The influence of epidemiological factors to prevalence of *Cryptosporidium parvum* of neonatal calves in northern Kazakhstan

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Abstract
Logistic regression analysis was conducted to assess the epidemiological factors influencing the *Cryptosporidium parvum* infection in bovine farms in Northern Kazakhstan. Faecal samples were collected on 24 farms from 245 neonatal calves and analysed using microscopy and immune chromatographic commercial kits. The prevalence of calves' infection ranged from 1.6% to 29.1%. In bivariate regression analysis four epidemiological factors, including "age of calves", "clinical appearance of diarrhoea", "calves housed without dam" and "large type of farm" were found to be significantly associated with infection; the associations were confirmed by the multivariate analysis. Moreover, the last analysis suggests also that the above first three epidemiological factors favoured *C. parvum* infection of calves.

Keywords: bivariate logistic regression analysis, multivariate logistic regression analysis, *Cryptosporidium parvum* infection, calves, Northern Kazakhstan

INTRODUCTION

Protozoa species of the *Cryptosporidium* Tyzzer, 1907 genus (Phylum Apicomplexa) are pathogens of many vertebrate animals, including humans (Xiao et al. 2004, Fayer 2010). The parasites have a global distribution and cause epidemiological outbreaks of the contagious disease among people with symptoms of diarrhoea, abdominal pain, nausea, vomiting and fever (Cacciò and Chalmers 2016). For those living in endemic areas, immunocompromised people such as infants, malnourished subjects or patients with T-cell immunodeficiency conditions (e.g. due to HIV infection), symptoms can be severe and even dangerous for life (Checkley et al. 2015, Hunter et al. 2013, Insulander et al. 2004). The recent occurrence of large outbreaks in several countries all over the world, as well as the results of many surveys of human and animal cryptosporidiosis, indicate that pathogens of this infection are widespread (Checkley et al. 2015).

Humans may acquire *Cryptosporidium* by direct contact with infected people or animals, and indirectly through contaminated water and food (Fayer 2010, Xiao 2010). Now, more than 20 species of *Cryptosporidium* have been described (Xiao et al. 2004, Fayer 2010). It was established that specific subtypes of the zoonotic

Cryptosporidium parvum and the anthroponotic *C.hominis* are responsible for the majority of human cases. Furthermore, many surveys of cryptosporidiosis indicate that cattle may be the main source of *Cryptosporidium* infection of people, and transmission of *C.parvum* from calves is common in outbreaks in human populations (Vieira et al. 2015). However, in Central Asia there is only sporadic information about cases of human infection with cryptosporidiosis (Borodina et al. 1994, Nurtaev et al. 2005), although prevalence of cattle's *Cryptosporidium* in northern regions of Kazakhstan is relatively high (Ibrayev et al. 2016, Ussenbayev et al. 2018). Therefore, studies of the epidemiology of the disease in the region in order to ensure the safety of livestock breeders and their families are considered relevant.

The present study was undertaken for analysis of the epidemiological features of *Cryptosporidium parvum*'s distribution in calves from farms of Northern Kazakhstan.

MATERIALS AND METHODS

Epidemiological data were collected in January-August 2019 by a cross-sectional investigation of 24 farms, including nine dairy, five fattening and 10 small house holding farms, in 13 districts of Northern Kazakhstan. In total, faeces were sampled from 245 neonatal calves under one month of age. Material for parasitological studies was collected individually at one-time trips to farms by per rectum sampling of faeces. Samples were examined microscopically for presence of *Cryptosporidium* oocysts after routine staining with carbolic fuchsin (Heine, 1982). Additionally, samples were tested for *C.parvum* with commercial FassisiBoDia immune chromatographic test (Fassisi GmbH, Germany).

Using the obtained factual field data, the value of the "Infected" variable was analysed and its correlation with the epidemiological variables was assessed statistically by the Logistic Regression Analysis Technique. A bivariate analysis for the association between variables and *C.parvum* infection, based on a logistic model, was performed (odds ratio) using R software. The significance of the variable association was tested with the Wald test. For evaluation of the efficiency of possible confounding variables, a multivariate analysis was performed by means of a logistic regression model. Only factors with a Wald's P-value smaller than 0.05 were included in the multivariate model and then there was performed a backward deletion process. The covariates included in the multivariate analysis were the following: age of calves in days, types of farms (Large and Middle sized), the presence of clinical signs of the disease (Diarrheal and Non-Diarrheal), and the methods of keeping the calves (With Dams and Without Dams). In both the bivariate and multivariate analyses, associations were considered as significant at $p < 0.05$.

To get a global basis for evaluation of the different variables of the *Cryptosporidium* infection's epidemiology, the quality of the statistical data was analysed by a "receiver operating characteristic curve", or ROC curve, and the quantity character of obtained results was assessed by "area under the curve" (AUC) of the ROC curve.

RESULTS

Studies have shown the presence of *Cryptosporidium parvum* in nine (69.2%) districts, where sampling was carried out. The species was found in 12 (50.0%) farms of the region, eight of which were large agricultural enterprises, where dairy herd included more than 150 cows, three middle-sized farms with less than 50 dairy cows and a small house holding farm with less than ten cows.

Average prevalence of cryptosporidiosis in neonatal calves' population in the studied area was 28.0%, including calves from one to 14 days with prevalence 24.2%-29.1%, and calves aged 22-31 days – 1.6%. According to calf-management practices, it was found that the infection level of herds has a significant correlation with calf housing without contact with its dam. So, in farms where calves were grown in individual pens without any contact with dams, the infection prevalence was 30.3%, but in farms where calves were kept with adult cows the invasion rate was 6%.

It was supposed that the studied variables (age of calves in days, types of farms, the presence of clinical signs of the disease and the methods of keeping calves) have not influenced the "infected" variable (null-hypothesis). If the impact of them on the "infected" variable with $p < 0.05$ was established, the correlation was evaluated as significant (alternative hypothesis). In other words, the significance of difference from 0 of each variable's effect on the "Infected" variable's prediction was tested.

Bivariate analysis of the "Infected" and "Age" variables have shown that the predicted probability of invasion depends on the age of calves in days, and demonstrates a smooth decrease in the level of infection to the 30th day, which corresponds to real field data (**Fig. 1**).

The values of y-axis Intercept and Slope were -0.0151 and -0.0844, respectively. Z-value (the estimated value divided by standard error) for the slope was -3.7450, which was confirmed by p-value <0.05 (**Table 1**). Thus, the effect of the "Age" variable on prediction of the infection prevalence is significantly different from 0.

For bivariate analysis it was accepted that the interaction of the "Infected" variable and "clinical appearance" variables that the latter variables have two cases: individuals with a diarrhoea clinic (DR) and individuals without signs of diarrhoea (NDR). The presence of diarrhoea was taken into account according to the faeces' consistency: only individuals with liquid faeces were considered as DR. In computer simulations the highest probability of diarrhoea symptoms was observed in individuals infected with cryptosporidia (**Fig. 2**). In these digital experiments, a Log (Odds Ratio) value of -2.3084 was obtained, i.e. the absence of diarrhoeal clinics 2.3084 times decreases the odds of being infected than the presence of diarrhoea in calves. Z-values (the Wald's Test values) were -5.1190 and -3.7690, thus estimated values are greater than five and three standard deviations away from 0 in normal distributed curves, respectively. Then these were confirmed by p-values <0.05 (**Table 1**).

In bivariate analysis, with the association of the "Infected" and "Farm types" variables, the probability of infection was at the highest level in large farms than in middle-sized livestock enterprises (**Fig. 2**). The value of Log (Odds) of Large farms was -0.9410 and Log (Odds Ratio) was -1.4868. Z-value for the Log(Odds) was -5.5290, which was confirmed by z-value. So, influence of the "Farm Type" variable on infection prediction was
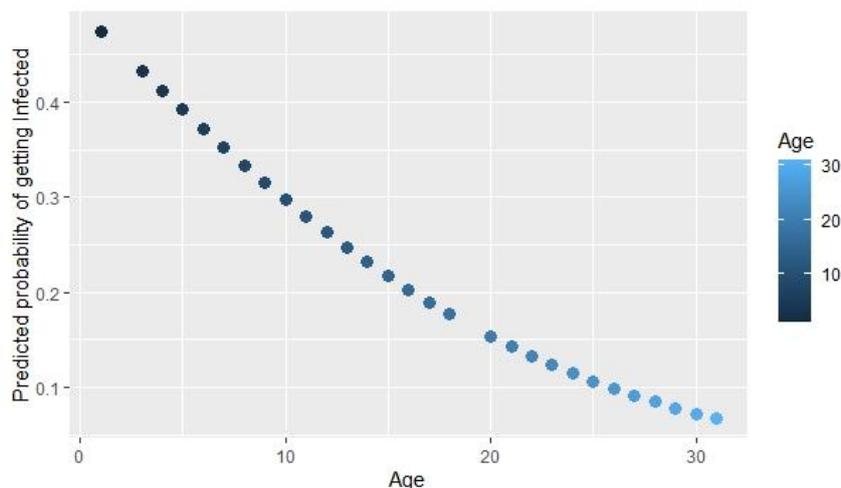
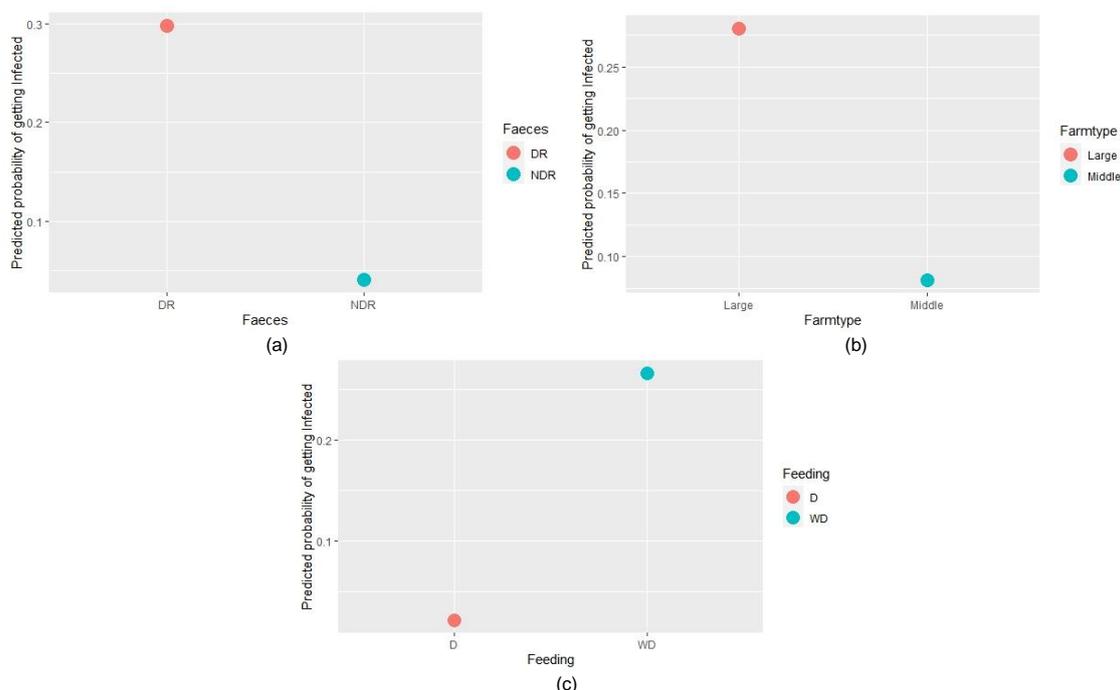**Fig. 1.** Predicted probability of being infected for calves

Fig. 2. Predicted correlations between the "Infected" variable and the epidemiological variables: a) clinical appearance of cryptosporidiosis (DR – diarrhoeal, NDR – non-diarrhoeal calves'); b) sizes of farms (Large, Middle); c) calf keeping technologies (D – with dams, WD – without dams)

Table 1. Computed LogReg values of epidemiological variables influenced the *Cryptosporidium* infection of calves

	Value	z-value	Pr(>|z|)
Infected (Ages)			
Intercept	-0.0151	-0.0440	0.9646
Log(Odds Ratio)	-0.0844	-3.7450	0.0001
Infected (Clinical Appearance)			
Log(Odds)_DR	-0.8557	-5.1190	3.07e-07
Log(Odds Ratio)	-2.3084	-3.7690	0.0001
Infected (Farm Types)			
Log(Odds)_Large	-0.9410	-5.5290	3.22e-08
Log(Odds Ratio)	-1.4868	-3.2420	0.0012
Infected (Calf keeping technologies)			
Log(Odds)_WD	-3.8071	-3.7650	0.0002
Log(Odds Ratio)	2.7930	2.7290	0.0064

also significantly different from 0. Accordingly, in calf keeping technologies, a significant correlation was established between the "Infected" and "Growing without dam calves" variables – WD (**Fig. 2**). A bivariate analysis Log (Odds)WD outcome was -3.8071 with Log (Odds Ratio) 2.7930 with significant z-values and p-values <0.05 of both these coefficients (**Table 1**).

The AUC of the "Infected" calve probability's dependence on all analysed four epidemiological variables lay in enough area for assessing the obtained computing results as significant (**Fig. 3**).

Therefore, bivariate analysis results had shown a significant association of cryptosporidiosis prevalence
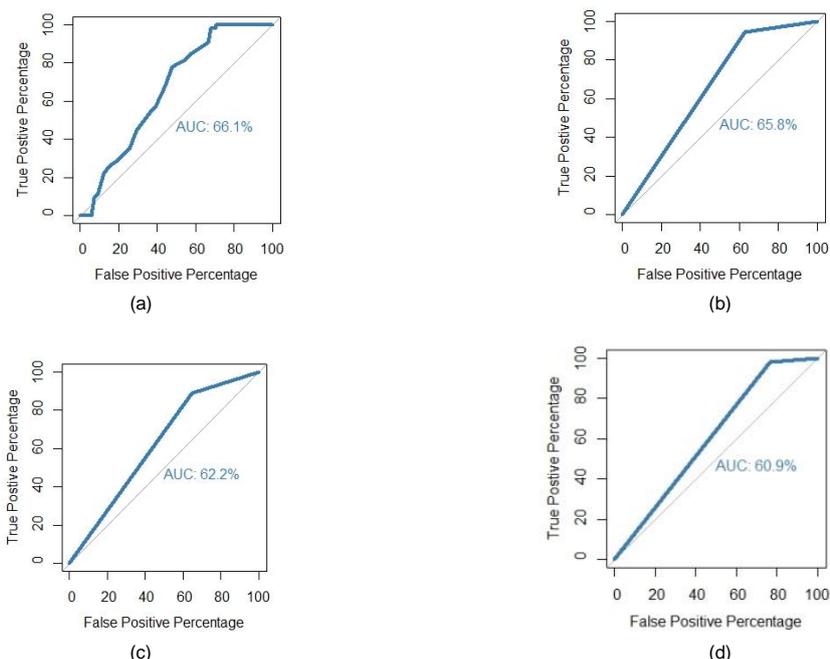
Fig. 3. AUC of the infected calves' probability associated with the following epidemiological variables: a) age of calves in days; b) diarrhoeal clinics of calves; c) size of farms; d) calf-management technologies
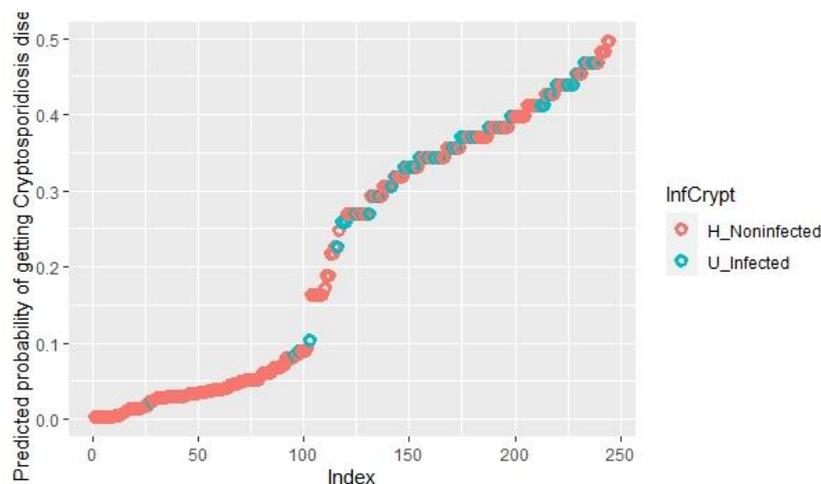

Fig. 4. The predicted logistic regression curve of the infection with Cryptosporidum dependence on the age, clinical conditions and keeping technologies of the studied individuals

with all investigated variables (age, clinical condition of calves, type of calf keeping and farm types).

At the next stage, there was performed a Multiple Logistic Regression analysis of the influence of the above variables on the calf infection rate. In this simulation the "Farm type" variable (Large farms), showing individually a significant association with cryptosporidiosis, became non-significant when it was included in a multivariate model; other epidemiological variables maintained a significant association with infection status. Therefore, with a backward deletion process, the non-significant factor was excluded from the model; the coefficients of the model were not affected substantially, as confirmed by z- and p-values

as well as the odds ratio. A significant correlation with infection status maintained only such variables as "age of calves", "clinical appearance of diarrhoea" and "calves housed without dam".

Moreover, the infection rate of the population, considering these variables, is predicted in the form of the following graph, shown in **Fig. 4**.

DISCUSSION

Although cryptosporidiosis is a dangerous zoonotic disease, a few studies of the parasitosis have been carried out in Central Asia. Results of these studies indicate that *C.parvum* is widespread in central

Kazakhstan. Thus, cryptosporidia were found in nine of the 13 studied districts of this region, namely in large agricultural enterprises and peasant farms. It is known that among the other four cattle *Cryptosporidium* pathogens, only the species *C.parvum* is contagious to humans and it is the main zoonotic agent of cryptosporidiosis among people (McLauchlin et al. 2000, Caccio` et al. 2005). Therefore, in the last decade, the number of reported cryptosporidiosis outbreaks, when *C.parvum* was identified as the etiological agent, has grown throughout the world (Fournet et al. 2013, Olson et al. 2004).

It was established that the main source of the pathogen for people is water (Smith et al. 2010), and the huge amount of oocysts excreted by the infected host has defined the increasing of environmental contamination (Ramirez et al. 2004). Cattle have been involved in the epidemiological process as a source of *C.parvum*, because cattle farms are located in watersheds (Smith et al. 2010).

Prevalence of *Cryptosporidium parvum* in neonatal calves of the studied region varies on different agricultural farms, in the range of 1.6-29.0%. Average infection with cryptosporidia of calves in our study was higher than in Canada (Gow et al. 2008), Spain (de La Fuente et al. 1999.), but lower than in Africa (Peter et al. 2015) and Germany (Velez et al. 2019). This, apparently, reflects the influence of many factors, including the climate and the level of housing technologies used in farms (for example, individual boxes, hygienic conditions of calf housing places); physiological state of animals at the time of sampling (diarrhoeal and non-diarrhoeal); used research and coprological diagnostic methodologies.

Our surveillance in Northern Kazakhstan suggests that the infection rate of the calves at the first two weeks of age is higher than that of calves of the next two weeks of life, and this is consistent with other studies (Kváč et al. 2006, Peter et al. 2015, Velez et al. 2019). Both bivariate and multivariate linear regression models fitted this data. This is due to the fact that calves are born immunologically defenseless, and their immunity increases with time, so older animals can generate a more intense primary immune response than individuals under four weeks of age (Tizard 2009).

In Kazakhstan, cow-calf-management in middle sized and small farms and large beef farms is described by the continuous contact between calves and dams that means the development of natural colostral immunity in neonatal animals. Such technology was assessed as protective against *C.parvum* infection in both bivariate and multivariate analyses. Analogical results were shown in the Czech Republic. Here the incidence level of *Cryptosporidium* invasion showed a greater increase in dairy farms where newborn animals were grown individually from birth, in comparison with beef farms, where neonatal calves were kept with their dams (Kváč et al. 2006). In our research, the multivariate analysis has not shown a significant correlation between the large herd size farming and the risk of *C.parvum* infection. But in some studies, calves were more infected with *Cryptosporidium* sp. on farms with large cow herds (Garber et al. 1994). It was suggested that larger herds produce higher density of calf populations, which come to be infected with *C.parvum* and increased contamination of environment and, therefore, risk infection to humans.

In our analysis, several epidemiological factors were found to be significantly associated with infection by the bivariate analysis, and the associations were confirmed by the multivariate analysis. Results of the analysis (best fitting model with three independent epidemiological variables) can be explained as there is a correlation between prevalence of *C.parvum* and the age, the clinically appeared scour, and the type of calf rearing (without dam) of neonatal animals. The multivariate analysis suggests also that the significant risk factor favoring *C. parvum* infection is the housing of calves separated from their dams, whereas the practice of calves being nursed by dams may be protective.

CONCLUSION

Our studies have shown that the most statistically significant relationship is observed between prevalence of *Cryptosporidium parvum* and the age, the clinical conditions and the types of keeping management of neonatal calves, which could be the main source of infection for farm staff and the environment in Northern Kazakhstan.

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