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Full Length Article

An Epidemiologic Study of Retained Fetal Membrane in Dairy Cows on Two Dairy Herds in China

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Abstract

In this research, the influencing factors of retained fetal membrane on dairy cattle on two dairy herds (herd No. 1 and No. 2) from Hebei Province in China were evaluated. The distribution of retained placenta was compared between the two dairy herds by means of Chi-square test and then analyzed by means of logistic regression of linear models. The following factors in the first lactation were not significant (P>0.05) or not associated with retained fetal membrane: stillbirth, twins, calving difficulty, previous abortion, milk fever. Twins were not significantly associated to retained fetal membrane (P>0.05) in the second lactation. Stillbirth, twins, calving difficulty, previous abortion and milk fever (P<0.01) in second lactation were significantly associated to retained fetal membrane. Univariate analysis showed that there was a significant correlation between risk factors for retained placenta. The variables for stillbirth and milk fever were associated with retained fetal membrane. The combined effect of calving season and previous abortion for retained fetal membrane was examined and the results displayed that cattle calved during hot months and had a history of previous abortion were 22 times higher possibility to experience retained placenta, by comparison of cows that calved in non-hot months and without a history of previous abortion. Therefore, Stillbirth, calving season, previous abortion and milk fever are the risk factors associated with retained fetal membrane. © 2019 Friends Science Publishers

Keywords: Dairy cattlel; Epidemiology; Retained placenta; Risk factors

Introduction

6-8 h after delivery, almost 77% of the cattle excreted membranes (Boro *et al.*, 2015). Retention of fetal membranes (RFM) is a condition in which membranes are not excreted within 12-24 h after delivery (Fourichon *et al.*, 2000). RFM is a major key factor for metritis and clinical endometritis (Maizon *et al.*, 2004; Gautam *et al.*, 2009), which is related to tissue injury, delayed uterine recovery, endometrial dysfunction and ovarian cycle disorder (Sheldon *et al.*, 2002; Herath *et al.*, 2009). 25% of RFM-bearing animals will suffer from endometrial inflammation (Opsomer *et al.*, 2000). The main reasons for reduced fertility are slower uterine involution and ovarian cycle recovery (Drillich *et al.*, 2003; Maizon *et al.*, 2004). The incidence of RFM in dairy cows is 5-10% with an average prevalence of 8.6% (Kelton *et al.*, 1998; LeBlanc, 2008). Collagenases play an important part in the elimination of fetal membrane, by destroying peptide bonds in collagen (Sheldon *et al.*, 2002). The economic losses due to RFM are serious and the causes of RFM are complicate. The purpose of the present study is to evaluate the critical factors related to the pathological condition of retained fetal membranes on two commercial cow herds in China.

Material and Methods

Two dairy herds (herd No. 1 and No. 2) from Hebei Province in China were included in the present study. The annual average temperature of hot months in Herd No. 1 was 27.2°C in 2015. The herds' cattle were feed in the light of the farm protocol developed by the boss and veterinarians of herd No.1. The annual average temperature of hot months in Herd No. 2 was 26.2°C in 2015. Cattle were feed on the basis of the scheme developed by the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products.

The same reproductive management protocols were used in Herd No. 1 and No. 2 for this study. Cows calved from January 1, 2015 to December 31, 2015 and within 30 d after their calving in herd No. 1 (n= 2184) and in herd No. 2 (n= 1649) were considered for inclusion in the study. In both study herds, diets were formulated on the basis of TMR (total mixed ration).

The temperature of each cow was taken in the first 10 d postpartum. Cows with rectal temperatures greater than 39.5°C are considered to have fever. Feverish cattle or cows that appeared to be sick but have no fever are checked to preclude mastitis, ketosis, metritis, and displaced abomasum.

Milk fever is mainly the disease of calving cows before birth. Typically, cows get excited at an early stage, as well as tremors in muscles of the head and limbs, after that the cows stumble and kneel down slowly and lie flat on their side, eventually the cows would shock and death. Acute puerperal metritis was investigated when the cattle had stench, reddish brown, watery vaginal discharge and rectal temperature ≥ 39.5°C within the first 10 DIM. Cows were then treated according to the established farm agreements. Cows with abnormal delivery (with or without RFM dystocia) should be carefully monitored because they are more susceptible to metritis, fever and hypocalcemia.

The present study was designed as a case-control study. Cows diagnosed and treated for RFM during the first 30 d postpartum were selected as case cows. Cows non-affected with RFM during the first 30 d post-partum were classified as control cows.

Farm records were used to collect the following data: herd identification, cow identification number, lactation number, previous history of abortion, calving date, calving difficulty (yes, no), twins (yes, no), diagnosis of milk fever (yes, no; date), diagnosis of metritis (yes, no; date) and diagnosis of clinical mastitis (yes, no; date).

The incidence risk for RFM was calculated for the two farms. The distribution of RFM was compared between farms by means of Chi-square test of association and then analyzed by means of logistic regression of linear models. The risk ratio (RR) was used an epidemiologic measure of association between an explanatory variable (*i.e.*, Retention fetal membranes) and the outcome of interest (previous abortion) in the present study. Descriptive statistics of qualitative and quantitative variables were calculated, before odds ratios (OR) were estimated using logistic regression. The risk factors tested were: herd identification, previous abortion, calving difficulty, calving season, stillbirth, twins and milk fever. The model-building strategy involved four steps. In the first step, all variables were screened using univariable conditional logistic regression with the data grouped by herd. When the subsequent univariable analysis of investigated exposure factors related to retained fetal membrane in first lactation cattle that calved in two commercial dairy herds had been finished and then their second or later lactations dairy cows were analyzed. The third step of analysis is the correlation matrix of investigated exposure factors associated with retained fetal membrane in their second or later lactations dairy cows. The fourth step was multivariable analysis with interaction of investigated exposure factors associated with retained fetal membrane in their second or later lactations dairy cows. All analyses were carried out using Analytical Software Statistics 10.0.

Results

Incidence and Impact of RFM of Cows in Herd No. 1 and Herd No. 2

379 of 3833 cows (9.89%) were diagnosed with RFM. The incidence of RFM at herd 1 and herd 2 were 11.26% and 8.07%, respectively. The odds of RFM were 0.69 times lower in herd 2 compared to herd 1; the odds of RFM were 0.59 times lower in lactation number 2+ compared to lactation number 1; the odds of RFM were 0.45 times lower in lactation number2+ compared to lactation number1 in herd 1 (Table 1, 2 and 3).

Incidence and Impact of RFM of Cows in their First Lactation

In univariate analysis, the variables of calving season and herd had a p value of ≤ 0.01 in their first lactation. Stillbirth, twins and calving difficulties had a p value of ≤ 0.20 and were further investigated in the multivariable analysis of RFM. Previous abortion, stillbirth, calving difficulty and milk fever had a p value of ≤ 0.80 and were further investigated in the multivariable analysis of RFM (Table 4).

Incidence and Impact of RFM of Cows in their Second or Later Lactations

In univariate analysis, the variables of calving season, previous abortion, stillbirth, calving difficulty and milk fever had a p value of ≤ 0.20 and were further investigated in the multivariable analysis of RFM (Table 5, 6, 7 and 8). We examined the combined effect of calving season and previous abortion for RFM, and the results demonstrated that cows that calved during hot months and had a history of previous abortion were 22 times higher possibility to experience RFM, by comparison of cows that calved in non-hot months and without a history of previous abortion (Adjusted OR=22.63; 95% CI = 6.30, 81.36) (Table 7 and 8).

Discussion

In this study, the odds of RFM were 0.69 times lower in herd 2 compared to herd 1. The first reason may be the lower level management induces the higher RFM. The bad management can increase the produce of abortion, stillbirth, milk fever, calving difficult and other diseases in parturition which were the critical element related to RFM (Bourne *et al.*, 2008; Kankofer *et al.*, 2010; Pontes *et al.*, 2015). The second reason may be the higher temperature in the farm 1whcih was located in more hot area. It is showed that the incidence of RFM during hot season was higher in hybrid and Friesian cows (Hossein-Zadeh and Ardalan, 2011). Kumari *et al.* (2015) also observed that during summer the incidence of RFM increased markedly by 1.75 times in Murrah buffalos.

In the univariable analysis, the variables for calving season and herd had a p value of ≤ 0.01 in their first lactation, the odds of RFM being 0.43 times lower in herd 2 compared to herd 1. The main reason may be relative to the bad management of herd 1, the unskilled workers and veterinarians would increase the incidence of RFM induced by the increasing stress and decrease the immunity of dairy cows, especially for the first lactation cows.

Table 1: Univariable analysis of investigated exposure factors related to retained fetal membrane in dairy cows that calved in two commercial dairy herds in China from 1 January to 31 December 2015

Variable	Category	Retained fetal membranes		OR	95% CI	*p*
		Yes N (100%)	No N (100%)			
Herd	1	246 (65)	1938 (56)	1.00	Reference	NA
	2	133 (35)	1516 (44)	0.69	0.55, 0.86	< 0.01
Lactation	1	122 (32)	752 (22)	1.00	Reference	NA
number	2+	257 (68)	2702 (78)	0.59	0.47, 0.74	< 0.01

N (100%), No. of cases; OR, Odds ratio; CI, Confidence intervals; NA, Not applicable

Table 2: Univariable analysis of investigated exposure factors related to retained fetal membrane in dairy cows that calved in two commercial dairy herds in China from 1 January to 31 December 2015

Variable	Category	Retained fetal membranes		Adjusted OR	95% CI	*p*
		YesN (100%)	NoN (100%)			
Herd	1	246 (65)	1938 (56)	-	-	-
	2	133 (35)	1516 (44)	0.67	0.54, 0.84	<0.01
Lactation	1	122 (32)	752 (22)	-	-	-
number	2+	257 (68)	2702 (78)	0.57	0.45, 0.72	<0.01

Table 3: Univariable analysis of investigated exposure factors related to retained fetal membrane in dairy cows that calved in two commercial dairy herds in China from 1 January to 31 December 2015

Variable	Category	Retained fetal membranes		OR	95% CI	*p*
		Yes N (100%)	No N (100%)			
HERD 1	Lactation 1	85(34)	375 (19)	1.00	Reference	NA
	Lactation 2+	161(66)	1563 (81)	0.45	0.34, 0.60	< 0.01
HERD 2	Lactation 1	37 (28)	381 (25)	1.00	Reference	NA
	Lactation 2+	96 (62)	1135 (75)	0.87	0.59, 1.29	0.49

In the second or later lactations, the odds of RFM were 0.82 times lower in herd 2 compared to herd 1. Risk factors like difficult calving, abortion, and stillbirth can increase the incidence of RFM in the two herds. Cows with abortion experience are 7.11 times more likely to have RFM than cows without abortion experience. Cows with calving difficult had a 2.79 times higher incidence of RFM than cows without calving difficult. Abortion, stillbirth and difficult calving may cause circulatory disorders that impair normal fetal detachment, leading to RFM. Hossein-Zadeh and Ardalan (2011) also discovered that calving problems including difficult calving and stillbirths were related to RFM, difficult calving might be due to the weakness of uterine contraction, delayed uterine involution and uterine mechanical damage. Our results are similar to other studies reporting increase of RFM incidence in cows with previous abortion, stillbirth and dystocia (Swai *et al.*, 2005).

In their second or later lactations, cows with stillbirth had a 2.79 times higher incidence of RFM than cows without stillbirth. During late cow pregnancy, several hormones are involved in maintaining and developing a successful pregnancy and parturition of healthy calves. The stillbirth may cause hormonal imbalances of pregnant female, which can damage normal membrane detachment and lead to RFM.

Table 4: Univariable analysis of investigated exposure factors related to retained fetal membrane in first lactation dairy cows that calved in two commercial dairy herds in China from 1 January to 31 December 2015

Variable	Category	Retained fetal membranes		OR	95% CI	*p*
		YesN (100%)	No N (100%)			
Herd	1	85 (69)	375 (49)	1.00	Reference	NA
	2	37 (31)	377 (51)	0.43	0.29, 0.65	<0.01
Calving	Cold months	66 (54)	513 (68)	1.00	Reference	NA
season	Hot months	56 (46)	239 (32)	1.82	1.24, 2.68	<0.01
Previous	No	121 (99)	752 (100)	1.00	Reference	NA
abortion	Yes	1 (1)	0 (0)	ND	ND	ND
Stillbirth	No	117 (96)	735 (98)	1.00	Reference	NA
	Yes	5 (4)	17 (2)	1.85	0.67, 5.10	0.23
Twins	No	119 (98)	743 (99)	1.00	Reference	NA
	Yes	3 (2)	9 (1)	2.08	0.56, 7.79	0.27
Calving	No	116 (95)	711 (94)	1.00	Reference	NA
difficulty	Yes	6 (5)	41 (6)	0.90	0.37, 2.16	0.80
Milk	No	118 (97)	752 (100)	1.00	Reference	NA
fever	Yes	4 (3)	0 (0)	ND	ND	ND

ND, No data

Table 5: Univariable analysis of investigated exposure factors related to retained fetal membrane in their second or later lactations dairy cows that calved in two commercial dairy herds inChina from 1 January to 31 December 2015

Variable	Category	Retained fetal membranes		OR	95% CI	*p*
		YesN (100%)	NoN (100%)			
Herd	1	161 (63)	1563 (58)	1.00	Reference	NA
	2	96 (37)	1139 (42)	0.82	0.63, 1.07	0.13
Calving season	No	146(57)	1983(73)	1.00	Reference	NA
(hot months)*	Yes	111(43)	719(27)	2.10	1.62,2.72	<0.01
Previous	No	243 (95)	2677 (99)	1.00	Reference	NA
abortion	Yes	14 (5)	25 (1)	6.17	3.17,12.02	<0.01
Stillbirth	No	241 (94)	2638 (98)	1.00	Reference	NA
	Yes	16 (6)	64 (2)	2.74	1.56, 4.81	<0.01
Twins	No	254 (99)	2668 (99)	1.00	Reference	NA
	Yes	3 (1)	34 (1)	0.93	0.28, 3.04	0.90
Calving	No	240 (93)	2661 (98)	1.00	Reference	NA
difficulty	Yes	17 (7)	41 (2)	4.60	2.57, 8.22	<0.01
Milk fever	No	248 (97)	2676 (99)	1.00	Reference	NA
	Yes	9 (3)	26 (1)	3.74	1.73, 8.06	<0.01

*Hot months are June, July and August

Table 6: Correlation matrixof investigated exposure factors associated with retained fetal membrane in their second or later lactations dairy cows that calved in two commercial dairy herds inChina from 1 January to 31 December 2015

	Previous abortion	Stillbirth	Calving difficulty	Milk fever
Previous abortion				
Stillbirth, n = 80	0.29			
Calving difficulty, n = 58	0.37	< 0.01		
Milk fever	0.49	0.95	0.70	

Kornmatitsuk *et al.* (2002) found that the patterns of the PGF2 metabolite, cortisol, progesterone and PAGs (pregnancy-associated Glycoproteins) were changed in the cases of calving difficulty and stillbirth. Cortisol was responsible for decreasing plasma and tissue progesterone concentration and rising estrogens concentration during the last stages of gestation (Power and Challis, 1987).

Table 7: Multivariable analysis of investigated exposure factors associated with retained fetal membrane in their second or later lactations dairy cows that calved in two commercial dairy herds inChina from 1 January to 31 December 2015

Variable	Category	Adjusted OR	95% CI	*p*
Herd	1	-	-	-
	2	0.78	0.60, 1.02	0.07
Calving season: hot months	No	-	-	-
	Yes	2.06	1.58, 2.69	< 0.01
Previous abortion	No	-	-	-
	Yes	7.11	3.61, 14.04	< 0.01
Stillbirth	No	-	-	-
	Yes	2.79	1.57, 4.95	< 0.01
Milk fever	No	-	-	-
	Yes	3.52	1.61, 7.71	< 0.01

Hosmer-Lemeshow statistic = 1.27; df = 3; $p = 0.73$

Table 8: Multivariable analysis with interaction of investigated exposure factors associated with retained fetal membrane in their second or later lactations dairy cows that calved in two commercial dairy herds inChina from 1 January to 31 December 2015

Ariable	Category	Adjusted OR	95% CI	*p*
Herd	1	-	-	-
	2	0.78	0.60, 1.03	0.07
Stillbirth	No	-	-	-
	Yes	2.79	1.57, 4.96	< 0.01
Milk fever	No	-	-	-
	Yes	3.53	1.61, 7.73	< 0.01
Calving season: hot months	Previous abortion			
No	No	-	-	-
No	Yes	5.89	2.55,13.57	< 0.01
Yes	No	2.02	1.54, 2.65	< 0.01
Yes	Yes	22.63	6.30,81.36	< 0.01

Kornmatitsuk *et al.* (2004) found that the levels of both oestrone sulphate and PAG were low during late pregnancy and at the time of parturition cases of stillbirth and low calf viability.

Various risk factors, including preterm birth or induced labor, dystocia, hormonal imbalances, and immunosuppression, can disrupt normal production processes and cause placenta retention (Beagley *et al.*, 2010). In their second or later lactations of the present study, cows with MF had a 3.53 times higher incidence of RFM than cows without MF. Milk fever was characterized by hypocalcemia during parturition because of a sudden increase in calcium demand and an inevitable delay in calcium metabolism. Many literature data indicated that comparing with cows without RFM, cows with RFM had a significant decrease in plasma calcium concentration immediately after calving (Risco *et al.*, 1994; Melendez *et al.*, 2004). Calcium activates many enzymes through phosphorylases and plays an important role at the cellular level (Brogley *et al.*, 1999). Hypocalcemia may affect the mechanism of detachment of cotyledon from uterine caruncles. Collagenase activity of cotyledon villi participates in fetal membrane release process. Collagen enzyme activity of healthy cow increased and that of RFM cow decreased. Collagenase is a calcium-dependent enzyme (Brogley *et al.*, 1999).

The analysis revealed that cows that calved during hot months and had a history of previous abortion were 22 times higher possibility to experience retained placenta, by comparison of cows that calved in non-hot months and without a history of previous abortion. In this study, cows with previous abortion had 5.89 times higher incidence of RFM than cows without previous abortion and not in hot months in their second or later lactations. The adjusted odds of RFM were 2.02 times higher in cows calving during hot months compared to cows without previous abortion and not calving during hot months. Cows with previous abortion and calving in hot months had a 22.63 higher incidence of RFM than cows without previous abortion and not calving in hot months. The first reason may be more serious heat stress to the cows calving in hot reasons than cows in non-hot seasons. The effects of heat stress and its adverse consequences on health, milk production, and overall productivity have been widely investigated (Bernabucci *et al.*, 2010). Heat stress reduces fertility in lactating cows (Lopez-Gatius, 2003). Early embryonic development is sensitive to thermal stress (Ealy *et al.*, 1993). The second reason may be increase in temperature of climate, affecting the normal physiological metabolism of dairy cows. Higher incidence of RFM during hot months has already been reported in crossbred and Friesian cows (Gröhn and Rajala-Schultz, 2000; Hossein-Zadeh and Ardalan, 2011). Gröhn and Rajala-Schultz (2000) observed that summer season markedly increased the incidence of RFM by 1.75 times in Murrah buffalos.

Conclusion

Stillbirth, calving season, previous abortion and milk fever are the risk factors associated with retained fetal membrane in dairy cows on two dairy herds (herd No. 1 and No. 2) from Hebei Province in China.

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