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Epidemiology of Parainfluenza Virus type-3 Infection in Cattle in North Kordofan, Sudan

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ABSTRACT

In this study the epidemiology of respiratory infection in cattle in North Kordofan State in three localities (Elobied, Omrowaba and Elnohood) as well as the seroprevalence of BPIV-3 were studied. Collected data about the reported cattle cases diagnosed as having pneumonia in veterinary clinics during 2006-2008 were statistically analyzed. In Elobied the reports showed that the pneumonic cases within the total animals brought to the clinics were 213 out of 5092 (4.2%), the highest incidence was in autumn then winter and summer. Data about diagnosed pneumonia cases and lungs condemned due to pneumonia in the three localities were analyzed as well. Out of 334 sera samples tested by indirect ELISA for BPIV-3 antibodies, 276 (82.6%) were found positive, the highest percentage of antibodies in adults were found in Elobied (93.7%), while the highest one in calves were seen in Omrowaba, there was a significant difference between age in Elobied (P = 0,001) where there were no significant difference between age in Omrowaba and Elnohood (p=0.644 and 0.574), respectively, there was no significant different between sex in Elobied,Omrowaba and Elnohood (p= 0.741 ,0.082 and 0.291, respectively).

INTRODUCTION:

Respiratory disease has had a major impact on the overall health of cattle and continues to be of great importance to date. Many of the diseases that have been shown to affect the respiratory tract of cattle have been grouped into an overall category

known as bovine respiratory disease (BRD) complex. This includes shipping fever syndrome, mucosal disease, enzootic calf pneumonia, acute respiratory distress syndrome, hemorrhagic syndrome, and atypical interstitial pneumonia (Baker, 1995, Ames , 1997, Apley , 2006).

Bovine Parainfluenzavirus type3 infections cause less serious disease than BRSV (Verhoeff and van Nieuwstadt, 1984), but are nevertheless significantly correlated with respiratory disease in cattle (Stott *et al*., 1980).

Clinical signs such as slight fever, coughing and nasal discharge are observed after PIV-3 infections in young animals (Bryson *et al*., 1978, Bryson *et al*., 1979, Bryson *et al*., 1979b, Verhoeff and Van Nieuwstadt, 1984), the virus is thought to have a predisposing role in shipping fever and enzootic pneumonia; it may be isolated from severe or fatal cases together with bacteria and Mycoplasma spp (Bryson *et al.*, 1978, Stroz *et al*., 2000).

The predisposing role of PIV-3 in bovine respiratory diseases is probably correlated to its immunosuppressive effects on leucocytes (e.g. decreased phagocytosis by alveolar macrophages and decreased lymphocyte proliferation), and destruction of mucociliary system (Hesse and Toth, 1983, Babiuk *et al*., 1988, Basaraba *et al*., 1994, Adair *et al*., 2000).

In Sudan, Eisa *et al* (1979) reported the occurrence of antibodies to PIV-3 in sera of some domestic animals. Khalifa and Khallafallah (1974) reported that the mortality rate in bull calves and heifer calves of Kenana cattle at Ombenin livestock improvement center during 1964-1973 was 26.4% and 20.7%, respectively, the most common causes of death were pneumonia and digestive troubles.

This study is intended to investigate the epidemiology of respiratory infection in cattle in North Kordofan state and the role of parainfluenza virus in causation of respiratory infection.

MATERIALS AND METHODS:

Retrospective study: Data about the incidence of bovine respiratory infection as well as the condemnation of lungs in the slaughter houses due to pneumonia during 2008-2010 were collected and statistically analyzed using SPSS software program.

The data were collected from the Annual records of Animal Wealth General Directorate, North Kordofan State and Elobied Teaching Veterinary Hospital.

Sample collection: A total of 334 blood samples were collected randomly from cattle of different age and sex showing respiratory signs as well as healthy ones in three localities (Elobied, Omrowaba and Elnohood) in North Kordofan State (Table 1).

Table 1: location and number of serum collected from three localities in North Kordofan State during 2006- 2008

Area	Male	Female	Total
Elobied	149	50	199
Omrowaba	30	35	65
Elnohood	16	54	70
Total	195	139	334

Blood (5 ml) was taken aseptically from jugular vein of each animal using anti coagulant free vacutainer tubes and transported on ice to the Elobied Veterinary Research Laboratory, serum was separated by centrifugation of blood at 3000 rpm for 10 minutes at room temperature, the aliquots were transferred into 1.5 sterile microtube (Eppendorf) and kept at -20^{o}C until

used.***Indirect ELISA for PIV3 antibody detection:*** Commercial indirect ELISA kits developed by Bio-X Diagnostics, Jemelle Belgium were used to determine the presence of antibodies against PIV-3.

Statistical analysis: SPSS version 11.5 was used for the analysis of the results of PIV-3 antibodies detection by indirect ELISA. Chi square test was used to compare the results for age, sex, and locality. The result is significant if the P-Value is less than 0.05.

RESULTS:

Prevalence of pneumonia in cattle in North Kordofan State: The reported cattle cases diagnosed as having pneumonia in veterinary clinics in North Kordofan State in the three localities (Elobied, Omrwaba, and Elnohood) during 2006-2008 was analyzed. In Elobied the reports showed that the pneumonic cases within the total animals brought to the clinics were 213 out of 5092 (4.2%), the highest incidence was in autumn then winter and summer. In Omrwaba also the highest incidence was in autumn then winter and summer. In Elnohood the highest incidence was in summer then winter and autumn.

Statistical analysis for reported cases of pneumonia in veterinary clinics in North Kordofan State, showed that there was significant difference (p =0.000) between seasons on different years (Table 2).

Table 2: Seasonal distribution of cattle pneumonia in North Kordofan state during 2006-2008

Years	Seasons	Pneumonia	Others	P-value Years
	Winter	44	142	
2006	Summer	48	3243	0.000
	Autumn	159	1154	
	Total	251	4539	
	Winter	37	239	
2007	Summer	34	373	0.000
	Autumn	42	901	
	Total	113	1513	
	Winter	42	228	
2008	Summer	25	229	0.000
	Autumn	75	244	
	Total	142	701	

In Elobied the reports showed that there was significant difference (p =0.000) between seasons on different years (Table 3).

Table 3: Seasonal distribution of cattle pneumonia in Elobied, North Kordofan during 2006-2008

Years	Seasons	Pneumonia	Others	P-value
	Winter	10	30	
2006	Summer	22	3058	
	Autumn	42	804	0.000
	Total	74	3892	
	Winter	17	53	
2007	Summer	13	179	
	Autumn	12	663	0.000
	Total	42	895	
	Winter	26	24	
2008	Summer	13	134	
	Autumn	58	147	0.000
	Total	97	305	

In Omrwaba there was also significant difference between seasons in 2006 and 2007 (p =0.000 and 0.004) while in 2008 there was no significant difference (p =0.945) between seasons on different years (Table 4)

Table 4: Seasonal distribution of cattle pneumonia in Omrowaba, North Kordofan during 2006-2008

Years	Seasons	Pneumonia	Others	P-value
	Winter	23	7	
2006	Summer	4	7	
	Autumn	22	57	0.000
	Total	49	71	
	Winter	12	19	
2007	Summer	13	91	
	Autumn	12	59	0.004
	Total	37	169	
	Winter	13	79	
2008	Summer	8	44	
	Autumn	16	85	0.945
	Total	37	208	

In Elnohood there was significant difference between seasons in 2006 (p =0,002), while in 2007 and 2008 There was no significant difference (p =0,228 and 0,240) between seasons on different years (Table 5).

Table 5: Seasonal distribution of cattle pneumonia in Elnohood, North Kordofan during 2006-2008

Years	Seasons	Pneumonia	Others	P-value
	Winter	11	105	
2006	Summer	22	178	
	Autumn	75	293	0.002
	Total	108	576	
	Winter	8	167	
2007	Summer	8	103	
	Autumn	18	179	0.228
	Total	34	449	
	Winter	3	125	
2008	Summer	4	51	
	Autumn	1	12	0.240
	Total	8	188	

Condemnation of lungs due to pneumonia lesions in North Kordofan State during 2008 -2010: Data about the reported condemned lungs showing pneumonia in slaughterhouses in North Kordofan State during 2008-2010 in three Localities (Elobied, Omrwaba and Elnohood) was analyzed. The reports showed that the total of condemned lungs due to pneumonia lesion were 14214 out of 27375 (52.3%) the highest condemnation was seen in autumn then winter and summer. Statistical analysis of condemned lungs showed that there is significant difference (p =0,000) between seasons on different years (Table 6).

Table 6: Seasonal distribution of condemned cattle lungs due to pneumonia in North Kordofan state durig 2008-2010

Years	Seasons	Condemnation of lung due to pneumonia	Condemnation of lung due to other lesions	Partial condemnation of other organs	p- value	
	Winter	1419	14	992		
	Summer	1183	44	1185		
	Autumn	2499	25	1148	0.000	
2008	Total	5101	83	3325		
	Winter	1165	17	1414		
	Summer	1113	24	1184		
	Autumn	1566	16	1656	0.000	
2009	Total	3844	57	4254		0.008
	Winter	1542	7	1552		
	Summer	1775	10	1528		
	Autumn	1952	9	2236		
2010	Total	5269	26	5316	0.000	

In Elobied the reports showed that the total of condemned lungs due to pneumonia lesion were 3849 in 2008 ,3736 in 2009 and 5113 in 2010. The highest condemnation was seen in autumn, summer and then winter. Statistical analysis of condemned lungs showed that there is significant difference (p =0,000) between seasons on different years (Table 7)

Table 7: Annual distribution of condemned cattle lungs in Elobied, North Kordofan during 2008-2010

Years	Seasons	condemnation of lung due to pneumonia	condemnation of lung due to other lesions	partial condemnation of other organs	p-value
	Winter	998	3	400	
	Summer	1122	34	539	
2008	Autumn	1729	12	755	0.000
	Total	3849	49	1694	
	Winter	1134	12	1044	
	Summer	1085	2	664	
2009	Autumn	1517	8	1218	0.000
	Total	3736	22	2926	
	Winter	1487	7	1097	
2010	Summer	1711	6	1245	
	Autumn	1915	5	1770	0.000
	Total	5113	18	4112	

In Omrwaba the reports showed that the total number of condemned lungs due to pneumonia lesion were 432 in 2008, 66 in 2009 and 61 in 2010. The highest condemnation were seen in winter, autumn and then summer in 2008 , autumn ,summer and winter in 2009 and summer, winter and autumn in 2010 . Statistical analysis of condemned lungs showed that there was significant difference between seasons in 2008 and 2010 (p =0,000),

while in 2009 there was no significant difference (p =0,745) between seasons (Table 8).

Table 8: Annual distribution of condemned cattle lungs in Omrowaba, North Kordofan during 2008-2010

Years	Seasons	condemnation of lung due to pneumonia	condemnation of lung due to other lesions	partial condemnation of other organs	p-value
	Winter	360	11	528	
	Summer	35	10	495	
	Autumn	37	13	372	
	Total	432	34	1395	
2008					0.000
	Winter	20	5	349	
	Summer	22	10	416	
2009	Autumn	24	8	341	0.745
	Total	66	23	1106	
	Winter	21	0	230	
	Summer	35	0	170	0.000
2010	Autumn	5	0	132	
	Total	61	0	532	

In ELnohood the reports showed that the total of condemned lungs due to pneumonia lesion was 130 in 2008, 42 in 2009 and 95 in 2010. The highest condemnation were seen in winter, autumn and summer in 2008, autumn, winter and summer in 2009 and winter, autumn and summer in 2010. Statistical analysis of condemned lungs showed that there was significant difference (p =0,000) in 2008 and 2009 between seasons, meanwhile in 2010 there was no significant difference between seasons (p = 0.017) (Table 9).

Table 9: Annual distribution of condemned cattle lungs in Elnohood, North Kordofan during 2008-2010

Years	Seasons	condemnation of lung due to pneumonia	condemnation of lung due to other lesions	partial condemnation of other organs	p-value
	Winter	61	0	64	
	Summer	26	0	151	
2008	Autumn	43	0	153	0.000
	Total	130	0	368	
	Winter	11	0	21	
	Summer	6	12	104	
2009	Autumn	25	0	97	0.000
	Total	42	12	222	
	Winter	34	0	225	
2010	Summer	29	4	113	
	Autumn	32	4	125	0.017
	Total	95	8	463	

PIV-3 antibodies detection using indirect ELISA: A total of 334 cattle sera were tested using indirect ELISA for PIV-3 antibodies. Antibodies against PIV-3 were detected in 276 sera (82.6%). In Elobied the antibodies were detected in 184 out of 199 (92.5%), while it were detected in 45 out of 65 (69%) and 47out of 70 (67%) in Omroaba and Elnohood, respectively, the details are presented in Table (10).

Table (10): PIV-3 antibodies detection using indirect ELISA in cattle sera collected from three localities in North Kordofan state

Localities	No. of Serum samples	Positive	%+ve
Elobied	199	184	92.5
Omroaba	65	45	69
Elnohood	70	47	67
Total	334	276	82.5

The antibodies detected against PIV-3 in adults and calves are shown in Table (11), the highest percentage of antibodies in adults were found in Elobied (93.7%), while the highest one in calves were seen in Omrowaba.

Table 11: Results of indirect ELISA for PIV-3 antibodies detection in cattle sera in three localities in North Kordofan (adults and calves)

Area	Adults			Calves		
	Tested	+ve	%	Tested	+ve	%
Elobied	191	179	93.7	8	5	62.5
Omrowaba	31	22	71	34	23	67.6
Elnohood	55	39	71	15	8	53.3
Total	277	240	86.6	57	36	63.1

Statistical analysis of the results using SPSS showed that there was a significant difference between age in Elobied ($p = 0.001$) but there were no significant difference between age in Omroaba and Elnohood ($p = 0.644$ and 0.574), respectively (Table12), Also there was no significant difference between sex in Elobied, Omroaba and Elnohood ($p = 0.741$, 0.082 and 0.291) (Figure 1).

Table 12: Results of indirect ELISA for PIV-3 antibodies detection in cattle sera in three localities in North Kordofan (different ages).

Area	Age groups	ELISA Test		P-value
		Positive	Negative	
Elobied	3 month-1 year	5	3	0.002
	1-2 year	44	1	
	2-4 year	79	9	
	4-10 year	56	2	
	Total	184	15	
Omrowaba	3month-1year	23	11	0.644
	1-2 year	15	5	
	2-4 year	4	1	
	4-10 year	3	3	
	Total	45	20	
Elnhood	3month-1year	8	7	0.574
	1-2 year	19	8	
	2-4 year	8	3	
	4-10 year	12	5	
	Total	47	23	

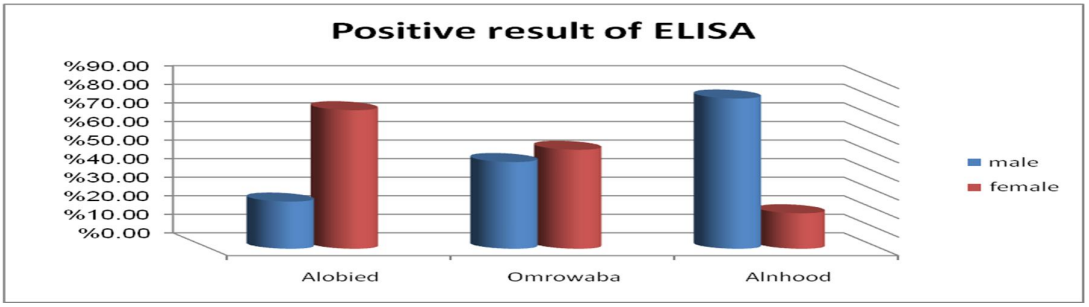

Figure 1: Results of indirect ELISA for PIV3 antibodies detection in cattle sera (males and females).

DISCUSSION:

Bovine respiratory disease (BRD) complex in terms of impact, both financially and in overall health, has been the most important contributor to losses in the beef and dairy cattle industries for many years, (Bateman et al., 2006). The most significant pathogen which is involved in the etiopathogenesis of BRD is parainfluenza virus type 3 (PIV-3) (Kita *et al*., 1994, Klimentowski *et al*., 1995, Jared *et al*., 2010a).

Para influenza virus 3 is one of the viruses known to cause respiratory infection. The disease itself is not serious if there are no complications because the infection generally runs a clinical course of 3–4 days with complete recovery (Murphy *et al*., 1999). However infection with PIV-3 appears to predispose the host to secondary bacterial infection and it has also been shown to play a role in shipping fever.

In the present study, the epidemiology of cattle respiratory infection in North Kordofan State during the period 2006-2008 was investigated. The results revealed that the high incidence of pneumonia reported at the Veterinary Clinics was in autumn and winter season in North Kordofan in Elobied and Omrowaba while highest incidence in Elnohood was in summer, Similar results were reported in Gezira State, Sudan (Nada et al., 2015). This indicated that respiratory defenses of cattle can be impaired by stress, as well as shortage of feed and water, this was previously described by Brigham *et al* (2008). The observation of this study was also in line with the previous reports describing the role of the Environmental factors including climate, ambient temperature, dust particles, and stocking densities (exposure to agents) in the increase of the incidence of respiratory infection (Callan and Garry, 2002, Duff and Galyean, 2007),

The analysis of data about the condemnation of lungs due to pneumonia from 2008-2010 in slaughterhouses showed that the highest condemnation was seen in autumn, summer and winter, statistically there was significant difference ($p = 0.000$) between seasons on different years. This is highly expected as it is suggested that during dry season, the dusty dry wind increase irritation of the respiratory tract, preparing the ground for microbial infection and the condition may be aggravated by stress due to lack of feed or inadequate feed (Raji *et al*., 2000), the same observation was reported

in Gezira state by Nada et al (2015), the results also agreed with Speidel *et al* (2008), they found that the dramatic changes in temperature extremes increased the probability of animals being identified with clinical BRD during the early feeding period. Commonly proposed predisposing factors (stressors) include transportation, commingling with other cattle, dust, cold, sudden and extreme weather changes, dehydration, hypoxia, exposure to endotoxin, cold coupled with wetness, and acute metabolic disturbance Antibodies against PIV-3 was detected in 82.6% of tested cattle sera in this study. Serological studies of bovine para influenza virus 3 are wide spread all over the world, Sakhaee *et al* (2009) detected antibodies to PIV-3 in serum samples of cattle in Iran. In Sudan in an early study, Eisa *et al* (1979) detected PIV-3 antibodies in 58% of cattle and 7% of 102 camel sera using HI. However higher prevalence of PIV-3 (81%) antibodies was detected in camel sera in Sudan (Bornstein and Musa 1987).

In the current study, the detected seroprevalence of PIV -3 (82.6%) is considered far higher than that detected by Eisa (1979) who found PIV-3 antibodies in 58% of cattle sera obtained from different localities in Sudan tested by HI. This indicates the wide spread of the virus beside the highly sensitive technique (indirect ELISA) used in this study. The same technique was used by Intisar *et al* (2010) they detected parainfluenza virus 3 antibodies in 82.2% of camel sera collected from different areas in Sudan. High seroprevalence of PIV-3 was detected in this study in the different localities, highest percentage of PIV-3 antibodies was observed in sera collected from Elobied (92.5%). Detected seroprevalence of PIV-3 in cattle in this study is comparable to that reported in different countries, 85.6% in Turkey (Avci et al., 2014), 90% in Iran (Roshtkhari et al., 2012), 69 % in Saudi Arabia (Yousef et al., 2013), however lower seroprevalence (27.8%), (20%), were detected in India (Goswami et al., 2017), and Kenya (Callaby et al., 2016), respectively.

The higher prevalence of PIV-3 antibodies was in adults (86.6%) while it was 63.1% in calves. The high prevalence of PIV-3 antibodies in adults compared with young animals indicated multiple previous infections in adults. This was in agreement with some work. Bryson (1990) reported that PIV-3 has subsequently been found to have a worldwide distribution with high serum antibody prevalence in adult animals, Valarcher and Hägglund (2006) found that PIV-3 antibodies tested by ELISA in 78 beef calves and their mothers were 97.7% and 100%, respectively in France.

The high titer of antibodies reflects that exposure to this agent is common in these regions, and detected antibodies in investigated apparently healthy cattle indicated that, parainfluenza type 3 virus infection is common and could be frequently symptom less. This is similar to some studies, Sakhaee *et al* (2009) detected antibodies in 181 serum samples of clinically healthy cattle in Iran, the samples were tested by commercial indirect ELISA kits against some viruses in which PIV-3 was found in 100% of samples. Frigeri and Arush (1979) examined sera from 1023 cattle for PI3 antibodies in Somalia land

using HI, positive reactions were found in 87.4%, for cattle.

This study showed the significant role of respiratory infection in health condition of cattle at North Kordofan as well as existence of PIV-3 in cattle, further work to characterize the virus is needed.

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