

Genetic diversity of *Cryptosporidium parvum* strains in cattle in Poland – a cross-sectional population study

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Cryptosporidium parvum (*C. parvum*) infections are reported in cattle worldwide. Ruminants are not only the reservoir of the parasite for other animal species, but they can also be a source of infection for humans. The aim of the study was an assessment of the prevalence and molecular identification of *Cryptosporidium parvum* subtypes circulating in the cattle population in Poland.

In total, 1601 faecal samples were collected from cattle at the age of 1 week to 4 months over a period of five years (2014–2018). The animals were kept on 267 farms across 16 Polish provinces. The cattle belonged to 14 breeds, among which the most numerous was Polish Black and White Holstein Friesian (HO), mixed exclusively meat breeds (MM), Simentaler (SM) and Limousine (LM) breeds. Detection of *C. parvum* strains in faecal samples was performed using the 18 small subunit ribosomal RNA (SSU rRNA) PCR-RFLP method, while the parasite subtypes were determined on the basis of microsatellite sequence analysis of the GP60 gene fragment. A chi-squared (χ^2) test was employed to show the relationships between *C. parvum* subtypes and their prevalence in cattle of different breeds, animals age (age group) and their geographical distribution in Poland. The analysis of variance (ANOVA) was conducted to assess frequency of infections in cattle caused by particular *C. parvum* subtypes.

The overall prevalence of *C. parvum* in cattle from Poland was estimated at 6.2% (100/1601). The infected animals were housed in 44 (16.5%) out of 267 monitored farms. *C. parvum* subtypes were successfully identified for 82 out of 100 detected

parasite strains, which belonged to the IIA and IID genetic families. In the genetic group IIA, the following subtypes were identified IIAA17G1R1 (n = 26), IIAA17G2R1 (n = 24), IIAA15G2R1 (n = 10), IIAA16G1R1b (n = 9), IIAA22G1R1 (n = 4), IIAA14G2R1 (n = 2), IIAA10G1R1 (n = 2), IIAA21G1R1 (n = 1), IIAA18G3R1 (n = 1) and IIAA21R1 (n = 1). The IID genetic family was only represented by IIDA24G1 subtype (n = 2). In this study, five new *C. parvum* subtypes (A21R1, A18G3R1, A22G1R1, A21G1R1 and A14G2R1) were detected. They have not been identified in cattle in Poland so far.

The analysis of variance showed that only *C. parvum* IIAA17G2R1 and IIAA17G1R1 infections occurred in cattle at significantly higher frequency compared to other parasite subtypes (F = 3.39; p=0.0003). The infections caused by IIAA17G2R1 (total number of infections 24) and IIAA17G1R1 (total number of infections 26) dominated in the studied animal population. In the case of remaining *C. parvum* subtypes (total number of infections from 1 to 10, LSD_{0.05} = 14.1) there were no significant differences observed in the frequency of their prevalence. The majority of infections were detected in dairy cattle of HO breed ($\chi^2=761.2$; p=0.000). Of note is that, ten out of 11 *C. parvum* subtypes were found in HO calves. There was no relationship observed between infections caused by particular *C. parvum* subtype and cattle age (age groups) ($\chi^2 = 29.4$; p=0.133). However, the relationship between *C. parvum* subtype and place of its detection was confirmed by χ^2 test ($\chi^2 = 312.0$; p=0.000). The IIAA17G1R1 strains prevailed

in Podkarpackie and Świętokrzyskie provinces, while the IIaA17G2R1 in Małopolskie, Mazowieckie and Warmińsko-Mazowieckie provinces. In cattle farms, respectively in Zachodniopomorskie and Lubuskie, IIaA16G1R1b and IIdA24G1 infections were mainly observed.

The prevalence of *C. parvum* infections in cattle herds in Poland is similar to that observed in different populations of this animal species across Europe. Furthermore, the results indicate at the large genetic diversity of *C. parvum* strains circulating in cattle in Poland.