# Phylogenetic Analysis of "*Candidatus* Mycoplasma turicensis" Isolates from Pet Cats in the United Kingdom, Australia, and South Africa, with Analysis of Risk Factors for Infection[∇]

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Two hemotropic mycoplasmas have been recognized in cats, *Mycoplasma haemofelis* and "*Candidatus* Mycoplasma haemominutum.*" We recently described a third feline hemoplasma species, designated "*Candidatus* Mycoplasma turicensis,*" in a Swiss cat with hemolytic anemia. This isolate induced anemia after experimental transmission to two specific-pathogen-free cats and analysis of the 16S rRNA gene revealed its close relationship to rodent hemotropic mycoplasmas. The agent was recently shown to be prevalent in Swiss pet cats. We sought to investigate the presence and clinical importance of "*Candidatus* Mycoplasma turicensis" infection in pet cats outside of Switzerland and to perform the molecular characterization of isolates from different countries. A "*Candidatus* Mycoplasma turicensis"-specific real-time PCR assay was applied to blood samples from 426 United Kingdom (UK), 147 Australian, and 69 South African pet cats. The 16S rRNA genes of isolates from different countries were sequenced and signalment and laboratory data for the cats were evaluated for associations with "*Candidatus* Mycoplasma turicensis" infection. Infections were detected in samples from UK, Australian, and South African pet cats. Infection was associated with the male gender, and "*Candidatus* Mycoplasma haemominutum*" and *M. haemofelis* coinfection. Coinfected cats exhibited significantly lower packed cell volume (PCV) values than uninfected cats. Phylogenetic analyses revealed that some Australian and South African "*Candidatus* Mycoplasma turicensis" isolates branched away from the remaining isolates. In summary, "*Candidatus* Mycoplasma turicensis" infection in pet cats exists over a wide geographical area and significantly decreased PCV values are observed in cats coinfected with other feline hemoplasmas.

Hemotropic mycoplasmas (also known as the hemoplasmas) are the causative agents of infectious anemia in several mammalian species. In felids, studies have revealed the existence of three different hemoplasma species: *Mycoplasma haemofelis*, "*Candidatus* Mycoplasma haemominutum*" (7, 8, 15–17, 24) and, most recently, "*Candidatus* Mycoplasma turicensis" (29). The latter was discovered in a Swiss pet cat with a history of severe hemolytic anemia, and phylogenetic analyses revealed that this agent is most closely related to rodent hemoplasma species, namely, *Mycoplasma haemomuris* and *Mycoplasma coccoides* (29).

There is still little knowledge of the epidemiology of hemoplasma infections, but the recent development of molecular methods has greatly facilitated the identification of these agents (1, 5, 10, 15, 23, 29). PCR analysis is now the diagnostic method of choice for hemoplasma infections, and sensitive and specific real-time PCR assays have been published for the

detection, differentiation, and quantification of all three feline hemoplasma species (23, 28).

In applying PCR-based methods, *M. haemofelis* and "*Candidatus* Mycoplasma haemominutum*" infections in pet cats have been diagnosed worldwide (4, 5, 10, 13, 22, 24, 26). The prevalence of these two agents varies greatly in different studies performed in different countries, which may partly be due to the different sample collection methods and/or differences in the sensitivity and specificity of the PCR assays used in these studies. We have recently shown that "*Candidatus* Mycoplasma turicensis" is present in the Swiss pet cat population at a low prevalence similar to that of *M. haemofelis* infection (28). The presence and clinical importance of "*Candidatus* Mycoplasma turicensis" outside of Switzerland has not been investigated to date.

Infections with *M. haemofelis* usually result in severe hemolytic anemia (1, 7, 27), characterized by apathy, pale mucous membranes, tachycardia, and tachypnea, and infections may be fatal. In contrast, "*Candidatus* Mycoplasma haemominutum*" infections do not usually induce clinical signs (7, 27), although some reports have documented mild or moderate anemia subsequent to infection (6, 9). Experimental transmission of "*Candidatus* Mycoplasma turicensis" to two specific-pathogen-free cats induced mild to severe anemia (29). However, a recent

cross-sectional study performed in Swiss pet cats suggests that cofactors, such as iatrogenic or retrovirus-induced immuno-suppression, are involved in the development of anemia in "*Candidatus* Mycoplasma turicensis"-infected animals (28). Importantly, most "*Candidatus* Mycoplasma turicensis"-infected cats in the latter study were coinfected with "*Candidatus* Mycoplasma haemominutum," a fact that complicates the interpretation of the pathogenic potential of this novel third feline hemoplasma.

We sought here to investigate the presence and clinical importance of "*Candidatus* Mycoplasma turicensis" infection in cats from three different continents and to perform the molecular characterization of "*Candidatus* Mycoplasma turicensis" isolates from different countries. A specific real-time PCR assay was applied to blood samples from United Kingdom (UK), Australian, and South African pet cats, and different signalment and laboratory variables were analyzed to investigate any associations present with "*Candidatus* Mycoplasma turicensis" infection.

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## MATERIALS AND METHODS

**Sample and data collection.** DNA samples extracted from a total of 642 feline EDTA-anticoagulated blood samples were available from previous studies (13, 21, 22). Frozen DNA was sent from the Department of Veterinary Clinical Science, University of Bristol, Bristol, United Kingdom, to the Clinical Laboratory, University of Zurich, Zurich, Switzerland by courier service. The samples comprised 426 blood samples collected from 306 sick and 120 healthy cats in the United Kingdom (21), 147 blood samples collected from 139 sick and 8 healthy cats in the Sydney area of Australia (22), and 69 blood samples submitted to a private veterinary laboratory in South Africa (13). Of the original 78 South African samples, 9 were excluded from the present study since no more material was available. The South African samples originated from cats that had shown a variety of disease conditions, and all had been suspected of having hemoplasma infection based on blood smear evaluation. Hemograms and blood smear examinations had been performed using EDTA-anticoagulated blood (13, 21, 22). Biochemical data had only been recorded for the South African cats (13).

**PCR assays.** The DNA samples had been analyzed previously for *M. haemofelis* and "*Candidatus* Mycoplasma haemominutum" infections with conventional (21) or real-time (13, 22) PCR assays. In the present study, a specific real-time PCR assay was used to detect and quantify "*Candidatus* Mycoplasma turicensis" in these samples (29). The conventional PCR generates PCR products of the same size with both *M. haemofelis* and "*Candidatus* Mycoplasma turicensis" infection (29); so, the one United Kingdom sample that tested positive for both agents (presumed *M. haemofelis* by conventional PCR and "*Candidatus* Mycoplasma turicensis" by real-time PCR) was also subjected to a *M. haemofelis*-specific real-time PCR assay (28). Coinfection with "*Candidatus* Mycoplasma turicensis" and *M. haemofelis* was thereby confirmed. In each run, the amplification buffer contained dUTP for the use with uracil-*N*-glycosylase to prevent carryover of PCR amplicons, and water was used as the negative control.

**Sequencing of 16S rRNA genes.** The near-complete 16S rRNA genes from "*Candidatus* Mycoplasma turicensis" isolates were cloned and sequenced using previously described methods (29), with the exception of D7 and D9, which were amplified with newly designed "*Candidatus* Mycoplasma turicensis" -specific primers (forward primer [5'-CGA ATT GTC GAA AGA CAA TTA GC-3'] and reverse primer [5'-AGA AGT TTC ATT CTT GAC ACA ATT TAA-3']). The sequences obtained were compared to known sequences held on the GenBank database, and the percent identity was calculated using GCG Wisconsin Package (Accelrys GmbH, Munich, Germany). The sequences were aligned using CLUSTAL W (25) and manually adjusted with Jalview 2.07 (3). Only positions where the nucleotide composition was known in all sequences being compared were used in the phylogenetic analysis. A phylogenetic tree was constructed with the neighbor-joining method (18) from a distance matrix corrected for nucleotide substitutions by the Kimura two-parameter model (11). The data set was resampled 1,000 times to generate bootstrap values.

TABLE 1. Sample characteristics: gender and breed distribution in the group of all examined cats and grouped by country of origin (United Kingdom, Australia, and South Africa)

| Variable | No. of cats (%) | | | | *P* (chi square) |
| | All | United Kingdom | Australia | South Africa[a] | |
|---|---|---|---|---|---|
| **Gender** | | | | | NS[b] |
| Female | 266 (47.4) | 198 (47.8) | 68 (46.3) | ND | |
| Male | 295 (52.6) | 216 (52.2) | 79 (53.7) | ND | |
| **Breed** | | | | | <0.0001 |
| Pedigree | 106 (23.8) | 46 (15.3) | 60 (58.9) | ND | |
| Nonpedigree | 340 (76.2) | 254 (84.7) | 86 (41.1) | ND | |

[a] ND, not determined.
[b] NS, not significant.

**Statistical evaluation.** Parameters for each cat were compiled and analyzed with Excel (Microsoft, Wallisellen, Switzerland), Analyze-it Clinical Laboratory (Analyze-it Software, Leeds, United Kingdom), Prism software (GraphPad, San Diego, CA), and Intercooled Stata v7 (Stata Corp., College Station, TX). The following variables were considered: breed and gender (both not available for the South African cats), age, packed cell volume (PCV), hemoglobin (Hb; not available for all of the Australian cats), red blood cell (RBC) count (not available for the Australian cats), and PCR results for *M. haemofelis*, "*Candidatus* Mycoplasma haemominutum" and "*Candidatus* Mycoplasma turicensis." Blood biochemistry data included total serum protein (TSP), albumin, globulin, blood-urea-nitrogen, creatinine, alkaline phosphatase (ALP), and alanine aminotransferase. Continuously measured sample characteristics including age, PCV, Hb and RBC count, most of them non-normally distributed within the sample groups (country), were compared between groups using the Kruskal-Wallis analysis of variance (ANOVA) on Ranks with post-hoc Kruskal-Wallis Z-tests and the Bonferroni correction for multiple comparison. For categorical variables (gender, breed) the chi-square test was used. The univariable association between "*Candidatus* Mycoplasma turicensis" status (binary outcome) and the variables age, breed, gender, PCV, Hb level, RBC count, and "*Candidatus* Mycoplasma haemominutum" and *M. haemofelis* infection status was assessed using a survey logistic regression model in Stata, with the country as the survey stratum variable. Initially, continuously measured variables were in addition categorized by quartiles in order to assess the assumption of the linearity in the odds. If fulfilled, then the continuous format was used. Correlation between the PCV, Hb level, and RBC count were checked using the Spearman rank correlation test ($r_{Sp}$), and the Hb level and RBC count dropped from further multivariable analysis due to a strong correlation with PCV ($r_{sp} > 0.90$). All other variables measured for all countries were included in a multivariable survey logistic regression model (age, PCV, and infection status for "*Candidatus* Mycoplasma haemominutum" and *M. haemofelis*) in order to derive final odds ratio (OR) estimates with 95% confidence limits.

Continuously measured blood biochemistry parameters were compared between groups by using the nonparametric Mann-Whitney U test. The PCV values of hemoplasma-negative and singly and doubly infected cats were found to be normally distributed in each group; *M. haemofelis*- "*Candidatus* Mycoplasma turicensis" -coinfected cats could not be tested for normal distribution due to low case numbers. PCV values were compared between the groups with one-way ANOVA and Tukey's test with corrections for multiple comparisons. The correlation between PCV and "*Candidatus* Mycoplasma turicensis" blood loads (copy numbers of DNA template per ml of blood) was assessed by the Spearman rank correlation test.

**Nucleotide sequence accession number.** The 16S rRNA nucleotide sequences generated from "*Candidatus* Mycoplasma turicensis" isolates have been submitted to GenBank (accession numbers DQ464417 to DQ464425).

## RESULTS

**Sample characteristics.** Sample characteristics are shown in Tables 1 and 2. Differences among the three sample groups were found for age, PCV, RBC count, and breed distribution. The Australian cats in the present study were significantly older and more frequently pedigrees than the United Kingdom cats, and the Australian cats had significantly higher PCV

TABLE 2. Sample characteristics (age, PCV, Hb level, and RBC count) in the group of all examined cats and by country of origin (United Kingdom, Australia, and South Africa)

| Variable and cat group | No. of cats | Median | 95% CI[a] | P[b] |
|---|---|---|---|---|
| **Age (yr)** | | | | |
| All cats | 606 | 7.0 | 6.0–8.0 | <0.0001 |
| UK cats | 402 | 6.0 | 5.0–7.0 A | |
| Australian cats | 147 | 10.0 | 9.0–11.1 A | |
| South African cats | 57 | 6.0 | 5.0–8.0 | |
| **PCV (%)** | | | | |
| All cats | 631 | 33.8 | 32.0–34.7 | <0.0001 |
| UK cats | 422 | 34.5 | 32.5–35.4 B | |
| Australian cats | 140 | 35.0 | 33.0–36.0 C | |
| South African cats | 69 | 29.0 | 25.0–31.0 B, C | |
| **Hb level (g/dl)** | | | | |
| All cats | 545 | 10.5 | 10.1–10.8 | NS |
| UK cats | 420 | 10.7 | 10.2–11.1 | |
| Australian cats | 56 | 10.1 | 9.6–10.8 | |
| South African cats | 69 | 10.2 | 8.7–11.0 | |
| **RBC count ($10^6$/μl)** | | | | |
| All cats | 480 | 7.3 | 7.1–7.5 | 0.011 |
| UK cats | 411 | 7.5 | 7.1–7.8 D | |
| Australian cats | | ND[c] | ND | |
| South African cats | 69 | 6.6 | 5.7–7.2 D | |

[a] That is, the 95% CI of the median (based on the standard error). Matching letters are placed next to entries whose means were significantly different.
[b] Overall P values as determined by Kruskal-Wallis ANOVA on Ranks to compare the age, PCV, and Hb values between three groups (UK, Australian, and South African cats) and the RBC count between two groups (UK and South African cats). Significant (S) group differences were identified using the Kruskal-Wallis Z-test with the Bonferroni correction for multiple comparison. NS, nonsignificant.
[c] ND, not determined.

TABLE 3. Number and percentage (sample prevalence) of cats that tested real-time PCR positive for "*Candidatus* Mycoplasma turicensis"[a]

| Group (n) | No. of PCR-positive cats (%)[b] | | |
|---|---|---|---|
| | "*Candidatus* Mycoplasma haemominutum" | *M. haemofelis* | "*Candidatus* Mycoplasma turicensis" |
| All cats (642) | 134 (21) | 24 (3.7) | 43 (6.7) |
| UK cats (426) | 73 (17)* | 7 (1.6)* | 10 (2.3) |
| Australian cats (147) | 35 (24)* | 7 (4.8)* | 15 (10) |
| South African cats (69) | 26 (38)* | 10 (15)* | 18 (26) |

[a] For comparison, the recently published sample prevalence for "*Candidatus* Mycoplasma haemominutum" and *M. haemofelis* is shown.
[b] *, "*Candidatus* Mycoplasma haemominutum" and *M. haemofelis* PCR results (13, 21, 22). For the South African cats, only the results from the 69 cats included in the present study are given.

values than the South African cats. The South African cats, on the other hand, had significantly lower PCV and RBC values than the UK cats in the study. Gender and Hb values were not significantly different among the three sample groups.

**Sample prevalence.** The real-time PCR results for "*Candidatus* Mycoplasma turicensis" are shown in Table 3. Among all cats analyzed, 6.7% tested PCR positive for "*Candidatus* Mycoplasma turicensis." The sample prevalence was clearly the highest for the South African cats (26%), followed by the Australian (10%) and UK (2.3%) samples. In all three groups, the number of "*Candidatus* Mycoplasma turicensis" PCR-positive cats was higher than the number of *M. haemofelis*-infected animals but lower than the number of "*Candidatus* Mycoplasma haemominutum" PCR-positive cats. Several "*Candidatus* Mycoplasma turicensis"-infected cats were coinfected with other feline hemoplasma species: of the 43 "*Candidatus* Mycoplasma turicensis"-infected cats, 23 cats also tested positive for "*Candidatus* Mycoplasma haemominutum" (6 UK, 8 Australian, and 9 South African cats), 4 also tested positive for *M. haemofelis* (1 UK, 1 Australian, and 2 South African cats), and 2 South African cats tested PCR positive for all three feline hemoplasma species.

**Sequencing of 16S rRNA gene.** To confirm the PCR results and to evaluate sequence variations among "*Candidatus* Mycoplasma turicensis" isolates from different countries, the nearly complete 16S rRNA gene from isolates derived from two UK, three Australian, and four South African pet cats was

sequenced. Most isolates revealed 99% identity with a published "*Candidatus* Mycoplasma turicensis" sequence from an isolate originating from a Swiss pet cat (DQ157150) with the exception of two isolates from South African (G5, 98.8% identity; D7, 97.5% identity) and two isolates from Australian pet cats (B3, 97.5% identity; D9, 97.5% identity). The latter three sequences (B3, D7, and D9) were almost completely identical to each other (≥99.9% identity) but exhibited 32 to 33 nucleotide differences within the 1,295 bp sequenced when aligned to the published Swiss "*Candidatus* Mycoplasma turicensis" sequence (DQ157150). The G5 sequence showed 16 nucleotide differences from that of the published Swiss "*Candidatus* Mycoplasma turicensis" sequence. The 16S rRNA sequences from G5, B3, D7, and D9 also contained one nucleotide mismatch within the binding site of the real-time PCR probe (position 5 of 21 nucleotides).

Phylogenetic analyses confirmed the close evolutionary homology of "*Candidatus* Mycoplasma turicensis" with rodent hemoplasma species, namely, *M. coccoides* and *M. haemomuris* (Fig. 1). In accordance with the sequencing results mentioned above, four "*Candidatus* Mycoplasma turicensis" isolates (G5, B3, D7, and D9) branched away from the remaining isolates. However, no obvious geographical clustering of "*Candidatus* Mycoplasma turicensis" isolates was evident.

**Characteristics of "*Candidatus* Mycoplasma turicensis" PCR-positive cats.** Of the eight variables evaluated with the univariable logistic regression model, six were significantly associated with PCR-positive status for "*Candidatus* Mycoplasma turicensis" (Table 4). "*Candidatus* Mycoplasma turicensis"-infected cats had significantly lower PCV, Hb, and RBC values. In addition, male cats were more likely to be infected than females, and "*Candidatus* Mycoplasma turicensis" infection was significantly associated with "*Candidatus* Mycoplasma haemominutum" and *M. haemofelis* coinfection, respectively. In multivariable analysis, PCV (OR = 0.96, 95% confidence interval [95% CI] = 0.93 to 0.98, P = 0.001), as well as coinfection with *M. haemofelis* (OR = 3.20, 95% CI = 0.94 to 10.81, P = 0.061) and "*Candidatus* Mycoplasma haemominutum" (OR = 5.27, 95% CI = 2.68 to 10.37, P < 0.001), respectively, remained moderately to strongly associated with "*Candidatus* Mycoplasma turicensis" PCR-positive status.

Biochemical data were only available from the South African cats. "*Candidatus* Mycoplasma turicensis" PCR-positive status was significantly associated with increased TSP (median =
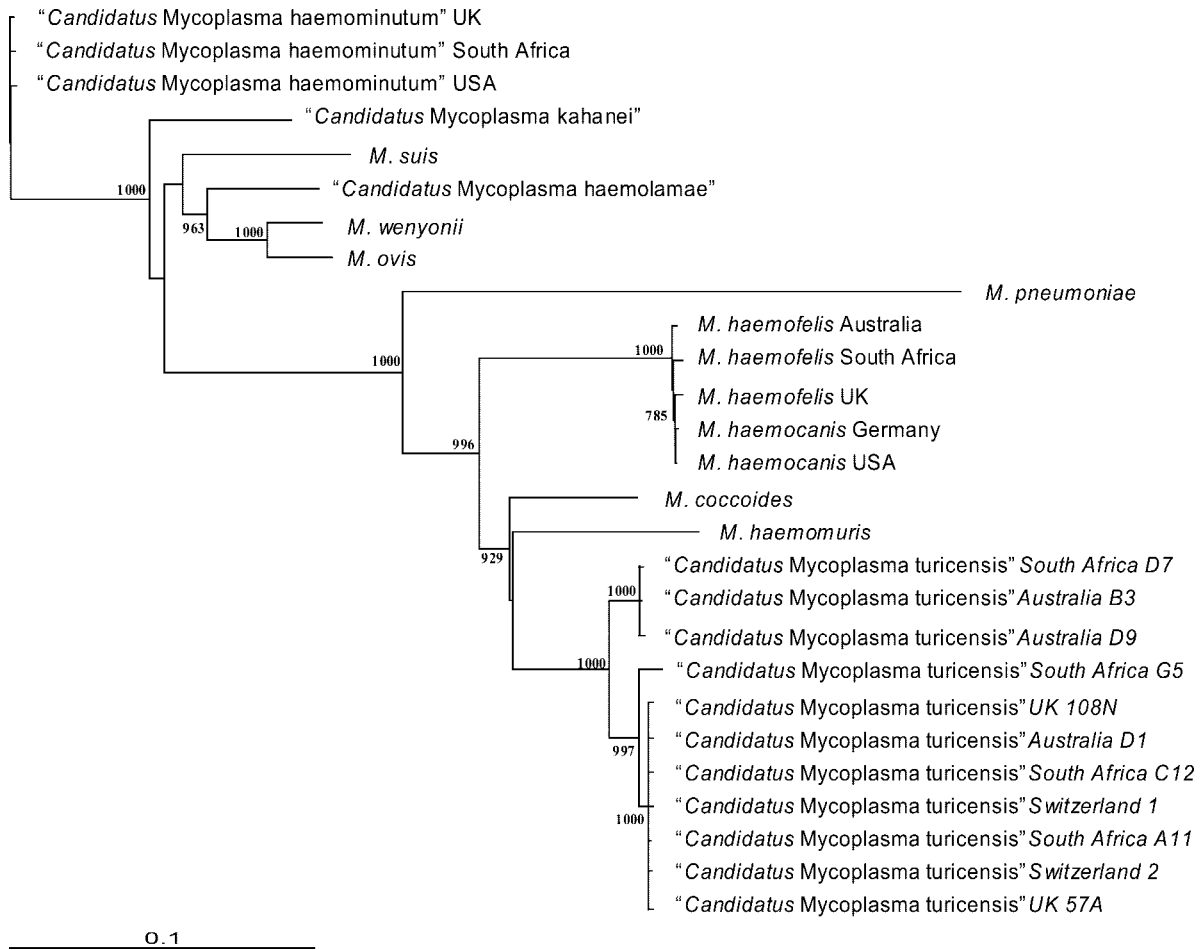
FIG. 1. Phylogenetic analysis of nearly complete 16S rRNA gene sequences from "Candidatus Mycoplasma turicensis" isolates from different countries and related organisms. Bootstrap values are given at the nodes of the tree; only values of ≥700 are shown. "Candidatus Mycoplasma haemominutum" (United Kingdom, AY150980; South Africa, AY150979; United States, U88564), "Candidatus Mycoplasma kahanei" (AF338269), Mycoplasma suis (U88565), "Candidatus Mycoplasma haemolamae" (AF306346), Mycoplasma wenyonii (AF016546), Mycoplasma ovis (AF338268), Mycoplasma pneumoniae (M29061), Mycoplasma haemofelis (Australia, AY150976; South Africa, AF548631; United Kingdom, AY150985), Mycoplasma haemocanis (Germany, AY150973; United States, AF197337), Mycoplasma coccoides (AY171918), Mycoplasma haemomuris (U82963), and "Candidatus Mycoplasma turicensis" isolates (D7, DQ464424; B3, DQ464423; D9, DQ464425; G5, DQ464422; 108N, DQ464421; D1, DQ464417; C12, DQ464419; Switzerland 1, DQ157150; A11, DQ464418; Switzerland 2, DQ157151; and 57A, DQ464420) are shown.

TABLE 4. Survey logistic regression model with country treated as the survey stratum: univariable relationship between possible predictor variables and "Candidatus Mycoplasma turicensis" PCR-positive status

| Variable | OR[a] | 95% CI[b] | Wald P value |
|---|---|---|---|
| Age (continuous) | 1.03 | 0.98–1.09 | 0.254 |
| Breed[c] (pedigree vs. nonpedigree) | 0.55 | 0.16–1.93 | 0.353 |
| Gender[c] (male vs. female) | 2.98 | 1.17–7.61 | 0.022 |
| PCV (continuous) | 0.95 | 0.93–0.97 | <0.001 |
| Hb level (continuous) | 0.88 | 0.81–0.96 | 0.002 |
| RBC count[d] (continuous) | 0.78 | 0.69–0.88 | <0.001 |
| "Candidatus Mycoplasma haemominutum" coinfection (positive vs. negative) | 6.24 | 3.28–11.89 | <0.001 |
| M. haemofelis coinfection (positive vs. negative) | 5.23 | 1.95–14.04 | 0.001 |

[a] That is, the OR for "Candidatus Mycoplasma turicensis" infection in the exposed category compared to the unexposed category (baseline value). For continuous variables, the change in odds for "Candidatus Mycoplasma turicensis" infection for a one-unit change in the respective continuous variable is given.
[b] That is, the 95% CI of the OR.
[c] Variable not recorded for the South African cats.
[d] Variable not recorded for the Australian cats.

74.0 g/liter, $P = 0.0195$) and globulin levels (median = 50.5 g/liter, $P = 0.0029$) and decreased ALP levels (median = 21.5 U/liter, $P = 0.0344$) compared to "Candidatus Mycoplasma turicensis" PCR-negative cats (TSP, median = 69.0 g/liter; globulin, median = 38.0 g/liter; ALP, median = 39.0 U/liter).

Since many of the "Candidatus Mycoplasma turicensis"-infected cats were coinfected with another feline hemoplasma species, PCV values were compared between hemoplasma-uninfected cats, singly positive cats, and animals coinfected with feline hemoplasma species (Fig. 2). Interestingly, only cats coinfected with "Candidatus Mycoplasma turicensis" and either "Candidatus Mycoplasma haemominutum" (mean PCV = 25.8%) or M. haemofelis (mean PCV = 16.5%) exhibited significantly lower PCV values than hemoplasma-uninfected cats (mean PCV = 33.9%). Only two cats were coinfected with all three hemoplasmas; these animals had PCV values of 18 and 31%, respectively. Due to the small number of triply infected cats, no statistical analysis was possible.
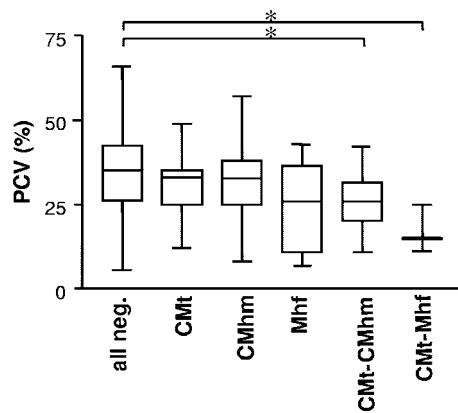
FIG. 2. PCV values of cats grouped by hemoplasma infection status. Boxes represent the 25th, 50th (median), and 75th quartiles, with whiskers extending to the greatest and smallest values. all neg., cats not infected with any feline hemoplasma (*n* = 468); CMt, cats PCR positive for "*Candidatus* Mycoplasma turicensis" alone (*n* = 13); CMhm, cats PCR positive for "*Candidatus* Mycoplasma haemominutum" alone (*n* = 103); Mhf, cats PCR positive for *M. haemofelis* alone (*n* = 13); CMt-CMhm, cats coinfected with "*Candidatus* Mycoplasma turicensis" and "*Candidatus* Mycoplasma haemominutum" (*n* = 23); CMt-Mhf, cats coinfected with "*Candidatus* Mycoplasma turicensis" and *M. haemofelis* (*n* = 4). Significant differences in PCV values between groups are indicated (one-way ANOVA, *P* < 0.0001; Tukey's multiple comparison test, *P* < 0.05 [*]).

"*Candidatus* Mycoplasma turicensis"-infected cats exhibited relatively low blood loads (median copy number = 900 copies/ml of blood; 5 to 95% percentile = 31 to 59,972 copies/ml of blood) comparable to those recently reported in Swiss pet cats (median copy number = 500 copies/ml of blood; 5 to 95% percentile = 22 to 126,300 copies/ml of blood) (28). There was no significant inverse correlation between PCV values and "*Candidatus* Mycoplasma turicensis" blood loads in infected animals (*n* = 42; $r_{Sp}$ = −0.17, 95% CI = −0.45 to 0.14, *P* = 0.27).

## DISCUSSION

This is the first study to report the presence of "*Candidatus* Mycoplasma turicensis" infections in pet cats from three different continents. Feline DNA samples were analyzed using a sensitive and specific quantitative real-time PCR assay (29). The prevalence of "*Candidatus* Mycoplasma turicensis" infection in UK cats was comparable to the recently published prevalence in Swiss pet cats (28). Interestingly, a high sample prevalence was found in South African and Australian pet cats; this can at least partly be explained by the fact that most of the investigated cats were clinically ill and that some of them were suspected of having hemoplasma infection. Since the present study was carried out using a convenience-sampled population, the limitations of which have been discussed previously (20), conclusions concerning the general prevalence of "*Candidatus* Mycoplasma turicensis" infections in UK, Australian, and South African pet cats cannot be drawn here. Nevertheless, it appears that "*Candidatus* Mycoplasma turicensis" infections are relatively common in pet cats of the countries under investigation. Furthermore, our results are consistent with reports of a higher feline hemoplasma prevalence in countries

with warmer climates (5, 13, 14, 22), suggesting that different blood-sucking arthropods may play a role in the transmission of hemoplasmas in different countries. Warmer climates harbor more and/or different blood-sucking arthropod species than colder regions. For example, only in southern and western Switzerland, regions known for higher mean annual temperatures compared to the northern and eastern areas of the country, has the tick *Rhipicephalus sanguineus* been reported (2).

The present study reports for the first time two cats concurrently infected with all three feline hemoplasma species. This finding suggests that no immunological cross-protection exists between the novel and the two better known feline hemoplasma species, an observation that has already been reported for "*Candidatus* Mycoplasma haemominutum" and *M. haemofelis* coinfection (27). The present study actually demonstrates a significant association between "*Candidatus* Mycoplasma turicensis" and *M. haemofelis* or "*Candidatus* Mycoplasma haemominutum" infections, respectively. The latter association has also been found in Swiss pet cats (28). These associations could be due to similar routes of transmission, such as via blood-sucking arthropods (12, 19, 30). Cats infected with one hemoplasma species could also exhibit an increased susceptibility to further hemoplasma infections. Alternatively, similar risk factors for infection for the three feline hemoplasma species could explain the finding of common coinfections with these agents.

Phylogenetic analyses based on the 16S rRNA gene sequence of "*Candidatus* Mycoplasma turicensis" showed that isolates from different countries exhibited >97% sequence identity. No clear geographical grouping of the isolates was evident. However, a different subcluster with one South African and two Australian isolates could be identified, branching away from the majority of the 12 isolates sequenced thus far in our laboratory (28; B. Willi, unpublished data). The 16S rRNA sequence of four isolates exhibited one nucleotide mismatch in the binding site of the real-time PCR probe. This could have resulted in a reduced PCR amplification efficiency for these isolates; however, such reduced PCR efficiency is likely to only interfere with accurate quantification of hemoplasma loads or qualitative analysis of samples with very low hemoplasma blood loads.

Interestingly, only "*Candidatus* Mycoplasma turicensis"-infected cats coinfected with either "*Candidatus* Mycoplasma haemominutum" or *M. haemofelis*, and not cats infected with only "*Candidatus* Mycoplasma turicensis," showed significantly lower PCV values than hemoplasma PCR-negative cats. An additive effect of feline hemoplasma infections on clinical disease has already been reported for cats coinfected with "*Candidatus* Mycoplasma haemominutum" and *M. haemofelis* (27). Our results support the hypothesis that cofactors, such as coinfection with other hemoplasma species or immunosuppression (28, 29), may be involved in the development of anemia in "*Candidatus* Mycoplasma turicensis"-infected cats.

In conclusion, we found that "*Candidatus* Mycoplasma turicensis" infection is present in pet cats from different countries, and coinfections with additional feline hemoplasma species are common. The pathogenic potential of "*Candidatus* Mycoplasma turicensis" seems to depend on cofactors, and a positive PCR result should be interpreted together with clinical and hematological findings.

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