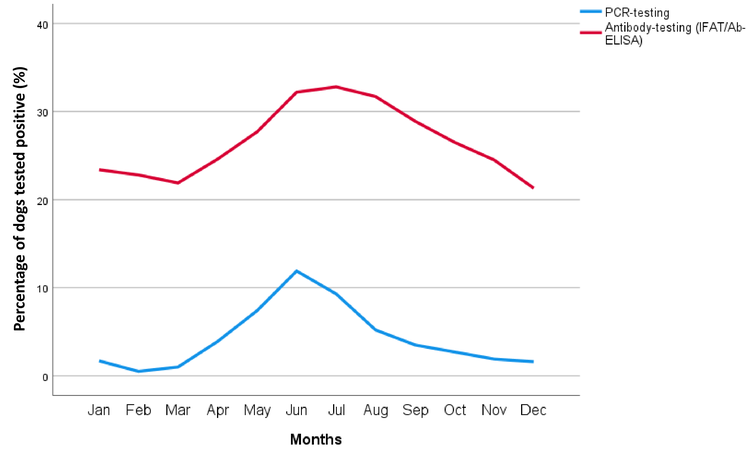


**Figure 2: Monthly distribution of dogs tested positive for *Anaplasma phagocytophilum* by direct (PCR; blue line) and indirect detection methods (IFAT, ELISA; red line) from 2008 to 2020; "Spring" = March to May, "summer" = June to August, "autumn" = September to November, "winter" = December to February**

## RESULTS

In total, 25,724/110,240 dogs (23.3%) tested positive by PCR (4.9%) and/or IFAT/ELISA (27.4%) (table 1). Male and elder dogs had a higher likelihood of being tested serologically positive (table 2, table 3). The months with highest PCR incidences overlapped with the ones with highest vector activity. Four weeks delayed to PCR-testing, peaks in serology were observed in positive tested dogs (figure 2).

## LIMITATIONS

There was no data available on ectoparasite prophylaxis, living conditions, possible stays abroad as well as the reasons for PCR and/or antibody testing. All these could have influenced the reported prevalence of *A. phagocytophilum* but might be of limited importance due to the large number of dogs included in the study.

## DISCUSSION AND CONCLUSIONS

Dynamic of canine infections with *A. phagocytophilum* in Germany was consistent with peaks in vector activity.[4] Positive test results in serological and PCR-testing showed prominent seasonality with peaks in summer. A trend of rising percentages of dogs tested positive by PCR or IFAT/ELISA throughout the years may indicate a growing importance of *A. phagocytophilum* infections in Germany. Sex and age had little or no effect on PCR results. Elder age corresponded with seropositivity. Regional differences in percentages of seropositivity and positive PCR results were recognized with highest likelihoods of being tested PCR positive in northeastern Germany. More research is necessary to examine possible reasons as e. g. climatic impact and/or prevalence of *A. phagocytophilum* in local tick populations.

## REFERENCES

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