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An updating on *Cryptosporidium parvum* in the water buffalo

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ABSTRACT: A cross-sectional survey of *Cryptosporidium parvum* infection in the water buffalo was carried out in central Italy. The survey was carried out on a sample of 90 farms, selected using a grid approach within a Geographical Information System, followed by proportional allocation. On each farm, faecal samples were collected from three to five asymptomatic buffalo calves, aged from 1 to 9 weeks (total number = 347). Each sample was tested for the presence of copro-antigens of *C. parvum* using a commercially available ELISA. Out of the 90 farms, 22 (24.4%) resulted positive. With respect to animals, out of the 347 faecal samples, 51 (14.7%) were found to have antigens of *C. parvum*. The results of the logistic regression model showed a positive association between the positivity to *C. parvum* and the high number of buffaloes on farms.

Key words: Parasites, *Cryptosporidium parvum,* Geographical Information System.

INTRODUCTION - The genus *Cryptosporidium* includes Apicomplexa protozoa which cause infections in farm animals, companion animals, laboratory animals, wild mammals, birds, reptiles and fish, as well as in humans. Until now, the following 14 species have been recognized: *C. hominis* (humans and monkeys), *C. parvum* (cattle, other ruminants and humans), *C. andersoni* (cattle), *C. muris* (rodents), *C. suis* (pigs), *C. felis* (cats), *C. canis* (dogs), *C. wrairi* (guinea pigs), *C. bailey* (poultry), *C. meleagridis* (turkeys, humans), *G. galli* (finches, chickens), *G. serpentis* (reptiles), G. *saurophilum* (lizards), *G. molnari* (fishes). In addition, several genotypes that show genetic diversity have been described (Xiao and Ryan, 2004; Cacciò *et al*., 2005). *C. parvum* is considered an important co-factor in neonatal diarrhoea in cattle, sheep, goats, and water buffaloes (*Bubalus bubalis*). This protozoon species has been detected in water buffaloes in Italy (Canestri-Trotti and Quesada, 1983; Canestri-Trotti *et al*., 1984; Galiero *et al*., 1994; Saralli *et al*., 2001), as well as in other countries, e.g. Spain (Gomez-Couso *et al*., 2005), Egypt (Iskander *et al*., 1987), Cuba (Rodriguez-Diego *et al*., 1991), India (Dubey *et al*., 1992) and Brazil (Araujo *et al*., 1996). However, data on the distribution of this protozoon in water buffaloes are quite fragmentary and have never been performed with a systematic epidemiological approach. For these reasons, a cross-sectional survey aimed to study the presence and distribution of *C. parvum* in water buffaloes of the

Italian Mediterranean breed was carried out in Latium, a region of central Italy where 18% of the Italian water buffaloes are bred (data from the National Institute of Statistics, ISTAT 2001). A Geographical Information System was constructed in order to plan the study and to display the results (Cringoli *et al*., 2005; Rinaldi *et al*., 2006).

MATERIAL AND METHODS - The survey was carried out in 20 contiguous municipalities (1,250 km^2 surface area) located in the provinces of Latina and Frosinone (Latium region). A Geographical Information System (GIS) was constructed utilizing as data-layers the topographic base map and the digital aerial photographs of the study area, as well as the geo-referenced points of all the buffalo farms. The survey was carried out on a sample of 90 farms (epidemiological units), selected using a grid approach followed by proportional allocation. For this purpose, a grid representing quadrants of 5 x 5 km was overlaid on the study area within the GIS. The number of farms sampled in each quadrant was proportional to the total number of study population in that quadrant (Cringoli *et al*., 2005; Rinaldi *et al*., 2006). On each farm, faecal samples were collected per rectum from 3 to 5 asymptomatic buffalo calves, aged from 1 to 9 weeks. Information on farm management (farm typology, number of water buffaloes on the farm, presence of animals other than buffaloes in the farm, e.g. cattle, sheep, goats, and dogs) were also recorded in each farm. Each faecal sample was tested for the presence of copro-antigens of *C. parvum* utilizing a commercially available enzyme linked immunosorbent assay (ELISA *Cryptosporidium* antigen screening, version: P00605/03, Institute Pourquier, 326 rue de la Galéra, Parc Euromédicine, 34097 Montpellier, France), following the manufacturer's instructions. The farm management data (all categorical) were analyzed by univariate (Pearson's chi-square test for independence) and multivariable (logistic regression) statistical analyses at farm level using as dependent variable the C. parvum coprological status (positive/negative). Only the independent variables that showed significance ($P<0.01$) in the univariate tests were used for the logistic regression model.

RESULTS AND CONCLUSIONS - Out of the 90 farms sampled, 22 (24.4%; 95% CI = 16.3-34.8%) had at least one tested positive calf for *C. parvum*. With respect to animals, out of the 347 faecal samples, 51 (14.7%; 95% CI = 11.2-19.0%) were found to have antigens of *C. parvum*. The results of the statistical regression model showed a positive association between the positivity to *C. parvum* and the number of buffaloes on farms (more than 100 water buffaloes, OR = 5.13, P = 0.008). The *C. parvum* prevalence values referred to animals (14.7%) are in line with those previously reported for water buffaloes in Italy: 14.2% in the Salerno province of southern Italy (Galiero *et al*., 1994) and 14.7% in the Latina province of Central Italy (Saralli *et al*., 2001). The only risk factor for *C. parvum* infection was the number of buffaloes on farm; the higher risk of infection in large size farms than in small size farms found in the present survey was also reported in sheep from Spain (Causapè *et al*., 2002). Intensive buffalo farming may stress the animals. In Italy, group-housing with a small number of animals per pen (7 to 10) is a common rearing system for buffalo calves. However, space restriction may decrease animal welfare (Fisher *et al*., 1997). In conclusion, the present survey added data to the parasitological scenario of water buffalo population in Italy, where the progressive transformation of farms has contributed to the increase of parasites having direct transmission from host to host, such as *Cryptosporidium*.

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