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Araştırma Makalesi ***Research Article***

Antibody Tracing, Seroepidemiology and Risk Factors of Bovine Respiratory Syncytial Virus and Bovine Adenovirus-3 in Dairy Holstein Farms

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Abstract

Antibody tracing, risk factors and seroepidemiology of bovine respiratory syncytial virus and bovine adenovirus-3 were investigated in 22 Industrial and Semi-Industrial dairy Holstein farms. Serum samples (n=736) from various ages of unvaccinated cows were collected from May to September 2012. Risk factors including age, past history of respiratory diseases, amount of milk production, husbandry type and herd size were considered. Data were analyzed by Chi-square and logistic regression. Results indicated that the infection with some of individual viruses was related to past history of respiratory disease and herd size. No specific pattern was seen on the effect of level of milk production on seropositivity of animals. The seroprevalence for BRSV and BAV-3 were 89.1% and 88%, respectively. The present study indicates that infections of bovine respiratory viruses frequently occur in cattle of Fars province and the main viral cause of primary occurrence of respiratory diseases may be due to aforementioned viruses.

Özet

Sütçü Siyah Alaca İşletmelerinde Sığır Respiratuvar Sinsityal Virüsü ve Sığır Adenovirüs-3'ün Antikor İzleri, Seroepidemiyolojisi ve Risk Faktörleri

Endüstriyel ve yarı-endüstriyel 22 sütçü Siyah Alaca işletmesinde sığır respiratuvar sinsityal virüsü ve sığır adenovirus-3'ün antikor izleri, risk faktörleri ve seroepidemiyolojisi araştırılmıştır. Çeşitli yaşlardaki aşılanmamış ineklerden Mayıs 2012 ile Eylül 2012 arasında serum örnekleri (n=736) toplanmıştır. Yaş, solunum hastalıklarıyla ilgili geçmişteki kayıtlar, süt üretim miktarı, işletme tipi ve sürü boyutunu içeren risk faktörleri dikkate alınmıştır. Veriler Ki-kare ve lojistik regresyon kullanılarak analiz edilmiştir. Elde edilen sonuçlar bazı bireysel virüslerle oluşan enfeksiyonların geçmişteki solunum hastalığına ve sürü boyutuna bağlı olduğunu gösterdi. Hayvanların seropozitiviteleri üzerinde süt üretim düzeyi etkisinin belirgin bir paterninin olmadığı görüldü. BRSV ve BAV-3 için seroprevalans sırasıyla %89,1 ve %88'di. Bu çalışma, sığır respiratuvar virüsleri enfeksiyonlarının Fars bölgesinde sıklıkla oluştuğunu ve solunum hastalıklarının primer oluşumunun başlıca viral sebebinin daha önce bahsedilen virüsler olduğunu göstermektedir.

Introduction

In spite of advances in veterinary medicine, animal husbandry, and animal welfare, respiratory disease among dairy cattle continues to be a major problem in the dairy industry (Gorden and Plummer, 2010). Bovine respiratory disease complex (BRDC) include largely single clinical entity of bronchopneumonia that occur with high morbidity accompanied with dramatic economic losses due to medication use and discarded milk, as well as cow fatalities (Radostits et al., 2007; Whitely et al., 1992). Effect of stress on viral-bacterial synergy in bovine respiratory disease can impair respiratory defense of cattle. The major etiological agents of this disease are the viruses (Lazić et al., 2009). Among many viruses the most significant role in BRDC is connected to the bovine respiratory syncytial virus

(BRSV), bovine parainfluenza-3 virus (BPI-3), bovine herpes virus type-1 (BHV-1) and bovine viral diarrhea virus (BVDV), while less important are bovine adenoviruses (BAV) and corona viruses (BCV) (Cortese et al., 1991).

Bovine respiratory syncytial virus (BRSV) is a RNA virus classified as a pneumovirus in the paramyxovirus family. Due to its frequent occurrence, predilection for the lower respiratory tract and ability to predispose the respiratory tract to secondary bacterial infections, the virus has significant role in the bovine respiratory disease complex. BRSV is distributed worldwide, and the virus is indigenous in the cattle population (Mahmoud and Allam, 2013).

Adenoviruses are nonenveloped double-stranded DNA viruses of the family Adenoviridae. BAV infection is widespread and is frequently subclinical (Smith, 2009).

Viral infections are very frequent in most cattle-rearing systems. Controlling viral respiratory disease in cattle requires a good understanding of factors that increase the susceptibility of animals to these viruses, as well as pathogen transmission characteristics within and between herds. Based on precise and complete knowledge of the epidemiology, virology and pathogenesis of each virus, control measures can be developed to decrease the economic impact of bovine respiratory diseases (Valarcher and Hagglund, 2006).

BRSV and BAV-3 have been detected in all continents (Mahmoud and Allam, 2013; Rabie et al., 2013; Roshtkhari et al., 2012; Sakhaee et al., 2009; Taylor et al., 2010; Valarcher and Taylor, 2007; Yavru et al., 2005). Sparse data is accessible on different relevant risk factors and their associations with seropositivity (Shirvani et al., 2011; Solís-Calderón et al., 2007) which may be critical for controlling and prevention of BRDC. To consider these risk factors, the effect of age, herd size, milk production level, husbandry type and past history of respiratory illness were evaluated.

Materials and Methods

Study design

Serum samples of apparently healthy female dairy Holstein cattle (3 month old calves to 7 year old cattle, n= 736) were collected from 22 industrial and semi-industrial dairy farms of different areas of Fars Province. Fars Province has approximately 350,000 cattle. Herd size was different from farm to farm with the most common cattle breed being Holstein/Friesian. A cross-sectional study was performed, with a random cluster sampling design, from May to September 2012. Herd and animal risk factors were acquired through a personal conversation. A questionnaire, which included variables such as health, management measures, etc., was filled out in each herd. None of the cattle herds in this study were vaccinated against BRSV and BAV-3.

Blood samplings and serological assays

Blood samples (5 ml) were collected aseptically from coccygeal vein of each animal, using plain tube and transported on ice to the laboratory. Serum was separated by centrifugation of blood at 3000 g for 10 min at room temperature, transferred into 1.5 ml sterile microtube and was kept at -20°C until analysis. Commercial indirect respiratory ELISA kit, developed by Bio-X diagnostic, (Jemelle, Belgique), was used to determine the presence of antibodies to BAV-3. Antibodies to BRSV were detected, using commercially available ELISA kits developed by SVANOVA, (Uppsala, Sweden). Microtiter plates coated with the respective viral antigens were used according to the manufacturer's instructions. The optical density (OD) was measured at 450 nm with an ELISA Reader (BDSL Immunoskan Plus).

Risk factors

Data on potential risk factors were obtained using a questionnaire presented to the owner or manager of each herd at the time of blood sampling. Explanatory variables were herd size (<50, 50-100, 100-300, 300-500, >500 animals), level of milk production [no, low (<15 L/day), moderate (15-25 L/day), high (>25 L/day)], husbandry type (semi-industrial, industrial), history of past respiratory disease (no, yes) and animal age group (≤2, 2-4, 4-6 and >6 years old).

Statistical analyses

The statistical program SPSS package version 16 was used for the statistical analysis. A primary screening test to identify explanatory variables significantly related to BRDC seropositivity was performed using Chi-square test. Only those explanatory variables with $P<0.05$ were analyzed by a standard logistic binary-regression model using a backward-selection procedure for clustered observations. Those variables with Wald statistic values with $P<0.05$ in the final model were considered as statistically significant.

Results

Our results indicated that 656 and 648 out of 736 serum samples were ELISA positive for BRSV and BAV-3, respectively. The prevalence was 89.1% (95% CI: 87-91%), and 88% (95% CI: 86-90%), respectively. Preliminary Chi-square test revealed significant associations between the presence of antibodies to BRSV with all respective risk factors ($P<0.05$), except age and type of husbandry (Table 1). This test revealed significant associations ($P<0.05$) between the presence of antibodies to BAV-3 and all risk factors, except type of

husbandry and herd size (Table 3). In multivariable logistic regression analysis, no statistically significant relationships was observed between type of husbandry and the prevalence of antibodies against two respective viruses (P>0.05). The effect of age on seroprevalence of BRSV and BAV-3 was not statistically significant in multivariable logistic regression analysis (P>0.05) (Tables 2 and 4). In the case of BAV-3, the cattle with higher milk production showed higher seropositivity (P<0.05). In BRSV, the cattle with lower milk production manifested higher seropositivity (Table 2). It was shown that the cattle with no history of respiratory disease, had significantly lower seropositivity to BRSV and BAV-3 (P<0.05) (Tables 2 and 4). The odds of being a seropositive animal for BRSV were greater in larger herds (Table 2).

Table 1. Chi-square results and risk factors of BRSV prevalence in studied cattle (n=730).

Tablo 1. Araştırılan büyükbaş hayvanlarda (n=730) BRSV risk faktörlerinin prevalansı ve Ki-kare sonuçları.

Risk Factors		Negative (n=80)		Positive (n=656)		P Value
		N	%	N	%	
Type of Husbandry	**S**	12	15	132	20.1	0.27
	I	68	85	524	79.9	
Age (year)	**≤2**	4	5	92	14	0.06
	2-4	36	45	316	48.2	
	4-5	32	40	200	30.5	
	>6	8	10	48	7.3	
Level of Milk Production (L)	**No**	4	5	92	14	0.04
	Low	12	15	132	20.1	
	Moderate	40	50	292	44.5	
	High	24	30	140	21.3	
History of Previous Respiratory Disease	**No**	64	80	300	45.7	<0.001
	Yes	16	20	356	54.3	
Herd size	**<50**	8	10	24	3.7	0.001
	50-100	4	5	108	16.5	
	100-300	48	60	288	43.9	
	300-500	16	20	196	29.9	
	>500	4	5	40	6.1	

P values <0.05 is significant, S: semi-industrial, I: Industrial

Discussion

Bovine respiratory disease complex (BRDC) is a worldwide problem in cattle and imposes considerable financial losses on beef industry. It is the most common cause of mortality in dairy cattle (Radostits et al., 2007). Bovine viral respiratory viruses are endemic in cattle populations in most parts of the world (Mahmoud and Allam, 2013). High seroprevalences for BRSV (89.1%) and BAV-3 (88%) infections in present study indicates that exposure to these agents were frequent in the region. This is likely to be due to the absence of control measures against these infections. In view of the fact that vaccination of cattle was not practiced at all, seropositive response against BRSV and BAV-3 agents reflects natural exposure. High individual seroprevalences of BRSV and BAV-3 have been reported in dairy cattle in various parts of the world (Algirdas et al., 2008; Kadir and Burak, 2008; Sakhaee et al., 2009; Yavru et al., 2005). Yavru et al. (2005), showed that the prevalence of BAV-3 and BRSV in cattle in Turkey were 20.07% and 46.06%, respectively. In comparison, higher prevalences of BAV-3 and BRSV were seen in our study. Algirdas et al. (2008) showed that serological prevalence

of BRSV in Litvania was 54.5%. In a serological study in dairy cattle in Turkey by Kadir and Burak (2008) seroprevalence values for BRSV and BAV-3 was 73% and 92.3%, respectively. In their study, herd size was a very important risk factor for respiratory viruses (Kadir and Burak, 2008). According to study of Solís-Calderón et al. (2007) detection of antibodies against BRSV in beef cattle of Yucatan, Mexico, seroprevalences was 90.8%. In the other study in Egypt, seropositivity rate was 75.5 % for BRSV in the sampled population (Mahmoud and Allam, 2013). In recent years, seroprevalence of several viruses causing bovine respiratory diseases was established in different studies in several regions of Iran. Roshtkhari et al. (2012) reported the seroprevalence values for BRSV (64.2%) and BAV-3 (61.9%) in Mashhad (Iran). A serological survey on BRSV in Chahar Mahal Bakhtiary province (Iran) showed the infection rate of 80.98% (Tajbakhsh and Momtaz, 2003). Sakhaee et al. (2009) reported the seroprevalence of BRSV (100%) and BAV-3 (100%) in Kerman (Iran). Our results revealed that type of husbandry had no statistically significant relationships with the prevalence of antibodies against two respective viruses. Although seroconversion occurred in all age groups against BRSV and BAV-3, the rate of seropositivity was not statistically significant among different age groups. Our results showed that the cattle without a past history of respiratory disease had significantly lower seroprevalence of BRSV and BAV-3. Moreover, it could be stated that previous history of respiratory disease was directly related to the higher seropositivity and the chief viral causes of respiratory diseases may be due to aforementioned viruses in the area. In general concept, viruses are the first invaders, whereas bacteria act as the second pathogen which worsen animal's health (Solís-Calderón et al., 2007; Valarcher and Hagglund, 2006). Although some studies have demonstrated that high-producing cows are at increased risk of clinical infectious diseases (Grohn et al., 1994), no specific pattern was seen on the effect of level of milk production on seropositivity of animals. It is probable that the level of milk production actually affect severity of the disease rather than seroconversion. Prevalence of BRSV in larger herds was higher than smaller ones. This is probably due to a complex of environmental conditions including overcrowding, unchecked transportation and arrival, higher humidity, etc, in such herds.

Table 2. Results of multivariable logistic regression analysis for BRSV seropositivity in studied cattle.
Tablo 2. Araştırılan büyükbaş hayvanlarda BRSV seropozitivitesi için çoklu değişken lojistik regresyon analizi sonuçları.

Risk factor		SE	Wald static	P-value	OR [a]	95% CI [b]
Constant		0.68	9.24	.002	8.02	-
History of Previous Respiratory Disease	**No**	0.31	25.02	<0.001	0.20	0.11-0.38
	Yes[c]	-	-	-	1.00	-
Level of Milk Production (L)	**No**	0.61	2.35	0.12	2.552	0.77-8.44
	Low	0.42	4.57	0.03	2.46	1.07-5.62
	Moderate	0.32	0.38	0.53	1.22	0.64-2.31
	High	-	6.16	0.10	1.00	-
Herd Size	**<50**	0.81	0.01	0.90	0.90	0.18-4.49
	50-100	3.8E3	0.00	0.99	3.93E8	0.00
	100-300	0.65	0.29	0.58	1.42	0.39-5.09
	300-500	0.68	4.94	0.02	4.56	1.19-17.43
	>500[c]	-	13.58	0.001	1.00	-

a: Odds Ratio, b: Confidence Interval, c: Reference category, P values <0.05 is significant, S: semi-industrial, I: Industrial

Table 3. Chi-square results and risk factors of BAV-3 prevalence in studied cattle (n=730).

Tablo 3. Araştırılan büyükbaş hayvanlarda (n=730) BAV-3 risk faktörlerinin prevalansı ve Ki-kare sonuçları.

Risk factors		Negative (n=88)		Positive (n=648)		P Value
		N	%	N	%	
Type of Husbandry	**S**	16	18.2	128	19.8	0.7
	I	72	81.8	520	80.2	
Age (year)	**≤2**	4	4.5	92	14.2	<0.001
	2-4	44	50	308	47.5	
	4-6	40	45.5	192	29.5	
	>6	0	0	56	8.6	
Level of Milk Production (L)	**No**	4	4.5	92	14.2	0.001
	Low	28	31.8	116	17.9	
	Moderate	44	50	288	44.4	
	High	12	13.6	152	23.5	
History Of Previous Respiratory Disease	**No**	60	68.2	304	46.9	<0.001
	Yes	28	31.8	344	53.1	
Herd Size	**<50**	4	4.5	28	4.3	0.9
	50-100	12	13.6	100	15.4	
	100-300	44	50	292	45.1	
	300-500	24	27.3	188	29	
	>500	4	4.5	40	6.2	

P values <0.05 is significant, S: semi-industrial, I: Industrial

Table 4. Multivariable logistic regression analysis of risk factors for BAV-3 infection in studied cattle.

Tablo 4. Araştırılan büyükbaş hayvanlarda BAV-3 enfeksiyonu için risk faktörlerinin çoklu değişken lojistik regresyon analizi.

Risk factor		SE	Wald static	P-value	OR[a]	95% CI [b]
Constant		5.2E3	.000	0.99	4.1E9	-
Age (year)	**≤2**	5.3E3	.000	0.99	.000	.000
	2-4	5.3E3	.000	0.99	.000	.000
	4-6	5.3E3	.000	0.99	.000	.000
	>6[c]	-	4.76	0.19	-	-
Level of Milk Production (L)	**No**	-	-	-	-	-
	Low	0.39	10.58	0.001	0.28	0.13-0.60
	Moderate	0.35	3.05	0.081	0.53	0.26-1.07
	High[c]	-	11.00	0.004	1.00	-
History of Previous Respiratory Disease	**No**	0.25	12.44	<0.001	0.40	0.24-0.66
	Yes[c]	-	-	-	-	-

a: Odds Ratio, b: Confidence Interval c : Reference category, P values <0.05 is significant

It could be concluded that the past history of respiratory problems and herd dimension may affect the prevalence of BRSV and BAV-3. No conclusive result was found when considering level of milk production. It was shown that BRSV and BAV-3 infections are very frequent in dairy farms of Fars Province, and are implicated in the appearance of bovine respiratory disease complex. Furthermore, it is proposed that deterrent measures including group vaccination, biosecurity and separation of diseased animals be established to lower viral causes of bovine respiratory infections in dairy farms of the area.

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