Detection of *E.coli K99* and *Rota virus* antigens in diarrheic and healthy buffalo of Babil Province, Iraq.

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Abstract

This study was conducted to detecte *E.coli K99* and *Rota virus* antigens in both diarrheic and healthy buffalo of Babil province (Iraq) by using ELISA kit.100 Fecal samples were collected from (12 calves aged 3-11 day, 13 calves aged 1-9 months and 75 adults at age 1-5 years) during November 2013-April 2014.Out of the 44 *E.coli* isolates which had growth on EMB agar, 7(58.3%), 5(38.4%) and 18(24%) isolates for calves aged 3-11 day and 1-9 months and adults at age 1-5 years respectively were found to be positive *E.coli K99* antigen. The percentage of infection with *rota virus* was 4(33.3%) in calves aged 3-11 day, 2(15.3%) in calves aged 1-9 months and 1 (1.3%) in 1-5 years. The results revealed a wide spread of *E.coli K99* and *rota virus* between calves and adults buffalo (diarrheic and healthy animals), in which buffalo considered a reservoirs and a potential source of more important zoonotic enteropathogens.

Key words: *E.coli K99, Rota virus,* ELISA, Buffalo, Babil.
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تثبيت مستضدات الاشيريشيا القولونية K99 وفايروس الدوار في الجاموس (المصاب بالإسهال والسليم ظاهريا) في محافظة بابل/ العراق

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الخلاصة

أجريت هذه الدراسة لتثبيت مستضدات الاشيريشيا القولونية K99 وفايروس الدوار في براز الجاموس المصاب بالاسهال والسليم ظاهريا في محافظة بابل (العراق) باستخدام فحص الاليزا (المقايسة المناعية المرتبط بالانزيم الممتز). جمعت 100عينة براز من (12 عجل بعمر 11–3 يوم، 13 عجل بعمر 9–1 شهر و75 بالغ بعمر 5–1 سنة) خلال تشرين الثاني 2013 – نيسان 2014. من مجموع 44 عزلة من الاشيريشيا القولونية والتي نمت على أكار الايوزين مثيلين الأزرق، 7 (58.3%)، 5 (38.4%) و18 (24%) عزلة للعجول بعمر 11–3 يوم و9–1 شهر وبالغ بعمر 5–1 سنة على التوالي كانت موجبة للاشيريشيا القولونية k99. كانت نسبة الإصابة بفايروس الدوار 4 (33.3%) في العجول بعمر 11–3 يوم، 2 (15.3%) في العجول بعمر 9–1 شهر و1(1.3%) في 5–1 سنة. النتائج تشير إلى الانتشار الواسع للاشيريشيا القولونية k99 وفايروس الدوار بين الجاموس (العجول والبالغ) المصاب بالاسهال والسليم ظاهريا، لهذا يعتبر الجاموس خازن ومصدر فعال لاهم الممرضات المعوية المشتركة بين الإنسان والحيوان.

الكلمات المفتاحية: الاشيريشيا القولونية K99، فايروس الدوار، الاليزا، جاموس، بابل.

Introduction

Diarrhea is a major problem in live stock production throughout the world (1). Enteritis in newborn calves causes high morbidity and mortality leading to significant economic loses, the principal known etiological agents include bacteria, viruses, protozoa (2,3). Diarrhea due to *Esherichia coli* is one of the most common diseases of

young calves, *E.coli K99* is seen in calves greater than 3-5 days old (4). Verotoxic *E.coli* associated with human diseases can also be isolated from feces of healthy cattle and buffalo(5). *E.coli* population are divided in to serotypes and serogroups according to antigenic composition (Somatic or O antigens, flagellar or H antigens and capsular or K antigens (6). *Rota virus*, the member of family *Reoviridae*, is the most important cause of human and animal diarrhea (2), birds worldwide (7,8). The group A *rota virus* is the most recorded in the cases of diarrhea, the virus has been reported in buffalo of India (9) and in buffalo calves of many countries like Turkey (10) and Iraq (11). The main purpose of the present study was to detect *E.coli K99* and *rota virus* in feces of calves and adult buffalo (diarrheic and non diarrheic animals) of Babil governorate.

Material and Methods

- Animals: The population of this study consisted of 100 buffalo (25 buffalo calves aged between 3-11 days old and 1-9 months) as well as 75 adults buffalo of local breed at Babil governorate, Iraq as in (Table 1).
- Isolation and Identification of *E.coli K99*: Aloopful of fecal sample was inoculated on 5% sheep blood agar, MacConkey agar, Eosin Methylen blue (EMB) agar(Oxiod).After overnight incubation at 37 ºC, colonies of *E.coli* were subculture on TSI, SIM and incubated aerobically at 35-37 ºC for 24 hr. The results of reactions read and compared with charts according to (12).
- Serological test: *Rota virus* was detected in feces of buffalo by commericial Kit (Latex agglutination test, plasmatic Laboratory Products/ United Kingdom).

Sandwich ELISA Kit (Enzyme Linked Immunosorbent Assay) was performed to detect antigens of *rota virus* and *E.coli K99* in fecal samples of buffalo as described by the Kit (*E .coli F5* (*K99*) and *Rota virus* ELISA Kit) Code L 11413.

Table (1) No. of exanimated samples of buffalo of Babil

Age of animals	No. of exanimated samples	Fecal status	
		ND	D
3-11 days	12	4	8
1-9 months	13	9	4
1-5 years	75	55	20

Results

The percentage of isolation of *E.coli* was 83.3% of buffalo calves aged (3-11 days), 77% of buffalo calves aged (1-9 months) and 32% of adults animals as in Table (2). All the isolates of *E.coli* which were growth on EMB agar were analyzed by Sandwich ELISA Kit to confirmed detection of this pathogenic microorganism. *E.coli K99* was found in 58.3% of calves aged (3-11 days), While the percentages was 38.4% at age (1-9 months) and 24% in adults buffalo as in Table (3). Concerning *rota virus* results of present revealed that out of 100 (diarrheic and non diarrheic) fecal samples collected from buffalo, *rota virus* was detected by Latex agglutination test and confirmed by Sandwich ELISA Kit as in Table (4,5) , yielding a prevalence rate of 33.3% and 15.3% in 3-11 day old calves and 1-9 months calves respectively, while the difference was occurred only at aged 1-5 years between the 2 tests, where the rate of infection was 1.33% (1 sample) for ELISA and 2.66% (2 sample) for Latex agglutination test.

Table (2) No. of *E.coli* isolates grown on EMB agar

Age of animals	No. of exanimated samples	*No. of *E.coli* isolates growth on EMB
3-11 days	12	10 (83.3%)
1-9 months	13	10(77%)
1-5 years	75	24(32%)

***Differences were significant (Chi-square value 9.87 P=0.007)**

Table (3) positive results for *E .coli K99* by ELISA Kit

Age of animals	No. of exanimated samples	No. of samples positive for ELISA*	Fecal status	
			ND**	D***
3-11 days	12	7(58.3%)	2(20%)	8(80%)
1-9 months	13	5(38.4%)	4(40%)	6(60%)
1-5 years	75	18(24%)	10(41.6%)	14(58.3%)

***Differences were not significant (Chi-square value 4.42 P=0.11)**
****Differences were not significant (Chi-square value 2.109 P=0.348)**
*****Differences were significant (Chi-square value 10.27P=0.006)**

Table (4) positive results for *Rota virus* by Latex agglutination test

Age of animals	No. of exanimated samples	No. of samples positive for **rota virus*	Fecal status	
			ND **	D***
3-11 days	12	4 (33.3%)	O	4(100%)
1-9 months	13	2 (15.3%)	1 (50%)	1 (50%)
1-5 years	75	2(2.6%)	2(100%)	O

***Differences were significant (Chi-square value 13.17 P=0.001)**
****Differences were not significant (Chi-square value 0.821 P=0.365)**
*****Differences were not significant (Chi-square value 2.05 P=0.152)**

Table (5) positive results for *Rota virus* by ELISA Kit

Age of animals	No. of exanimated samples	No. of samples positive for **rota virus*	Fecal status	
			ND**	D***
3-11 days	12	4 (33.3%)	O	4(100%)
1-9 months	13	2 (15.3%)	1 (50%)	1 (50%)
1-5 years	75	1(1.3%)	1(100%)	O

***Differences were significant (Chi-square value 16.16 P=0.00001)**
****Differences were not significant (Chi-square value 1.97 P=0.16)**
*****Differences were significant (Chi-square value 16.89 P=0.00001)**

Discussion

Out of the 44 *E.coli* isolates which had growth on EMB agar, 7(58.3%), 5(38.4%) and 18(24%) isolates for calves aged 3-11 day and 1-9 months and adults at age 1-5 years respectively were found to be positive *E.coli K99* antigen. The prevalence rate of *E.coli K99* was 8(80%) of diarrheic and 2 (20%) of non diarrheic calves aged (3-11 day), the infection rate was 6(60%) of diarrheic and 4 (40%) of non diarrheic calves aged (1-9 months), these finding was do not agree with (13) in England who record ETEC in 7.51% of diarrheic calves, but not from clinically normal calves. (14) confirm that ETEC and other serogroups had the highest frequency in 1-7 day old calves in winter season and reported prevalence rate 28.41% for ETEC among diarrheic calves in Iran. (15) in Egypt recorded that infection rate of *E.coli K99* was 57.1% in calves aged 0-4 day while the rate was 20.8% in calves aged between (5-14 day, 15-21 day and > 21 day). In a study of (10) done for diarrheic calves in Turkey it is estimated that the prevalence of *E.coli K99* was 9.4%. In another study (16) performed among healthy diary cattle herd in Van in Turkey, out of 235 isolates (28 isolates were found to be positive for *K99*). (17) reported that *E.coli K99* was detected not only as 13.4% in the diarrheic calves but also as 5.6% in the healthy calves by ELISA technique. In a study of (18) recorded 4.95% prevalence rate among healthy water buffalo in India. Results of present study do not agree with (19) who suggesting that *E.coli K99* as the major cause of neonatal diarrhea occurring in the first 4 days of life; however, it rarely lead to diarrhea in older calves or adults animals. In a study of (20) done for diarrheic buffalo calves in Egypt, have recorded the most common *E.coli* serotypes in isolated samples were O26 (23.52%), O103(19.6%) and O119(17.64%). Percentage of infection with

rota virus was 4(33.3%) in calves aged 3-11 day, 2(15.3%) in calves aged 1-9 months and 1 (1.3%) in 1-5 years. The highest rate of infection with *rota virus* in present study was recorded in diarrheic calves at age (3-11 day and 1-9 months), this findings in agreement with (11) suggesting that bovine *rota virus* is the essential cause of neonatal calf diarrhea, on the other hand this findings is different from those reported in diarrheic calves in Turkey (10) and India (21) which recorded low percentage of infection 25% and 4.76% respectively, while the values confirmed in present study in adult buffalo (1-5 years) was lower than (9) who recorded 22.01% in buffalo of India. These differences in incidence rates between two pathogens (*E.coli* and *Rota virus*) among the studies may be attributed to different diagnostic methods used, farm management practices exercised in different regions and related to aging of calf and stress factors. The results obtained in this study indicate the wide spread of *rota virus* and *E.coli K99* between calves and adult buffalo of Babil (diarrheic and non diarrheic), so more studies are required in order to establish precisely the identity and prevalence of these zoonotic pathogens in Iraq, such studies will provide important epidemiological data about this microorganisms.

References

1. Ibrahim, E. D. (2007). Studies on microbial causes of diarrhea in calves. M.V.Sc. Thesis, Fac. Vet. Med Kafr El-Sheikh Univ.
2. Estes, M. & Kapikian, A. (2007). *Rotaviruses*. In: Knipe, D. M.; Howley, P. M.; Griffin, D. E.; Lamb, R. A.; Martin, M. A.; Roizman, B.; Straus, S. E. editors Fields virology. Philadelphia: Kluwer Health/Lippincott, Williams and Wilkins. PP: 1917-1974.
3. Dhama, K.; Chauhan, R. S.; Mahendran, M. & Malik, S. V. S. (2009). *Rotavirus* diarrhea in bovines and other domesticated animals. Vet. Res. Comm., 33(1):1-23.
4. Gyles, C. L. (1993). *Escherichia coli*. In: Gyles, C. L.; Thoen, C. O.; eds. Pathogenesis of Bacterial Infections in Animals, 2nd ed., Ames, Iowa: Iowa State Univ Pr, PP: 164-187.
5. Kahn, C. M. (2010). The Merck Veterinary Manual. Ed. Scott line. 10th Ed. White house station, NJ. Merck, P. 223.
6. Campos, L. C.; Franzolin, M. R. & Trabulsi, L. R. (2004). Diarrheogenic *Escherichia coli* categories among the traditional enteropathogenic *Escherichia coli* O serogroups-a review Mem. Inst. Oswaldo Cruz., 99(6): 545-552.
7. Fenner, J. F; Paul, E.; Gibbs, J.; Murphy, F. A.; Rott, R.; Studdert, M. J. & White, D. O. (1993). In: Veterinary Virology. 2nd ed. Academic press Inc, USA, 538: 550-552.
8. Kapikian, A. Z.; Hoshino, Y.; Chanock, R. M. (2001). *Rota* viruses. In: Knipe, D. M.; Howley, P. M.; Griffin, D. E.; Martin, M. A.; Lamb, R. A.; Roizman, B. & Straus, S. E. (ed.), Fields virology, Vol. 2.4th ed. Chapter 55. Lippincott Williams and welkins, Philadelphia, USA, PP. 1787-1833.
9. Malik, Y. S.; Kumar, N.; Sharma, K.; Sharma, R.; Kumar, H. B.; Anupamlal, K.; Kumari, S.; Shukla, S. & Chandrashekar, K. M. (2013). Epidemiology and Genetic Diversity of *Rota virus* strains associated with acute gastroenteritis in Bovine, Porcine, population of Madhya Pradesh, Central India, 2004-2008. Adv. Anim. Vet. Sci., 1(4):111-115.
10. Icen, H.; Arserim, N. B.; ISIK, N.; Ozkan, C. & Kaya, A. (2013). Prevalence of Four Enteropathogens with Immunochroma tographic Rapid Test in the Fecese of Diarrheic Calves in East and Southeast of Turkey. Pak. Vet.J.,1-4.

11. Robaiee, I. A. & Al-Farwachi, M. I. (2013). Prevalence of *rota* viral infection in diarrheic neonatal calves in Mosul city, Iraq. Vet. World., 538-540.
12. Quinn, P. J.; Carter, M. E.; Markey, B. K. & Carter, G. R. (1994). Clinical Veterinary Microbiology Mosby. Yearbook Europe Limited.
13. Sherwood, D.; Snodgrass, D. R. & Lawson, G. H. K. (1993). Prevalence of *Escherichia coli* in calves in Scotland and northern England. Vet. Res., 113:208-212.
14. Shahrani, M.; Dehkordi, F. S. & Momtaz, H. (2014). Characterization of *Escherichia coli* virulence genes, pathotypes and antibiotic resistance properties in diarrheic calves in Iran. Biol. Res., (47):28.
15. ABou El-Ella, G. A.; Mohamed, A. M. & Amer, A. A. (2013). Prevalence of Enteropathogens associated with neonatal calf scour in cattle and buffalo calves using (fastest® strips) Rapid field test. Search Journal, 59(138).
16. Cabalar, M.; Boynukara, B.; Gulhan, T. & Ekin, I. H. (2001). Prevalence of *Rota virus*, *Escherichia coli K99* and *O157:H7* in healthy dairy cattle herds in Van, Turkey. Turk. J. Vet. Anim. Sci., 25:191-196.
17. Ok, M. L.; Güler, K.; Turgut, U.; Ok, İ.; Şen, İ. K.; Gündüz, M. F. & Güzelbekteş, H. (2009). The studies on the etiology of diarrhoea in neonatal calves and determination of virulence gene markers of *Escherichia coli* strains by multiplex PCR. Zoonoses Public Hlth., 56:94-101.
18. Mahanti, A.; Samanta, I.; Bandyopadhyay, S.; Joardar, S. N.; Dutta, T. K. & Sar, T. K. (2014). Isolation, Molecular characterization and antibiotic resistance of Enterotoxigenic *E.coli* (ETEC) and Necrotoxigenic *E.coli* (NTEC) from healthy water buffalo.
19. Foster, D. M. & Smith, G. W. (2009). Pathophysiology of diarrhea in calves. Vet. Clin. North Am. Food Anim. Pract., 25: 13-36.
20. Abd- Elrahman, A. H. (2011). Colibacillosis in Newly born buffalo calves and role of Lacteol fort in preventing recurrence of calf diarrheia. Life Sci. J., 8(4): 497-502.
21. Udaykar, A.; Sharda, R.; Malik, Y. S.; Sharma, V. & Shrivastava, N. (2013). Occurance of group A *rota virus* in diarrheic buffalo and cow calves, Madhya Pradesh, India. Adv. Anim. Vet. Sci., 1(4s):51-53.