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Lapin *Escherichiae coli* K_1 Vaginosis

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Abstract

We used two groups of female rabbits .First group was used to study the pathogenesis of *Escherichiae colli* serotype K_1 ,second group was used to study the some immunological parameters .Second group was divided into three sub groups ,first was intravaginal inoculation with live bacteria ($0,5*10^4$),second was intravaginal inoculation with capsular antigen and third group was used as control .After suitable period blood and autiopsies were collected from all subgroups to study humoral immunity (titration ,immunoglobulin isotype ,total protein concentration) and cellular immunity (Leukocytte inhibition factor ,Macrophage migration inhibition factor ,skin test) . Specific antibody titer was showed with capsuller antigen and cell free culture antigen and specific leucocyte inhibtion factor and macrophage migration inhibition factor were showed in test group and not seen in control group . We concluded from this study that vaginal of rabbit female is part of common mucosal immune system because specific titer and cellular immunity were seen in intestinal parts.

الخلاصة

استعملت مجموعتين من الحيوانات اناث ارانب) فالمجموعة الاولى استخدمت في دراسة الامراضية لبكتريا الاشريشيا القولونية النوع المصلي K_1 واما المجموعة الثانية فقد استخدمت في دراسة بعض المعايير المناعية وقد قسمت الى ثلاثة مجاميع الاولى لقحت عن طريق المهبل ببكتريا وبتركيز $0.5*10^4$ وحدة/ مل اما الثانية فلقحت بمستضد المحفظة بتركيز (10 وحدات دولية) اما المجموعة الثالثة استخدمت كسيطرة . وبعد فترة حضانة معينة يتم جمع الدم ومقاطع نسيجية وهي اجزاء من الجهاز التناسلي واجزاء من القناة الهضمة من كل المجاميع لدراسة المناعة الخلطية وهي المعايرة واصناف الكلوبيولين وتركيز البروتين الكلي والمناعة الخلوية وتشمل عامل تثبيط هجرة الخلايا البيض وعامل تثبيط هجرة البلعم البير حيث اظهرت الدراسة اضداد متخصصة مع مستضد المحفظة ومستضد المزرعة الحر من الخلايا وكذلك بينت النتائج وجود فروق معنوية في عامل تثبيط هجرة الخلايا وعامل تثبيط هجرة البلعم الكبير . نستنتج من هذه الدراسة انالطبقة المخاطية المهبلية في اناث الارانب هي جزء من الجهاز المناعي المخاطي العام حيث لوحظت عيارات متخصصة ومناعة خلوية في اجزاء من الجهاز الهضمي .

Introduction

Vaginal colonization of *E.coli* is associated with various genitourinary, obstetric and neonatal complications and early onset neonatal septicemia and meningitis (Schiffer *et al.*, 1976). Vaginal *E.coli* have been reported to be sexually transmissible to male partner (Hebelka *e tal.* ,1993)vaginal *E.coli* is reservoir along the fecal ,vaginal ,urinary / neonatal course of transmission .the extraintestinal *E. coli* infection ,and most dominant strains of *E. coli* have the K1 antigen (Yasuoka *et al.*, 2002 ;Watt *et al.* ,2003).There is relation between the K1 capsular polysaccharide of *E.coli* and invasiveness (Schiffer *et al.*,1976; Yasuoka *et al.*,2002). Several reports suggest that the K_1 capsular polysaccharide confers invasiveness upon *E. coli* and there are indications that polysaccharide exerts an antiphagocytic effect similar to that observed with other encapsulated bacteria (Pluschke *et al.*, 1983).the aim of study as follow

1- Using animal model to study the pathogenesis of bacteria and immunological properties after intravaginal immunization with capsular k1 antigen and live bacteria of *E.coli*. .
2- Detection of certain cytokines including chemokines ,the IL-1α and IL-8 concentration in serum of rabbit .

Materials and Methods :

- Animals

Rabbits were elected as test experimental animals .They were brought from local market and of local breed (*Orcyctalagus cuniculus*) the sexing were females(1-1.5)kg.

II- Antigens

1. Viable antigen (live bacteria of *E. coli* K_1) dose (0.5×10^4)cfu/ml was used in immunization protocol.
2. Non viable antigen (capsular antigen) was prepared as (Kwapinsiki,1972) .The dose was 10 international unit was prepared with compared with WHO standard tube 1975, and this antigen was used in immunization protocol.

III- Immunization protocol

1. First group of animals that inoculated with live bacteria by inoculated (0.1)ml of (0.5×10^4)cf4/ml of live bacteria intravaginal and this inoculation was done at intervals of 2 days up to 14 days of ten the 14 days, the animals left one week and one of them was taken after 6 days and study the immunologic investigation. Second animal was taken after 12 days and third was taken after 18 days.

2. Second group of animals that inoculated with capsular antigen by inoculated 0.1ml of (10Iu capsular ag) in vaginal and intervals 2 days. Until 14 days and the animals were left for 32 days.

3. Macrophage stimulation.

Macrophage were stimulated by using casein digest (1.2g for 100ml D. W. and sterilized by autoclave) 5ml of casein digest was injected in peritoneal cavity (Bloom and Bennett, 1966).

This stimulation was occurred in second group of animals and after 72hrs the peritoneal fluid was collected and used in macrophage migration inhibitory study.

4. skin test

0.1 ml of capsular antigen (prepared as 3.8.1) was injected in the skin by using sterile syringe (size 1ml) and the erythrema and induration were recorded after (4)hrs, (24)hrs,(48)hrs , (72)hrs. (Tompkins *et al.*, 1973).

Three groups of animal were killed and autiopsies of genital tract (vagina, horns, fallopian tubes and ovary) intestinal tract (duodenum and appendix),these autiopsies were opened by clean scissor and laid in clean Petri dishes and the mucosa were scrabed with 10 ml formal saline and laid in clean tubes ,centrifuged at 3500 rpm/30 minute .then solution was formed

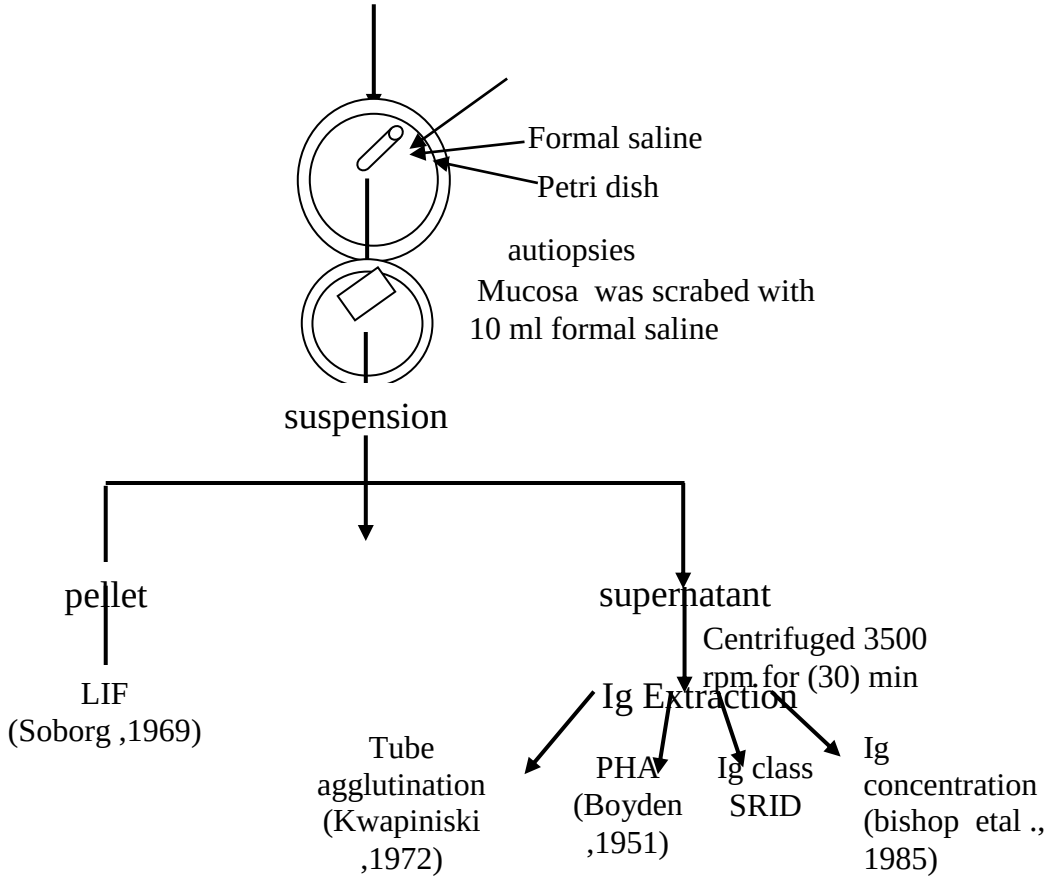

Figure (1) The study on immunological methods on rabbits mucosa

Results :

1-Infection Model

E. coli K_1 experimental lapin vaginosis were produced in its mild from where, fever, vaginal congestion and or slight secretions were noticed. *E coli* K_1 was isolated in 1/3 of the infected rabbits (table 1).

2- Immunological Model

E.coli K_1 stimulate mucosal and systemic humoral immune responses as well as mucosal and systemic cellular immune responses when rabbits were immunized with lives and capsular material as specific immunologic test were indicated.Specific antibodies appeared in serum and mucosal parts in rabbits that inoculated with bacteria and capsular antigen (tables 2,3).

The titers was not showed in normal rabbits and the mean of protein concentration was 122g/L and the mean of mucosal Ig 3.35g/L .

Immunoglobulin isotypes was determined in serum rabbit by using single radial immunediffussion plates and also was tried to determine isotypes in mucosal Igs but no results were appeared.

The percents of leucocyte migration inhibition were studied in rabbits in three sites the peritioneal fluid, peripheral blood and vaginal mucosa. The antigens that were used in this test were capsular antigen (cap) and cell free culture antigen (cfc) (table 4) the present of inhibition was calculated as the equation

Percent inhibition=1-[(mean area of migration with antigen)/(mean area of migration without antigen)].,1973).*al et*(Tompkin 100 ×

Delayed type hypersensitivity reaction was studied in rabbits by using capsular antigen. Positive results were determined using various calibration double fold thickness was measured in the rabbit after 24 hr , 48hr and 72hr and the signs were erythrema and indurations and the indurations 10mm to 28 mm .

IL-1α and IL-8 ELISA were used to determine the concentration of IL-1α and IL-8 in the rabbits groups and used the equations for standard showed that concentrations of IL-1α in test groups (live bacteria and rabbits with capsular ag) were higher than normal, and the concentrations of IL-8 in test group had higher concentrations than normal.

Table (1): The parameter that used to determine the infection model

Parameters	Group1	Group2	Group3
Dose	0.5×10^5	0.5×10^5	0.5×10^4 /ml
Volume	0.1	0.1	0.1
Fever	+	+	+
Pus cell	-	+	+
Redness of mucus membrane	+	-	+
E. coli	-	-	+
secretion	+	+	+

Table (2): Specific antibody titers and correlate with concentration in rabbit treated live bacteria

	Anticapsular		Anti cfc		Concentration g/L	
	serum	mucosal	serum	mucosal	serum	mucosal
Rabbit 1	640	D=64	320	D=64	104.43	1.26
		A=64		A=32		0.52
		O=64		O=32		8.6
		V=32		V=32		4.9
		H=32		H=32		4.9
Rabbit 2	640	D=64	640	D=64	111.805	0.52
		A=64		A=32		0.89
		O=64		O=32		8.6
		V=64		V=32		0.89
		H=32		H=32		3.47
Rabbit 3	320	D=64	640	D=64	119.17	0.158
		A=64		A=32		1.26
		O=64		O=32		1.26
		V=64		V=32		0.15
		H=32		H=32		4.94
					$\bar{x}$ =111.8	$\bar{x}$ =2.82

Table (3): Specific antibody titers and correlate with concentration in rabbit that received capsular antigen.

	Anticapsular		Anti cfc		Concentration g/L	
	serum	mucosal	Serum	mucosal	serum	mucosal
Rabbit 1	1280	D=64	640	D=32	119.17	1.26
		A=32		A=32		0.52
		O=64		O=32		4.49
		V=32		V=32		0.52
		H=32		H=32		16
Rabbit 2	640	D=64	640	D=32	119.17	4.94
		A=64		A=32		1.26
		O=32		O=32		6.79
		V=32		V=64		23.6
		H=32		H=32		16
Rabbit 3	320	D=32	640	D=64	119.17	23.6
		A=64		A=32		8.63
		O=64		O=32		23.6
		V=64		V=32		19.6
		H=32		H=32		16
					$\bar{x}$ =119.17	$\bar{x}$ =11.12

D=duodenum . H=horn. A=Appendix. I=Immunoglobulin
O=Ovary and fallopian tubes $\bar{x}$ = the mean V=Vagina

Table (4): Immunoglobulin isotypes in rabbits

Type of treatment	IgG mg/dL	IgA mg/dL	IgM mg/dL
live bacteria	2937.9	130.8	238.7
	2864.4	130.8	332.8
	2937.9	229	64.3
	$\bar{x}$ =2913.4	$\bar{x}$ =163.53	$\bar{x}$ =208.6
capsular antigen	2173.7	229	322.8
	2937.9	130.8	322.8
	2864.4	130.8	64.3
	$\bar{x}$ =2658.66	$\bar{x}$ =163.52	$\bar{x}$ =236.6
Control normal saline	2173.7	130.8	64.3
	2937.9	23.2	322.8
	$\bar{x}$ =2555.8	$\bar{x}$ =77	$\bar{x}$ =193.5

Table (5) :Leucocyte migration inhibition study rabbits

	peritoneal		Peripheral blood		Vagina mucosa	
Type of treatment	cfc	cap	cfc	cap	cfc	cap
live bacteria	40	50	65	86	43	58
	34	59	25	50	29	65
	40	50	59	75	42	59
	$\bar{x}$ =38	$\bar{x}$ =53	$\bar{x}$ =49.6	$\bar{x}$ =70.3	$\bar{x}$ =38	$\bar{x}$ =60.6
capsular antigen	44	50	44	50	29	50
	25	50	44	50	44	59
	40	50	40	60	39	62
	$\bar{x}$ =36.3	$\bar{x}$ =50	$\bar{x}$ =42.6	$\bar{x}$ =53.3	$\bar{x}$ =37.3	$\bar{x}$ =57
	S=10.01	S=0	S=2.3	S=5.7	S=7.6	S=6.2
Normal saline	10	3	5	5	5	5
	15	5	9	9	5	9
	$\bar{x}$ =12.5	$\bar{x}$ =4	$\bar{x}$ =7	$\bar{x}$ =7	$\bar{x}$ =5	$\bar{x}$ =7
	S=3.5	S=1.4	S=2.8	S=2.8	S=0	S=2.8

$\bar{x}$ = mean s= standard deviation

Table(6): IL-1α and IL-8 concentrations in rabbits

Type of treated	IL-1α concentration pg/ml	IL-8 concen pg/ml
live bacteria	161.7662	118.798
	164.20528	121.312
capsular antigen	82.90288	73.6832
	101.060416	173.2146
normal saline	33.3398	34.07
	25.44918	65.5012

Discussion:

A mild form of *E. coli* k1 experimental lapin vaginosis was noticed with the formation of fever vaginal congestion and or slight secretion,it appears that $0.5* 10^4$ colony forming units is the dose required for production of vaginosis by *E. coli* . Pus cells ,clue cells ,vaginal secretion and the organisms were detected .These signs of bacterial vaginosis were noticed after 2 days from intravaginal inoculation . This is probably related to the essential barrier provided by the epithelial cells at the end of cevix uterus and fallopian tubes ,this single cell layer was initially thought to reside in a sterile environment that was only infrequently exposed to bacteria constitutively present in the ectocervix and vagina for example viable free and sperm associated bacteria are found in the uterine lumen of the mouse . Clearance of viable bacteria occurred within approximately 48 hrs following mating and was associated with phagocytosis of bacteria as well as the presence of antimicrobial product (Parr and Parr ,1985; Thaper *et al.* , 1990 ; Johansson *et al.* ,1997 ;Kozlowski *et al.* ,2002)

E.coli k1 antigen stimulate local and systemic humoral responses. The specific antibody against capsule antigen or cell free culture antigen in both systemic and local, Inoculation of antigen intravaginally induced specific antibody .The nature of antigen crucial role in immune response of female genital tract (Wassen *et al.* ,1996). Successful local immunizations have been reported with mostly live

microorganisms as vectors while in soluble antigen have been found to be poorly immunogenic (Russell, 2002) .

This has been explained to be consequence of the lack of organized lymphoid tissue and M cells in the female genital tract .Its thought that replicating antigen provide better delivery of antigen because they infect local tissue and may be more easily taken up by antigen processing cells ,such as phagocytic cells as compared to soluble antigen alone ,however ,except for defined up take mechanism , the requirements for antigen presentation to T cells in female reproductive , well met by the presence of Langerhans dendritic cells ,macrophages and even epithelial cells that express MHC class I and I I and which specifically activate T cells , whether these mucosal antigen presenting cell can initiate a local IgA response or whether is a dependence for trafficking of APC to immunocomptent cells T and B cells in regional lymph nodes is not well understood (Wassen *etal*. ,1996) capsular antigen of B streptococcal was not found elicit antibody in mice after intravaginal inoculation (Hordness *etal* .,1997), while in rabbits that intravaginal inoculation *E.coli* K 1 capsular antigens was found to elicit the production of specific antibody with high titers in the present study.

Little variation in Ig isotypes was detected in rabbit serum immunized with live and capsular antigens ,the concentration of IgG,IgA and IgM were higher in immunized than control .This result was not unusual, since infection stimulate the lymphocytes responsible for production of antibodies (Wu *etal* .,2000), mice intravaginaly inoculated with protein of *Streptococcus mutans* coupled to the B subunit of Cholera toxin lead to increased the serum IgA,IgG (Wu *etal.* ,2000) . Study of LIF and MIF in rabbits were demonstrated in three groups of rabbits one group was considered as control groups and other as tested groups,No migration inhibition in control group and the means of MIF and LIF were (12.5 , 4, 7 , 7, 5,7) while in test groups showed inhibition of migration and the means of MIF and LIF were (36, 50,42.6,53.3,57) these results were similar to that reported by (Tompkins *et al.* ,1973). Hypersensitive skin reaction was used as an in vivo to study cell mediated immunity in rabbits against capsular antigen and the result of this test was positive and this indicate that capsul;ar antigen stimulated cell mediated immunity in vivo as well as in vitro, this similar to (Tompkins *et al.* ,1973). **t**he rabbits inoculated with live bacteria showed an IL-1α concentration were(161.766, 164.2052) pg/ml and that with capsular antigen were (82.9028 and 101.06) pg/ml while in control group were (33.339 and 25.44)pg/ml, this also demonstrated the concentration of IL-8 in these groups of rabbits and found its concentrations in test groups were higher than in control groups . this is probability due to the activity of IL-8 as chemotactic agent in inflammation (Ko *et al.* ,1993).

From this study we concluded that vaginal of female rabbit is part of common mucosal immune system .

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