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Herd-level risk factors for *Cryptosporidium* infection in dairy-goat kids in western France

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Abstract

We conducted a cross-sectional study of risk factors for herd-level kid positivity for *Cryptosporidium parvum* oocysts in dairy-goat farms (Deux-Sèvres, western France). From January to March 2003, faeces from a convenient sample of 879 5- to 30-day-old goat kids from 60 herds were examined microscopically after staining with carbol fuschin. Oocyst shedding was scored semi-quantitatively (0 to 4+) allowing us to obtain a cumulative score per herd. Standardized questionnaires with information about management practices were collected in each farm. We found positive kids in 32 of 60 herds (53.3%) and in 142 animals out of 879 (16.2%). We used logistic regression for two risk-factor model: (1) simple positive (case: herd score $\geq$1+, at least one positive kid in the herd, versus control: herd score = 0), (2) strongly positive (case: overall herd score $\geq$3+ versus control: herd score $<$3+). Risk factors associated with simple positive herds were period of sampling compared to the peak of births (After versus Before, OR = 4.2, 95% CI 1.2, 15.3) and practice of kid grouping by age or weight (Yes versus No, OR = 4.4, 95% CI 1.0, 19.1). Risk factors associated with strongly positive herds were period of investigation (February/March versus January, OR = 12.7, 95% CI 2.1, 76.6), exposure to graminaceous plants in forage (OR = 11.6, 95% CI 1.7, 81.0) and type of ventilation in the goat premises (Vertical versus Wind effect, OR = 14.7, 95% CI 2.1, 106.1). No important association was found between

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kid-management practices and herd positivity. These results suggest a major role of the environment of kids during their first hours of life in the adult-goat premises regarding the transmission of *C. parvum* infection.

Keywords: *Cryptosporidium parvum*; Dairy-goat farms; France; Logistic regression

1. Introduction

Cryptosporidium parvum infection is one of the principal enteropathogens in neonatal goats (De Graaf et al., 1999) and has been reported in French dairy goats since 1983 (Polack et al., 1983). In both natural and experimental conditions, cryptosporidial infection is associated with diarrhoea and mortality in kids aged 1–2 weeks (Nagy et al., 1984; Thamsborg et al., 1990; Koudela and Jiri, 1997; Vieira et al., 1997). As demonstrated in natural conditions in calves, oocyst excretion mainly occurs beginning on day 3–6 post-infection, continuing for 6–9 days and usually persisting at a detectable but asymptomatic level until 1 month of age (Olson et al., 1997; Uga et al., 2000; Castro-Hermida et al., 2002). However, clinical cryptosporidiosis occasionally occurs in goats >4 week old (Johnson et al., 1999). Because no fully satisfactory chemoprophylaxis is available to control neonatal cryptosporidiosis (De Graaf et al., 1999), a better knowledge of the main risk factors of *C. parvum* infection in kids is needed to allow the implementation of preventive hygienic measures both reducing the environmental oocyst burden and preventing the transmission to neonates. Such epidemiological information is relatively scarce in dairy-goat farms compared to farms with other ruminants except for some data from Spain (Matos-Fernandez et al., 1994) and from Poland (Majewska et al., 2000). Our aim was to assess herd-level risk factors for two different levels of oocyst shedding from kids.

2. Material and methods

We did this study between January and March, 2003.

2.1. Target population and sampling

The target population consisted of all dairy-goat farms of Deux-Sèvres, western France. The sampling frame contained herds from 850 owners (90% of all dairy-goat farms in Deux-Sèvres in 2003) who had a kidding period in winter.

A computed-generated list of 60 farms was selected (Epi Info Version 6.04) to be 95% confident that estimated herd-level prevalence was 40% ± 12.

Fifteen 5- to 30-day-old goat kids were selected from each herd as a convenience sample, based on a 95% confidence in selecting at least one positive kid, should the within-herd prevalence be 20% and test sensitivity (Se) be 50% (in Toma et al., 1996).

Faecal samples were collected directly from the rectum, identified and examined by microscopy after staining the smear with carbol fuschin. Slides were examined by a single

experienced microscopist who did not know the kid's values on any of the other variables. Oocysts were counted in 20 fields (1000×) and samples were recorded as negative when no oocysts was found.

Cryptosporidium oocyst shedding was scored semi quantitatively (0 to 4+) according to the number of oocysts per microscopic field: 0: absence of oocyst in 20 fields, 1+: <1 oocyst/field, 2+: 1–10 oocysts/field, 3+: 11–20 oocysts/field, 4+: >20 oocysts/field (Heine, 1982).

Although some faecal samples contain small numbers of non *C. parvum* particulates (Maldonado-Camargo et al., 1998), specificity (Sp) of Heine technique was assumed to have perfect animal-level specificity (leading also to perfect herd-level specificity). In contrast, the animal-level sensitivity is low because the method cannot detect light infections (Farrington et al., 1995).

2.2. Data collection

Standardized questionnaires with information about management practices were systematically collected. A personal interview of the farm owner/manager was performed during the visit. Factors hypothesized to be associated with the risk of *C. parvum* infection in kids were selected after a review of the scientific literature (Garber et al., 1994; Atwill et al., 1998; Maldonado-Camargo et al., 1998; De Graaf et al., 1999; Mohammed et al., 1999; Sischo et al., 2000; Huetink et al., 2001; Castro-Hermida et al., 2002). Factors were divided into three management categories: general, adult-goat and kid-goat management (Tables 1 and 2). The questionnaire was tested on five farms (not among the 60) to identify potential sources for misinterpretation of the questions and to further refine the questions. Individual data (age, sex, breed, presence of diarrhoea) were collected for each goat sampled.

2.3. Data analysis

Individual results of the Heine technique were added to obtain a score at the herd level (cumulative score). The outcome variables in the study were the case-control status of a herd in two situations: (1) simple positive (case: cumulative herd score ≥1+, at least one positive kid in the herd, versus control: cumulative herd score = 0); (2) strongly positive (case: cumulative score ≥3+, i.e. three kids with 1+ or one with 1+ and one with 2+ or one with 3+, versus control: cumulative herd score <3+).

The approach used to deal with large numbers of independent variables was to investigate potential associations fully between the independent and dependent variables. Factors that were associated ($p < 0.10$) with the likelihood of infection in the bivariable analysis were further considered in the multivariable analysis. The choice of a screening criterion of 0.10 was designed to limit multicollinearity but to ensure that potentially important variables were included in the next analytical step, the multivariable analysis (Dohoo et al., 1996).

The bivariable association between each hypothesized factor and each *C. parvum* infection situation (1, simple positivity; 2, strong positivity) was obtained from Student *t*-test, Wilcoxon test or Chi-square test depending on whether the independent variable had a continuous distribution (Gaussian or non-Gaussian) or categorical distributions. Normality of the continuous variables was tested using the Shapiro–Wilk test (Royston, 1995).

Table 1
Categorical variables offered for the multivariable logistic-regression models using the full data set of 60 dairy-goat herds (Deux-Sèvres, France, 2003)

Variable	Definition	Levels	No. of herds	Prevalence (%)	
				Simple positivity	Strong positivity
General management					
Period[a]	Season sampling	January	30	43	23
		February/March	30	63	53
Birth peak[a,b]	Sampling after the peak of births	No	23	35	22
		Yes	37	65	49
Breed	Breed type dominating	Saanen	29	45	34
		Alpine	31	61	42
Change	Change in herd size (in the last 5 years)	No	33	52	39
		Yes	27	56	37
Purchase	Goats purchased the previous year	No	49	49	35
		Yes	11	73	56
Separation 1	Separation goat/others ruminants	No	25	48	32
		Yes	35	57	43
Separation 2	Separation adults/kids housing	No	20	40	30
		Yes	40	60	43
Separation 3	Separation lactating goats/others	No	38	53	37
		Yes	22	55	41
Water[b]	Water source (municipal)	No	20	35	25
		Yes	40	63	45
Employee	Employee in the farm	No	49	51	37
		Yes	11	64	45
Adult goat management					
Autumn kidding[a]	Autumn kidding	No	42	50	29
		Yes	18	61	61
AI	Use of the artificial insemination	No	45	49	36
		Yes	15	67	47
Grouping[a]	Birth grouping	No	50	50	40
		Yes	10	70	70
Grazing	Use of pastures	No	53	53	40
		Yes	7	29	29
Leguminous	Leguminous plants in the diet	No	17	71	47
		Yes	43	47	35
Graminaceous[a]	Graminaceous plants in the diet	No	19	39	5
		Yes	41	61	51
Corn silage	Corn ensilage in the diet	No	38	55	39
		Yes	22	50	25

Table 1 (*Continued*)

Variable	Definition	Levels	No. of herds	Prevalence (%)	
				Simple positivity	Strong positivity
Cereals	Cereals in the diet	No	11	64	36
		Yes	49	51	39
Concentrates	Presence of commercial concentrates in the diet	No	15	33	9
		Yes	45	60	44
Floor	Type of floor in goat housing	Cement	19	42	26
		Ground	41	59	44
Wall[b]	Type of wall in goat housing	Wood/sheet	14	29	10
		Cement/stone/brick	46	61	43
Roof	Type of roof in goat housing	Sheet	41	51	37
		Wood	19	58	42
Insulation	Heat insulation in goat housing	No	52	52	40
		Yes	8	63	50
Ventilation[a,b]	Type of ventilation	Only wind effect	44	43	30
		Vertical (ridge tile)	16	81	63
Disinfectant	Regular use of disinfectant in goat housing	No	46	59	43
		Yes	14	38	21
Kid goat management					
Fattening	Fattening of the male kids	No	41	51	34
		Yes	19	58	47
Bovine colostrums	Distribution of bovine colostrum	No	53	57	40
		Yes	7	29	29
Colostrum bottle	Distribution of colostrum with feeding-bottle	No	43	47	33
		Yes	17	71	53
Colostrum bucket	Distribution of colostrum with bucket	No	45	51	36
		Yes	15	60	47
Milk dispenser	Distribution of milk with automatic milk dispenser	No	24	58	42
		Yes	36	50	36
Milk bucket	Distribution of milk with bucket	No	38	45	37
		Yes	22	68	43
Kid grouping[a,b]	Grouping of kids by age or weight	No	14	29	14
		Yes	46	61	46
Kid floor	Type of floor in kid housing	Cement	40	58	40
		Ground	20	45	35
Kid wall	Type of wall in kid housing	Wood/sheet	11	46	27
		Cement/stone/brick	49	55	41

Table 1 (*Continued*)

Variable	Definition	Levels	No. of herds	Prevalence (%)	
				Simple positivity	Strong positivity
Kid roof	Type of roof in kid housing	Sheet	25	48	28
		Wood	35	57	46
Kid insulation	Heat insulation in kid housing	No	46	54	39
		Yes	14	50	36
Auxiliary heating	Auxiliary heating in the kid housing	No	47	55	40
		Yes	13	46	31
Kid disinfectant	Regular use of disinfectant in kid housing	No	36	56	42
		Yes	24	50	33

[a] $p < 0.10$, therefore offered to the second multivariable model (strong positivity).
[b] $p < 0.10$, therefore offered to the first multivariable model (simple positivity).

Multivariable analysis consisted in a backward stepwise logistic regression with a procedure minimising the Akaike Criterion (AIC) (Akaike, 1974). The stepwise method based on AIC that we used was based on the stepAIC function (Venables and Ripley, 1999) (STEP procedure for glm models, R statistical software). The R function calculates the AIC according to the formula (R Documentation): AIC = $-2 \times$ log-likelihood + $k \times$ npar, where npar represents the number of parameters in the fitted model, and $k = 2$ for the usual AIC.

The starting candidate model was based on all of the predictors with $p < 0.10$ in the screening. Subsequent models were based on omitting a variable from the current candidate model or adding a variable that was not in the model, with the choice based on minimizing AIC. The final model was found when adding or omitting a variable did not reduce AIC further.

The linearity assumption for continuous variables selected for testing in the models was assessed by categorizing each continuous variable into multiple dichotomous variables of equal units and plotting each variable's coefficient against the midpoint of the variable. We also performed the Chi-squared test for trend in proportions (R statistical software).

For dealing with potential multicollinearity, the bivariable associations between factors retained and not retained in the logistic models were studied from Student *t*-test, Wilcoxon test, Chi-square test or Rank Correlation test depending on the distributions of the variables (Table 4).

3. Results

3.1. Descriptive epidemiology

In total, 879 goat kids were tested by Heine's technique. Of these, 142 (16.2%) were positive from 32 herds, out of a total of 60 herds sampled (53.3%). The number of goat kids

Table 2
Continuous variables offered for the multivariable logistic-regression models using the full data set of 60 dairy-goat herds (Deux-Sèvres, France, 2003)

Variable	Definition	Quartiles					Normality
		Minimum	25%	50%	75%	Maximum	
General management							
Goat	Number of adult goats	41	131	200	292	830	No
Cattle	Number of cattle	0	0	12	36	150	No
Sheep	Number of sheep	0	0	0	0	300	No
Adult goat management							
Kidding[a]	Duration of the kidding period (in months)	1	3	3	4	7	No
Surface[a,b]	Surface available per goat (in m^2)	0.8	1.2	1.5	1.7	2.4	Yes
Volume	Volume available per goat (in m^3)	3.1	8.1	9.8	13.7	36.2	No
Bedding	Adding bedding (number by week)	1	3	7	7	14	No
Daylight	Natural daylight in the goat housing (ratio transparent sheet surface/surface on the ground)	0.005	0.030	0.050	0.080	0.290	No
Change	Frequency of bedding change (number for year)	2	4	5	6	365	No
Kid goat management							
Dam	Contact duration between kids and their dam (in days)	0.1	0.2	0.5	3.0	10.0	No
Colostrum	Duration of colostrum feeding (in days)	0.2	1.0	2.0	7.2	30	No
Kid surface	Surface available for the kids (in m^2 per adult goat)	0.02	0.15	0.22	0.33	0.60	No
Kid volume[b]	Volume available for the kids (in m^3 per adult goat)	0.1	0.70	1.40	1.90	20.50	No
Kid bedding	Adding bedding (number per week)	2	7	7	7	14	No
Kid daylight	Natural daylight in the kid housing (ratio transparent sheet surface/surface on the ground)	0	0.01	0.03	0.07	0.48	No
Kid change[b]	Frequency of bedding change (number per year)	0.2	0.5	1.0	2.0	60.0	No

[a] $p < 0.10$, therefore offered to the second multivariable model (strong positivity).
[b] $p < 0.10$, therefore offered to the first multivariable model (simple positivity).

sampled was <15 in 7 farms (10 in 1 farm; 11 in 2 farms; 12 in 1 farm; 13 in 2 farms and 14 in 1 farm).

One hundred and three (11.7%), 24 (2.7%), 13 (1.5%) and 2 kids (0.2%) had respectively a score of 1+, 2+, 3+ and 4+.

Twenty three herds (38.3%) were classified as strongly positive (cumulative herd score ≥3+).

The highest prevalence for diarrhoea (51.9%) was detected in strongly positive herds compared with 17.0 and 8.1% in the low-positive (cumulative herd score <3+) and negative herds, respectively ($\chi^2 = 442$, $p < 0.001$).

3.2. Analytical epidemiology

Categorical and continuous variables are presented in Tables 1 and 2.

Four variables were retained in model 1 (simple positivity) and 5 in model 2 (strong positivity). Only one variable was selected in both models (Table 3).

Mainly retained factors were general management or adult goat management factors. Herds sampled in February/March (versus January) or After (versus Before) peak of births were at higher risk of infection. Risk also increased with characteristics of goat housing (wall in cement–stone–brick, vertical ventilation, large surface available by goat) and with breeding practices (birth grouping, forage with graminaceous plants) (Table 3). The only kid goat management factor identified was the practice of kid grouping by age or weight (Table 3).

Although water and kid change were not retained in model 1 (Table 3), each was associated with another risk factor that was retained (Table 4); so, these have not truly been ruled out as having association with simple herd positivity. Similarly, birth peak, autumn kidding, and kid grouping (Table 4) were associated with factors retained in the final version of model 2.

Table 3
Factors associated with simple[a] and strong[b] positivity to *C. parvum* in 60 dairy-goat herds (Deux-Sèvres, France, 2003)

Variable	*b*	*p*	Odds ratio	95% CI
Model 1 (simple positivity)				
Intercept	−3.13	0.002	–	–
Birth peak	1.46	0.02	4.2	1.2, 15.3
Wall	1.23	0.09	3.4	0.8, 14.1
Ventilation	1.46	0.08	4.3	0.8, 22.6
Grouping kids	1.48	0.05	4.4	1.0, 19.1
Model 2 (strong positivity)				
Intercept	−8.24	0.001	–	–
Period	2.54	0.005	12.7	2.1, 76.6
Birth grouping	1.41	0.13	4.1	0.7, 26.2
Graminaceous	2.45	0.01	11.6	1.7, 81.0
Ventilation	2.69	0.008	14.7	2.1, 106.1
Surface[c]	2.35	0.04	10.5	1.1, 98.8

[a] Model 1: residual deviance with 51 degrees of freedom: 63.76, AIC: 73.76.
[b] Model 2: residual deviance with 54 degrees of freedom: 48.40, AIC: 60.40.
[c] Linearity assumption for continuous variable (trend test, $\chi^2 = 4.96$, $p = 0.03$).

Table 4
Significant associations between factors retained and not retained in the multivariable logistic-regression models for *C. parvum* in 60 dairy goat herds (Deux-Sèvres, France, 2003)

Variable not retained	Variable retained	*p*	Direction of the association
Model 1 (simple positivity)			
Water	Birth peak	<0.01	Positive
Kid change	Ventilation	0.04	Negative
Model 2 (strong positivity)			
Birth peak	Period	<0.01	Positive
Autumn kidding	Period	0.01	Positive
Autumn kidding	Birth grouping	<0.01	Positive
Autumn kidding	Surface	0.03	Negative
Kid grouping	Graminaceous	<0.01	Positive

4. Discussion

Extrapolation of our results to kidding in the autumn should be done only with care. Many other agents (*Escherichia coli*, rotavirus, coronavirus) are involved in diarrhoea of neonatal ruminants. It would have been relevant to include these factors in our statistical analysis. However, this information in goat is extremely scarce (Millemann et al., 2003). In a few herds, the sample of 15 5- to 30-day-old kids was not achieved with an increased risk of misclassification in the negative group (sensitivity of the diagnosis at the herd level lower than the expected value of 0.95).

One of the objectives of our study was the identification and quantification of risk factors for *C. parvum* infection at the herd level. Many factors were investigated. In such a situation, the possibility of finding associations 'due to chance alone' goes up substantially (Dohoo et al., 1996). The other problem is multicollinearity which occurs when predictor variables are not statistically independent and which results in unstable estimates of regression coefficients in logistic-regression models (Dohoo et al., 1996).

Several variables eliminated by the backward-elimination process were highly collinear with variables retained by the modelling (Table 4). The information from the variables ''Birth peak'' and ''Period'' was probably redundant because the birth peak generally occurs before the end of the winter period (February/March). In the same way, ''Autumn kidding'' and ''Birth grouping'' variables included similar information because birth in autumn is allowed by non natural methods (photoperiod control or hormonal treatments) on groups of goats. To simplify our statistical approach, we could have preserved only one of the redundant variables, and discarded the other one, before the backward-elimination process. On the other hand, no obvious relationship appeared between the variables ''Water'' and ''Birth peak'' on the one hand and ''Kid grouping'' and ''Graminaceous'' on the other hand.

Our strategy for reducing the number of independent variables was to screen potential predictor variables using simple (unconditional) statistics and then select a subset of independent variables for inclusion in the final analysis. Another approach would be to create indices or scores which combine data from multiple factors into a single variable or to create a smaller set of independent variables through the use of multivariable techniques

such as principal-components analysis or factor analysis (Dohoo et al., 1996). However, those techniques do not assess the statistical significance of the direct associations between specific independent variables and the dependent variable.

Variable-selection methods attempt to balance the goodness-of-fit of a model with considerations of parsimony. However this is done, in theory one could look at all possible logistic-regression models to find the ''best'' one, but this becomes computationally prohibitive when p is large. For this reason, it is more classical to use a stepwise procedure, where candidate models are based on adding or removing a term from the current ''best'' model.

A flaw in this approach is that it does not directly address the crucial balance of goodness-of-fit versus parsimony. Akaike (1974) proposed a measure (AIC) based on information considerations that explicitly quantifies this balance. Model selection using AIC does better, more often resulting in improved performance relative to using the full logistic-regression model, particularly for smaller samples sizes (Perlich et al., 2003).

We acknowledge that we did not have random sampling at each step of our selection process. However, the specificity was good enough that we are confidant that, where positive kids were found, the herds truly were infected. The problem remaining is that because of uncertain sensitivity and the convenient sampling of kids, there might also be infected herds among those used as controls in our models. The effect, we feel, would have been to diminish the contrast between the control and the positive herds. With such large ORs as we found especially in the model for strong positivity, we believe that we have identified a few risk factors worthy of additional examination.

The period of sampling was linked to the risk of *C. parvum* infection with February/March period being more at risk than January (OR = 12.7). Monthly variations in prevalence were also recorded by Bourgoin (1996) and Lefay et al. (2000) in cattle in France and by Causape et al. (2002) in lambs in Spain. One of the likely explanations is the hygienic degradation of the neonates' environment and the simultaneous increase in disease incidence during the whole kidding or calving period especially when parturition is highly seasonal. This is particularly the case in dairy-goat breeding where kidding period mainly extends from November to March with the neonates' premises not being cleaned during this period. Otherwise, changes in crowding of premises or multiple peaks of newborn animals may account for seasonal variations in *Cryptosporidium* oocyst shedding (Huetink et al., 2001).

When examining the risk factors of *Cryptosporidium* infection in farms, none of the parameters related to the management of goat neonates, except the practice of kid grouping by age or weight, was significant in our study. This surprised us, because an extensive previous study of Atwill et al. (1998) performed in a similar system where dairy calves are immediately separated from their dams brought some evidence of the key role of environment in the contamination with *Cryptosporidium*. Those authors demonstrated the role of the floors and the walls of calf hutches in the transmission of cryptosporidial infection to neonates whereas the dam excretion was undetectable. Similarly, significant relationships between some calf-environment factors such as ventilation, frequency of disposal or addition of bedding, feeding, calf-to-calf contact and the odds of shedding *C. parvum* oocysts have been described (Maldonado-Camargo et al., 1998; Mohammed et al., 1999; Sischo et al., 2000).

In contrast, other results suggest an important role of the dam in the direct contamination of their offsprings. According to both the prepatent period of *C. parvum* infection and the breeding conditions of dairy calves in the Netherlands (immediate removal of calves from their dams, rearing in individual hutches), Huetink et al. (2001) considered that direct or indirect cow-to-calf transmission was probably the most important route of infection followed by indirect calf-to-calf transmission through vectors (caretakers, insects). Asymptomatic adult cattle or sheep can be inapparent carriers especially around parturition when an increase in oocyst output generally occurs (Xiao et al., 1994; Ortega-Mora et al., 1999; Ralston et al., 2003; Faubert and Litvinsky, 2000). Similarly, >25% of the goats >1 year old shed oocysts (Noordeen et al., 2001). Thus the risk factors we found (which mainly involved adult-goat management) have to be interpreted in relation with oocyst contamination, i.e output and persistency in adult premises. The vertical ventilation (ridge tile) appeared as a risk factor and this might be related to a more efficient system (compared to horizontal ventilation) leading to a paradoxically more favourable environment for the oocyst survival due to a lower level of ammonia in the litter (Jenkins et al., 1999; Walker et al., 1998). We also found that the type of the diet given to the adult goat was associated with an increased risk of *C. parvum* infection prevalence in kids. Forages might be involved through a direct (contaminated feed) or an indirect effect. Very little information is available on survival of oocysts in forage except the data of Merry et al. (1997) on ryegrass ensilage showing a 30% survival after 3 months. Except for contamination with rodent or pet faeces after collection, the survival of occysts on hay is expected to be very low if any because of the dryness of such a material. The impact of graminaceous versus leguminous in the diet on oocyst excretion remains to be investigated.

Knowledge of transmission routes of *C. parvum* infection remains essential for the development of a control program on farms. Our results suggest a major role of the environment of the first hours of kids life regarding the transmission of *C. parvum* infection in the adult-goat premises.

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