PREVALENCE OF FAILURE OF PASSIVE TRANSFER OF IMMUNITY IN DAIRY CALVES IN THE CZECH REPUBLIC

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Abstract

Prevalence of failure of passive transfer (FPT) of immunity remains relatively high worldwide. The aim of this study was to estimate the FPT prevalence in Czech dairy calves and to evaluate the selected factors – breed, herd size, sex of calves, single versus twin births and the influence of the season of birth. A total of 1,175 serum samples were taken from calves of Czech Fleckvieh and Holstein breed from 33 herds between October 2015 and October 2017. Serum IgG concentration was determined by reference method for IgG determination – radial immunodiffusion. Statistical evaluation was performed by Kruskal-Wallis test. The concentration of IgG ranged from 1.5 to 46.6 g/L with average value 13.7 g/L and was significantly influenced by breed, size of the herd and season. Using the criterion IgG < 10 g/L, it was found that 34.6 % of calves had FPT. The prevalence of FPT by breed was 42.9 % vs. 24.2 % (Czech Fleckvieh vs. Holstein), by size of the herd 45.0, 44.4, 25.5 and 22.0 % (< 200, 200–399, 400–599 and ≥ 600 cows per herd, respectively) and by season 25.3, 34.6, 29.9 and 52.5 % (spring, summer, autumn and winter, respectively). The sex of calves was not found to be a statistically significant factor. The study in newborn calves showed that FPT is still an important problem in Czech dairy herds, especially in the Czech Fleckvieh breed. In smaller herds and especially in the winter, the prevalence of FPT was very high.

Keywords: calves, failure of passive transfer, immunity, immunoglobulin G, radial immunodiffusion

INTRODUCTION

Calves are born agammaglobulinemic and, therefore, they are completely dependent on the passive transfer of immunity from colostrum (Weaver *et al.*, 2000; Godden, 2008; Beam *et al.*, 2009; Morrill *et al.*, 2015). Colostrum is the initial secretion from the mammary gland after parturition and represents a key source of immunoglobulins, nutrients, growth factors and other components important for newborn calves (Bielmann *et al.*, 2010). Good quality colostrum provides passive immunity to the calf, if ingested in the short period of macromolecular transport during the first 24 h of life (Davenport *et al.*, 2000). There are several factors influencing the successful passive transfer of maternal immunity, mainly the volume of ingested colostrum, timing of colostrum feeding, immunological and microbiological quality of colostrum (McGuirk and Collins, 2004; Godden, 2008). An adequate amount of IgG required by calf is > 100 g of IgG/ dose to achieve satisfactory transfer of passive immunity, meaning serum IgG concentration of > 10.0 g/L at 24 h after birth (Quigley *et al.*, 2001). This should be ensured when the calf receives the amount of colostrum that corresponds to 8.5 % of its body weight (Conneely *et al.*, 2014), which means the amount of about 4 litres of colostrum in large breeds of dairy cattle. Failure of passive transfer (FPT) occurs when calf does not ingest a satisfactory amount of maternal antibodies, resulting in a serum IgG concentration of less than 10 g/L (Godden, 2008; Radostits *et al.*, 2007). The standard examination of passive transfer of immunity in calves is the measurement of IgG in serum by radial immunodiffusion – RID (Beam *et al.*, 2009). As alternative method for rapid and simple monitoring of passive transfer was used serum total protein (TP) estimation with limit of ≤ 50 g/L assed by refractometry (Vogels *et al.*, 2013; Lawrence *et al.*, 2017) or TP ≤ 52 g/L determined photometrically (Cuttance *et al.*, 2017) or plasma TP < 56 g/L assed by refractometry (MacFarlane *et al.*, 2015). Failure of passive transfer of maternal immunity is connected with negative effect on health, morbidity and mortality (e.g. Lora *et al.*, 2018) and long-term negative effects of FPT on future productivity of dairy cows (Faber *et al.*, 2005). Despite all this knowledge, the FPT prevalence remains relatively high worldwide, e.g. 19.2 % in the US (Beam *et al.*, 2009), 24.8 % in New Zealand (Lawrence *et al.*, 2017), 26 % in the United Kingdom dairy farms (MacFarlane *et al.*, 2015), 38 % in Australian dairy herds (Vogels *et al.*, 2013) and 43.5 % in Swiss dairy herds (Reschke *et al.*, 2017). The reported range of prevalence of FPT on farms involved in studies is very wide, e.g. from 5 to 83 % in the New Zealand survey (Cuttance *et al.*, 2017).

Current research also deals with the risk factors associated with insufficient passive transfer, mainly poor colostrum quality (Beam *et al.*, 2009; Abdel-Salam *et al.*, 2014; Cabral *et al.*, 2016; Elsohaby *et al.*, 2016; Reschke *et al.*, 2017), inconvenient management factors (e.g. Vogels *et al.*, 2013, Cuttance *et al.*, 2017) and additional risk factors, for example breed, herd size, housing system, month of calving, duration of gestation, dry period length, parity of the dam or individual farm influence (McGuirk and Collins, 2004; MacFarlane *et al.*, 2015; Reschke *et al.*, 2017).

The aim of this study was to estimate the prevalence of FPT in calves from Czech dairy herds and to evaluate the selected factors e.g. breed, herd size, sex of calves, single versus twin births and the influence of the season of birth.

MATERIALS AND METHODS
Animals

Serum samples from calves were collected from 33 dairy farms in the Czech Republic from October 2015 to October 2017. Mean herd size was 415 (± 258) cows (median 310). Altogether 1,175 samples of calf serum (Tab. I) were obtained, with 651 samples (55.4 %) of the Czech Fleckvieh breed (C) and 524 samples (44.6 %) of the Holstein breed (H). The involved farms were divided into four groups: small (< 200 cows), medium (200–399 cows), large (400–599 cows), and very large (≥ 600 cows) herds to evaluate the impact of herd size on the status of immunity in calves. The numbers of calf serum samples from different groups of herds were as follows: 171 samples from small herds, 432 samples from medium herds, 322 samples from large and 250 samples from very large herds. There were 542 samples from heifers, 603 samples from bulls and 30 samples from twins of the same or different genders. The four seasons of the year were astronomic seasons.

Collection of serum samples

Blood samples were collected by local veterinarians as part of the calves' health check. All blood samples of calves were taken by jugular venepuncture at 1 to 6 days of life. Blood samples were collected into disposable plastic test tubes without the addition of anticoagulants. After the blood was coagulated, the samples were

centrifuged and the blood serum was frozen at –20 °C until laboratory examination.

Laboratory examination

Blood serum samples were transported into our laboratory at the Veterinary Research Institute in Brno (Department of Immunology) in freeze boxes (–20 °C) and then analysed. Serum IgG concentrations were determined by radial immunodiffusion (RID) as the reference method, in a modified way to the form described by Krejčí *et al.* (2016). RID plates were prepared by dissolving 1.5 % agarose in phosphate buffered saline and boiling in a water bath. Rabbit antibovine IgG (1 %) was added to the agarose solution tempered to 56 °C, and 22 mL of the final solution was added to 20 cm diameter Petri dishes. After the agarose had solidified, 2.5 mm diameter wells were cut-off in the agar. Serum samples were thawed at room temperature, vortexed for 10 s and diluted 1 : 20 with deionized sterile water, then 5 µL of each sample, was pipetted into a well. The diameter of the zone of precipitation was recorded after 24 hours of incubation at 23 °C. Sample IgG concentrations were determined by comparing the diameters of zones of precipitation with a standard curve generated with serial dilutions of bovine IgG standard. Calves were classified as having FPT if the IgG level was < 10 g/L, passive transfer status was classified as adequate and excellent with IgG range of ≥ 10 to < 15 g/L and ≥ 15 g/L, respectively (USDA, 2010).

Statistical analyses

Descriptive statistics of all obtained data were calculated. The normality of the data distribution was assessed using the Saphiro-Wilk test. Because the data of IgG concentration in serum was not normally distributed (Fig. 1), nonparametric tests (Kruskal-Wallis) were used for the statistical evaluation. Statistical analyses were performed using Statistica 10 software (2011; Statsoft Inc., Tulsa, OK).

I: *Descriptive characteristics of serum IgG levels in Czech newborn calves*

| Survey item	Samples		Immunoglobulin G (g/L)				
	No.	%	Mean	Median	Min.	Max.	SD
All herds	1175	100	13.7	12.5	1.5	46.6	7.5
Breed							
Czech Fleckvieh	651	55.4	12.5[a]	11.4	1.5	43.1	7.8
Holstein	524	44.6	15.1[a]	14.4	1.5	46.6	7.0
Herd size (cows per herd)							
< 200	171	14.5	12.7[a,b]	11.2	1.5	46.6	8.9
200–399	432	36.8	14.4[c,d]	10.8	1.5	36.5	7.7
400–599	322	27.4	14.9[a,c]	13.4	1.9	43.1	7.0
≥ 600	250	21.3	15.1[b,d]	14.7	1.8	33.1	6.3
Sex/number of calves							
Heifers	542	46.1	13.7	12.5	1.5	43.1	7.6
Bulls	603	51.3	13.7	12.7	1.5	46.6	7.5
Twins	30	2.6	12.4	11.4	1.5	29.4	6.4
Season							
Spring	194	16.5	14.8[a]	14.0	1.9	33.5	6.9
Summer	191	16.3	14.9[b]	13.5	1.6	41.7	8.6
Autumn	548	46.6	14.4[c]	13.0	1.5	46.6	7.4
Winter	242	20.6	10.3[a,b,c]	9.5	1.5	33.6	6.5

SD–Standard deviation; the same letters in individual parts show statistical significant difference between groups a, b, c, d P < 0.001

RESULTS

Levels of IgG in 1,175 evaluated serum samples ranged from 1.5 to 46.6 g/L (Fig. 1), the mean IgG content was 13.7 g/L in all tested samples and median was 12.5 g/L (Tab. I). Poor level of IgG saturation, meaning FPT (< 10 g/L IgG), was found in 34.6 % of calves (Tab. II), adequate saturation (IgG ≥ 10 and < 15 g/L) was found in 26.5 %, and

excellent saturation (≥ 15 g/L IgG) was found in 38.9 % of calves.

Prevalence of FPT (Tab. II) was higher in Czech Fleckvieh calves than in Holstein calves (42.9 % vs. 24.2 %). Significantly lower levels of IgG (P < 0.001) were found in calves of C breed – mean 12.5 g/L of IgG compared to calves of H breed – mean 15.1 g/L (Tab. I; Fig. 2). Statistically significant (P < 0.001) were also the differences in both
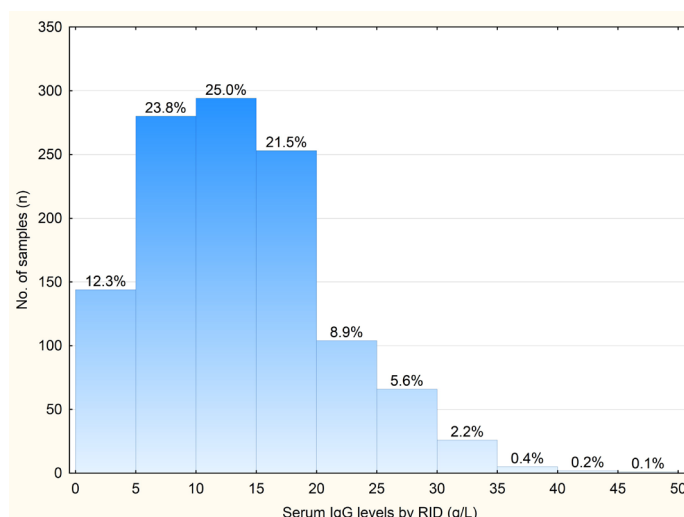
1: *Distribution of IgG levels in Czech newborn calf serum determined by radial immunodiffusion (RID)*

II: *Prevalence of failure of passive transfer (FPT) according to different factors*

Survey item	No. of samples	IgG < 10 g/L (FPT)		IgG ≥ 10 g/L	
		No.	%	No.	%
All herds	1175	406	34.6	769	65.4
Breed					
Czech Fleckvieh	651	279	42.9	372	57.1
Holstein	524	127	24.2	397	75.8
Herd size (cows per herd)					
< 200	171	77	45.0	94	55.0
200–399	432	192	44.4	240	55.6
400–599	322	82	25.5	240	74.5
≥ 600	250	55	22.0	195	78.0
Sex/number of calves					
Heifers	542	192	35.4	350	64.6
Bulls	603	203	33.7	400	66.3
Twins	30	11	36.7	19	63.3
Season					
Spring	194	49	25.3	145	74.7
Summer	191	66	34.6	125	65.4
Autumn	548	164	29.9	384	70.1
Winter	242	127	52.5	115	47.5

breeds when the gender of the calves was used as the categorical variable. The heifers of the C breed had significantly (P < 0.001) lower IgG levels (mean 12.8 g/L; n = 300) than the heifers of the H breed (14.8 g/L IgG; n = 242) and bulls of C breed had significantly (P < 0.001) lower serum IgG content (mean 12.4 g/L; n = 333) than bulls of H breed (mean 15.4 g/L; n = 270).

Concerning the herd size (Tab. I; Fig. 3), the test revealed significant (P < 0.001) differences between small and medium herds in comparison with large and very large herds. Numerically, the mean IgG content grew with the herd size, the highest IgG level was found in calves from very large herds (mean 15.1 g/L) but there were no significant differences between small and medium or between the large and very large herds. FPT prevalence in calves from small and medium herds (FPT 45.0 % and 44.4 %, respectively) was almost twice as high as that in calves from large and very large herds (25.5 % and 22.0 %, respectively) (Tab. II).

In addition, the influence of sex of calves and the number of siblings (heifers, bulls or twins of the same or different gender) on the serum IgG level was evaluated, but this variable was not found to be statistically significant (P = 0.28). The mean IgG level of 13.7 g/L was found in heifers and bulls, while in the twins the mean IgG level was 12.4 g /L (Tab. I; Fig. 4). The proportion of calves with FPT was very similar in bulls, heifers and twins ranging from 33.7 – 36.7 % (Tab. II).
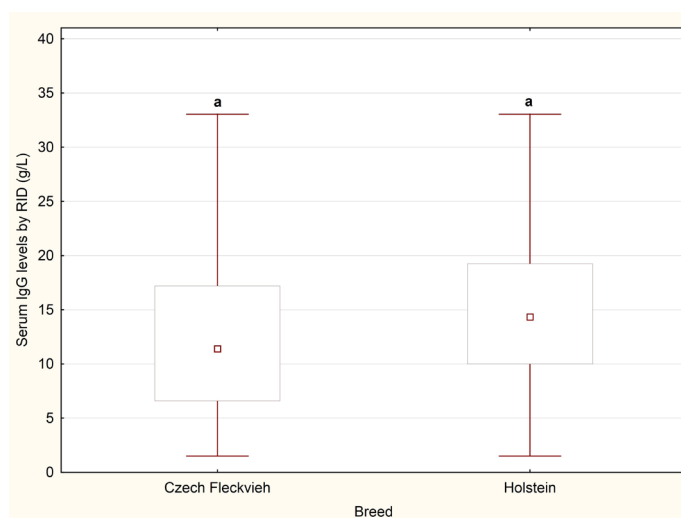
2: *IgG levels in Czech newborn calf serum by breed*
[a]P ≤ 0.001, the same letters show statistical significant difference between groups.
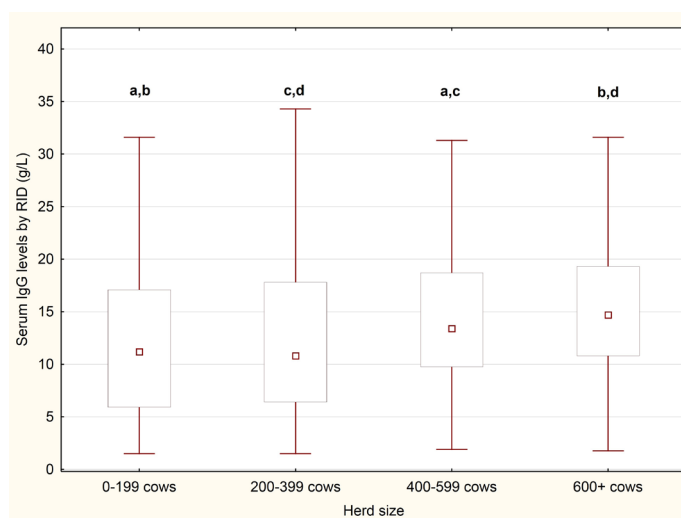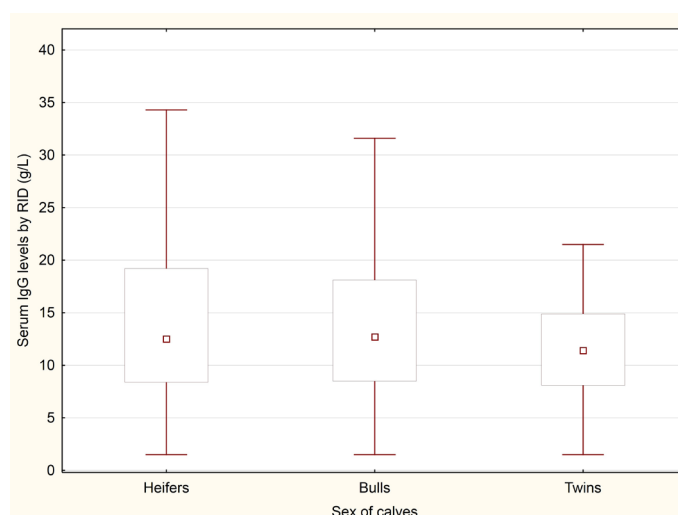

3: *IgG levels in Czech newborn calf serum by herd size*
[a,b,c,d]P ≤ 0.001, the same letters show statistical significant difference between groups.

Finally, the factor of the year season when the calves were born was considered. The significantly lowest level of serum IgG was found in calves born in winter (mean 10.3 g/L) compared to calves born in spring, summer and autumn (mean IgG level 14.8, 14.9 and 14.4 g/L, respectively) (Tab. I; Fig. 5). As expected, the proportion of FPT was higher in calves born during winter months (52.5 % of calves) than in those born in spring (25.3 %), summer (34.6 %) or autumn (29.9 %) months (Tab. II).

DISCUSSION

The results of this and previous studies (Podhorský *et al.,* 2007; Šlosárková *et al.,* 2014) showed that transfer of passive maternal immunity in Czech dairy herds is still an important problem. The current study indicated the FPT (serum IgG < 10 g/L determined by the RID assay, the first study in the Czech Republic based on this reference method) in 34.6 %, while Beam *et al.* (2009) published a lower value of FPT prevalence in US dairy herds determined by RID assay – 19.2 % (serum IgG < 10 g/L). When comparing other levels of the achieved passive immunity, good saturation (IgG ≥ 10 and < 15 g/L) was achieved in 26.5 % calves in our study, whilst only in 14.1 % of US calves (Beam *et al.,* 2009). However, excellent IgG saturation was achieved in 38.9 % of Czech calves, whereas in 66.7 % of US calves (Beam *et al.,* 2009). Similarly, the lowest FPT prevalence assessed by RID, 4.75 % was published by Deelen *et al.* (2014) in 5 Canadian herds. In their study, the mean IgG

4: *IgG levels in Czech newborn calf serum by sex of calves*
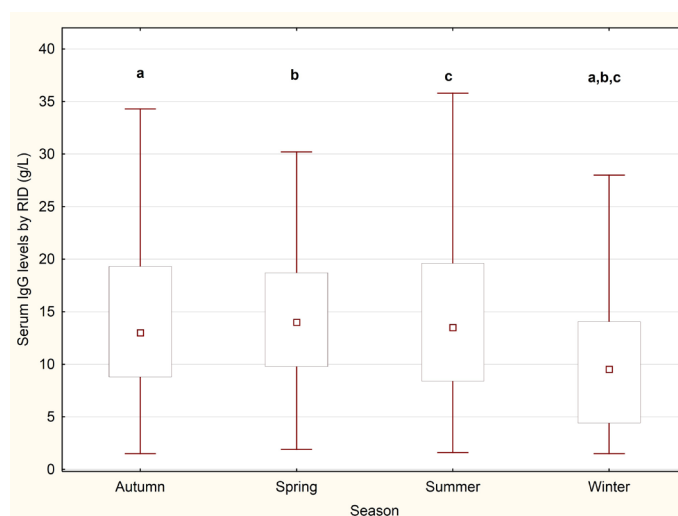
5: *IgG levels in Czech newborn calf serum by the season of birth*
[a,b,c]P ≤ 0.001, the same letters show statistical significant difference between groups.

concentration was 24.1 g/L with a range from 2.1 to 59.1 g/L. Our survey showed a similar range of IgG concentration (1.5 to 46.6 g/L), but with much lower mean value of IgG 13.7 g/L. The FPT prevalence found in our study is closer to the results of the studies that examined serum TP to estimate passive transfer status, e.g. 38 % calves with FPT found in Australian dairy herds (Vogels *et al.,* 2013) and 24.8 % in New Zealand (Lawrence *et al.,* 2017), both determined by refractometric evaluation of serum total protein (FPT = TP < 50 g/L). Using higher cut-points of serum TP of 52 and 57 g/L assed by refractometry, the incidences of FPT were 11 and 32 %, respectively in 19 Canada and USA herds (Windeyer *et al.,* 2014). The lowest prevalence of FPT indicated by TP evaluation (FPT = TP < 52 g/L), 8.4 % showed Trotz-Williams *et al.* (2008) in their first study (11 farms) contrary to their second study (112 farms) with FPT 37.1 %.

Even higher prevalence of FPT than in our survey was published by Reschke *et al.* (2017) in Swiss dairy herds, namely 43.5 % determined by electrophoresis. Using the same method, Lora *et al.* (2018) reported exactly the same percentage of FPT prevalence as we found in our present study (34.6 %) for dairy calves from three Italian farms.

As it is shown, there are big differences not only between different countries but also between published results from the same country. We suppose that one of the reasons for these differences is the selection of farms included in the studies and thus different colostrum management. The next factors could be e.g. number of evaluated samples, season or method which was used for the evaluation of FPT. With the aim to analyse some factors which can influence the occurrence of FPT in Czech dairy herds, we analysed some animal husbandry factors. According to our results, the occurrence of FPT was significantly influenced by breed, herd size and season.

In our survey, lower occurrence of FPT was shown in Holstein calves versus Czech Fleckvieh calves (24.2 % vs 42.9 %). Similarly as we did, significant differences between various breeds were found by Vogels *et al.* (2013), both at the calf and herd level. They reported that Jersey and Jersey-cross calves were less likely to have FPT than Holstein calves (26.9 % and 30.7 % versus 42.6 % determined by the same serum TP cut-off level). These authors assumed that it could be related to the Jersey' smaller size, as plasma volume (and hence absolute mass of immunoglobulin requirement) is closely related to body weight. Their results, and thus also interpretation, are questionable because the more recent study (McCraken *et al.,* 2017), found that

Jersey calves need a different, i.e. – lower cut-off level of TP for indication of FPT. Thus our differences found between breeds based on the reference method - RID assay are more representative. Another explanation can be the different quality of colostrum. The important role of this factor supports our previously published survey of colostrum quality in these Czech dairy herds where we found a higher IgG content in the colostrum of Holstein herds (median 80.3 g/L IgG) versus the Czech Fleckvieh herds (median 74.4 g/L IgG). As expected, similar differences were also found in the proportion of inadequate quality colostrum samples, i.e. 19.5 % for Holstein colostrum and 25 % for Czech Fleckvieh colostrum (Staněk *et al.,* 2017b). All of these findings are consistent with another fact revealed previously, that the quality of colostrum administered to the calves is routinely evaluated in 50.9 % of Holstein farms, but only in 39.0 % of Czech Fleckvieh farms in the Czech Republic (Staněk *et al.,* 2014).

According to our study, the effect of herd/farm size on the FPT prevalence was found to be significant. In calves from small and medium herds, FPT prevalence was nearly twice as high (45.0 % and 44.4 %) as that in calves from large and very large herds (25.5 % and 22.0 %). The same variable was also considered in the study of Vogels *et al.* (2013), but no significant differences were found. Only a slightly higher prevalence of FPT was found in calves born in large (501–700 cows) and very large (> 700 cows) herds, 46.4 % and 39.5 %, in comparison with the reference group (151–300 cows) 35.8 % found in medium operations. Similarly USDA (2010) reported no distinctive differences in FTP prevalence in US dairy heifers according to herd size (19.4 % in small herds with < 100 cows per herd vs. 22.1 % in large herds with ≥ 500 cows per herd). The reason why bigger herds in our study had a lower occurrence of FPT is not completely clear. We suppose that one of the factors could be that on large farms, there is a specialized farm staff, that take care mainly/only for the parturitions and newborn calves, including colostrum management. This specialization may also involve personal economic motivation which can be an important factor in the care for newborn calves.

The sex of calves is one of the many factors believed to play a critical role in FPT incidence (McGuirk and Collins, 2004). We also investigated the influence of sex of calves on the serum IgG level in the present research, but based on the direct IgG examination by RID method, this variable was not found to be statistically significant, as the proportion of calves with FPT was very similar in heifers, bulls

and twins, ranging from 33.7 %–36.7 %. The same conclusion was reached by Trotz-Williams *et al.* (2008), who found no statistically significant difference in passive transfer status in 1 day old or older bull and heifer calves based on the serum TP levels examination. However, opposite result was found by Vogels *et al.* (2013). They found the sex of calves associated with agammaglobulinaemia using multivariable analysis. Bull calves had twice the odds of agammaglobulinaemia as heifer calves. FTP prevalence according to serum TP levels (limit < 50 g/L) was 36.6 % in heifer and 44.0 % in bull calves. The explanation can be that, contrary to our study, the attitude to newborn calves according to their sex was partially different in their study.

The last factor which had a significant effect on the occurrence of FPT in calves was the season of the year. The absolutely highest prevalence of FPT, 52.5 % was found in calves born during the winter months vs. 25.3 %, 34.6 % and 29.9 % in calves born during spring, summer and autumn months. These results are not in agreement with the findings of Reschke *et al.* (2017). They found the highest prevalence of FPT in calves born during the spring and summer months (35.4 %–43.8 %). Also, in the study of Morin *et al.* (2001), the month of calving markedly affected the colostrum quality (specifically colostral specific gravity values), with the highest values occurring in autumn and the lowest values in summer. These findings correspond to the hypothesis that high ambient temperature during late pregnancy is associated with poorer colostrum composition and therefore worse maternal immunity transfer to the calves (Godden, 2008). The main causes of higher occurrence of FPT are low quality of colostrum and colostrum feeding pattern. Taken into account our survey of colostrum quality in Czech dairy herds (Staněk *et al.*, 2017a), where we found the highest IgG content in the colostrum in autumn and winter (significant difference in comparison with spring) we can say that colostrum quality was not the reason for the higher prevalence of FPT in winter in this study. We assume that main reason was the failure of human factor and the feeding pattern, probably less comfortable work during the hard winter months and thus worse care for the newborn calves.

CONCLUSION

The first Czech study which used a reference method for IgG determination showed that FPT is still an important problem in dairy calves, especially in the Czech Fleckvieh breed. Prevalence of FTP was also significantly associated with the size of the herd and the season of the year. In smaller herds and especially in the winter, the prevalence of FTP was very high. Further focus on colostrum management in Czech dairy cattle would be desirable.

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