GLOBAL JOURNAL OF MEDICAL SCIENCES VOL 6, NO. 1, 2007: 17 - 23

 ISSN 1596-2911

PATHOLOGY OF EXPERIMENTAL ESCHERICHIA COLI INFECTION IN MICE: A LABORATORY ANIMAL MODEL FOR RESEARCH IN MATITIS COMPLEX

J. T. ABRAHAM, P. A. AKPAN AND E. M. IKPEME

(Received 5 March 2005; Revision Accepted 8 March 2007)

ABSTRACT

Bacterial mastitis frequently causes serious depressions of milk production and in certain cases may result in death in the dairy herd. Experimental bacterial mastitis served as a prelude to studying the actual infection in dairy cows. Cultures of nine serotypes of Escherichia coli isolated from various bacterial infections of animals were introduced into the gland cistern of normal lactating teats of mice. Each sereotype was inoculated in five log doses ranging from 10 to 10^5 colony-forming units (CFU). Each dose level was inoculated into three mammary glands of each of three mice. Mice were sacrificed after 72hrs and the glands examined bacteriological and histologically. Positive bacteriological and histological results were required for a diagnosis of infection. The infective dose fifty (ID_{50}) for the nine stereotypes ranged from 39 to 12,000 CFU.The nine sereotypes designated as (1) highly virulent (2) moderately virulent and (3) weakly virulent. Histologically, the mastitis produced in mice resembles naturally occurring coliform mastitis in the cow.

KEYWORDS: Escherichia coli, mice, Pathology, Lactating, Mastitis

INTRODUCTION

Mastitis means inflammation of the mammary gland. Inflammation is the body's reaction to an injury, which may be caused by a physical, chemical or biological agent, or by combination of such agents. While any of these agents are capable of causing mastitis, infections by bacteria are by far the most prevalent (Adinarayana, 1968, Anderson, 1972). The bacterial causing mastitis are varied. Bacteriological investigations of mastitis have shown that about 90 per cent of the disease are caused by the gram positive cocci and only 5 per cent or less by the gram negative bacilli mostly coliform (Bushnell, 1974). Mastitis is the most economically damaging disease complex affecting the dairy industry. Most serious losses are due to decreased milk production from subeclinically-infected cows. Research into this complex infection required development of a suitable inexpensive laboratory animal model. The experimental mouse mastitis offers the possibility of examining a large number of strains of E coli or other coliforms by the technique that induced an active infection and a pathological response. The technique of inoculation of E coli serotypes into the mammary gland of mice was used to demonstrate that E coli of different serotypes differ in their virulence as evidenced by clinical response, the number of bacteria recovered from the infected glands and histological lesions induced.

MATERIALS AND METHODS

Swiss-Webster albino mice supplied by the department of Veterinary Sciences of the Pennsylvania State University USA were used throughout the investigation. Pregnant mice were maintained in individual plastic cages and rat pellets and water was available at libitum. The mice sucked their young for three days after parturition, after which the young were removed. Inoculation of the mammary glands was done on the second day after removal of the young.

BACTERIAL INOCULUM

Just before inoculation, the mice were lightly anesthetized with either, restrained on their backs and the teats swabbed with 70 per cent ethanol. The teats were held lightly with forceps and the inoculum was introduced through the teat canal with a blunted 30-guage – hypodemic needle. Three glands on one side of the animal were infused with the suspension while the corresponding contralateral glands received an equal volume of sterile saline. The volume of the inoculum was 0.5ml in all cases.

J. T. Abraham, Department of Biological Science, Cross River State University of Technology Calabar. Nigeria.
P. A. Akpan, Department of Biological Science, Cross River State University of Technology Calabar. Nigeria.
E. M. Ikpeme, Department of Biological Science, Cross River State University of Technology Calabar. Nigeria.

DOSAGE

Five dose levels of E coli serotypes were used and each dose level was administered to three glands of each of three mice. The pattern of inoculation for each E coli serotype is indicated below.

POST-INOCULATION OBSERVATIONS

Inoculated mice were observed three times daily for obvious signs of abnormality. Wen death occurred, before the end of the 72 – hour observation period, the animals were removed immediately and examined as indicated below. At 72 hours after inoculation, survivors were killed, and the mammary glands were examined microscopically. Inoculated glands were excised for bacteriological examination.

BACTERIOLOGICAL METHOD

Excised mammary glands were touched with a sterile loop and streaked immediately on Violet Red Bile Agar (VRB). Colonies having the appearance of E coli were transferred to trypticase soy agar slants. They were subsequently tested in triple sugar iron agar and on Simons Citrate agar. Isolants with reaction characteristics of E coli were subsequently identified serologically. The objective of serological identification was to assure that E Coli recovered from an infection was the same as that inoculated. Only the 'O' antigen was determined. In this procedure, a sterile trypticase soy broth was inoculated from an agar slant and incubated overnight at 37°C. The broth culture was heated at 100 °C for one hour. The culture was then diluted to an appropriate density with formal saline. One drop of diluted antigen and three drops of specific antiserum were mixed on a glass slide. The slide was rotated for 10minites and then read. The presence of fine particles in the mixture was a positive result, indicative of the presence of that specific 'O' antigen.

HISTOLOGICAL METHODS

Excised mammary glands were fixed in 10 per cent formalized saline, dehydrated in alcohol, cleared in xylol, and embedded in paraplast. Sections were cut at five microns and stained with hemataxylin and eosin. They were then mounted, cover slipped, and examined microscopical.

RESULTS

HISTOLOGICAL EXAMINATION

The histological findings are described under four headings

(1) No reaction (Fig 1)
(2) Mild reaction (Fig 2)
(3) Moderate reaction (fig 3) and
(4) Severe reaction (fig 4)

1. **NO REACTION**: No Pathological changes were seen in these tissues. This condition was found in all control glands.

2. **MILD REACTION**: There were few histological changes in these tissues. Mixed inflammatory cells were scattered throughout the tissue and in the alvelar lumina (fig2).

3 **MODERATE REACTION**: In theses sections there were greater accumulation of inflammatory cells. There were mixed inflammatory cells in a alveoli and ducts interalveolar infiltration of neutrophils and accumulation of inflammatory exudates and desquamated epithelial cells in the lumina (fig 3).

4 **SEVERE REACTION** The inflammatory changes in these sections were more severe than those already described. The alveolar lumina and lactiferous ducts were tightly packed with inflammatory cells and cellular debris. Degenerative changes were often seen in secretory.

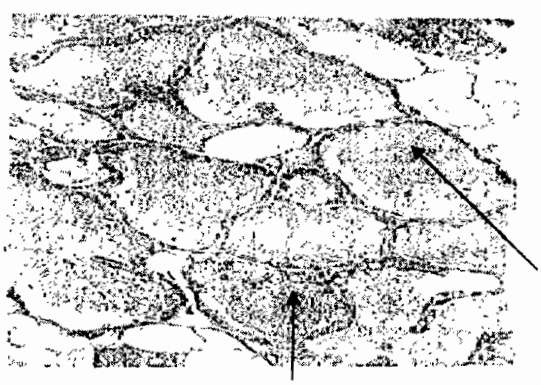

Fig 1 Mouse mammary gland control No reaction. Notice absence of inflamatory cells indicated by arrows (H & E Stain, 150X).

Fig 2: Mouse mammary gland, three days after infusion with 10^5 CFU of E coli 09. Mild reaction, Notice inflammatory cells within alveolar lumina. Indicated by arrow some lumina have proteinaceous material (H&E Stain, 350X)

Fig 3: Mouse mammary gland, moderate reaction three days after infusion with 10^5 CFU of E coli 0137 Notice inflammatory cells within alveolar lumina and in inter-alveolar spaces as indicated by arrows (H & E Stain, 350X).

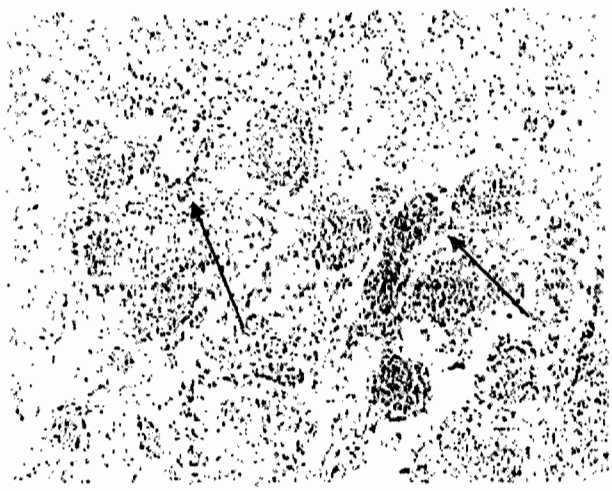

Fig 4 Mouse mammary gland, severe reaction three days after infusion with 10^5 CFU of E coli 0111. Notice mixed inflammatory cells within alveolar lumina and surrounding epithelial tissue. Indicated by arrows (H & E Stain, 150X)

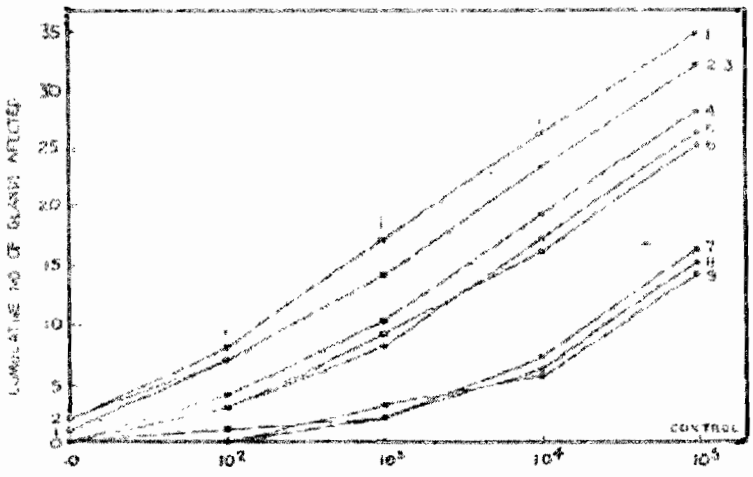

Fig. 5 Dose –Response curve for nine E coli serotypes, 1 = 0111, 2 = 02a, 3 = 0101, 4 = 046, 5 = 0137, 6 = 062, 7 = 09, 8 = 037, 9 = 0109.

Epithelial cells. There was heavy infiltration of interlveolar and interlobular spaces by inflammatory cells (fig 4).

The percentage of glands which developed mastitis and which were judged infected by the above criteria after inoculation with different doses of the nine E Coli serotypes are presented in Table 2. The serotypes are listed here in order of decreasing infectivity as determined by subsequent analysis of the data. The ability to establish infection was dose dependent and is shown clearly in this data (Table 2).

With a dose of 10^0cfu, infectivity was 100 percent for all serotypes except 0109 at the lowest dosages, 10^0cfu, establishment of infection was sporadic and was limited to a few serotypes. Mortality was low and tended to occur in mice given the highest dosage levels of the more virulent strains (Table 2). The five cases of negative cultural and negative histological results were considered not infected.

Histological examination revealed three types of inflammation, which was categorized as mild, moderate, and severe. There was considerable variability in the degree and intensity of the inflammatory changes. With the highly virulent stains, low doses did produce some severe inflammatory changes whereas with the less virulent ones inflammatory changes were observed mainly at the high dose levels.

The inflammatory changes seen, namely cellular infiltration of the alveolar lumina, interlobular and interalveolar tissues and some necrosis are also characteristic of coliform infection in the cow (Anderson, 1976, 1974B, 1974C, Bushnell, 1974).

The variation in the responses of various mice infused with identical doses of same serotype points to the resistance of the host as a major factor in the infection process.

The results indicate that E Coli mastitis can be established in lactating mice, that the responses is dose dependent, and that histologically, the disease produced resembles naturally occurring coliform mastitis in the cow. The infectivity ratio for the less virulent strains (table 3) at the lower levels was usually zero. The proportion of infected glands in all cases increased with increase in size of inoculum (table 3).

The more virulent organism gave raise to more rapid increase in the infectivity ratio of inoculum. Thus the proportion of infected glands is related to both the size of inoculum and the virulence of the organism.

Positive bacteriology and positive histological results were the two criteria for diagnoses of infection. These two criteria were necessary because the presence of one without the other may create some doubt concerning either the identity of the etiologic agent, or its capability to infect. Glands, which were positive for culture but negative for histology, were not counted among those infected because the presence of microorganism alone without any evidence of infection is an indication that the organisms are not virulent. Glands, which were culturally negative but positive histologically, were not counted as infected. Dose response curves for the nine E Coli serotypes are plotted in figure 1. These data suggests that the nine serotypes tended to fall into three groups of three strains high, moderate and low virulence. Infective dose fifty (ID_{50}) for the nine serotypes were calculated from the infectivity data. A mastitis index for each serotype (Ratio of glands infected to glands inoculated X100) was also calculated. These are presented in table 4. The

ID_{50} for the various strains ranged from 39 to approximately 12.000cfu indicating large difference in virulence among the serotypes.

DOSAGE

Five dose levels of each E Coli serotype were used and each dose level was administered to three glands of each of three mice. The pattern of inoculation for each E Coli serotype is indicated below.

TABLE 1

Dose level CFU in 0.05ml	Number of mice	Number of Glands
10	3	9
10^2	3	9
10^3	3	9
10^4	3	9
10^5	3	9

Thus each of the nine serotypes was infused at five-long dose levels into 45 glands of 15 mice.

TABLE 2: Summary of bacterological and histological diagnosis in mouse mammary glands inoculated with different stranins of E Coli

E. coli Inoculate	Positive Culture/ Positive Histology	Positive Culture/ Negative Histology	Negative Culture/ Positive Histology	Negative Culture/ Negative Histology
0111	35	10	0	0
02a	32	13	0	0
0101	32	13	0	0
0146	28	16	1	0
0137	26	17	0	2
062	25	19	1	0
09	16	28	0	1
037	15	30	0	0
0109	13	30	0	2

For each serotype a total of 45 glands were inoculated.
E. coli are arranged in decreasing order of virulence (up-down)

Table 3: Summary of glands infected and mortality following intramammary inoculation of different dosages of nine. E. Coli strains. Nine glands (three in each of the three mice were inoculated with ease dosage of each strain.

E. coli Inoculated	Does (CFU)	Number of Glands which Developed Mastitis	Infectiveity Ratio	Number of Mice which died
0111	10^5	9	100.0	1
	10^4	9	100.0	1
	10^3	9	10010	0
	10^2	6	72.7	0
	10	2	16.7	0
02a	10^5	9	100.0	2
	10^4	9	100.0	1
	10^3	7	81.5	0
	10^2	6	58.3	0
	10	1	7.1	0
0101	10^5	9	100.0	1

	10^4	9	100.0	0
	10^3	7	87.5	0
	10^2	5	53.8	0
	10	2	13.3	0
0146	10^5	9	100.0	0
	10^4	9	100.0	0
	10^3	6	76.9	0
	10^2	4	33.3	0
	10	0	0.0	0
0137	10^5	9	100.0	1
	10^4	9	100.0	0
	10^3	5	66.7	0
	10^2	3	23.1	0
	10	0	0.0	0
062	10^5	9	100.0	1
	10^4	7	88.9	0
	10^3	3	64.3	0
	10^2	6	21.4	0
	10	0	0.0	0

E coli are arranged in decreasing order of virulence (up-down)

E. coli Inoculated	Does (CFU)	Number of Glands which Developed Mastitis	Infectiveity Ratio	Number of Mice which died
09	10^5	9	100.0	0
	10^4	5	63.6	0
	10^3	1	14.3	0
	10^2	1	4.8	0
	10	0	0.0	0
037	10^5	9	100.0	0
	10^4	4	54.5	1
	10^3	2	14.3	0
	10^2	0	0.0	0
	10	0	0.0	0
0109	10^5	8	93.3	0
	10^4	3	46.2	0
	10^3	3	18.8	0
	10^2	0	0.0	0
	10	0	0.0	0

$$\frac{\text{Cumulative number of glands infected}}{\text{Cumulative number of glands infected + cumulative not infected}} \times 100$$

TABLE 4: Summary of experience on infectivity in the mouse mammary gland and susceptibility to killing by normal bovine serum of nine E coli strains.

E. coli Inoculated	ID in Mouse ^{50}Mammary Gland (CFU)	Mastitis Index [1]	Percentage Survival in normal Bovine Serm (1:32	Percentage survival in normal Bovine serum with added complement (1:32)
0111	39	78	95	75
02a	69	71	75	94
0101	81	71	66	64
0146	242	62	51	55
0137	417	58	37	50
062	465	56	42	68
09	5,300	36	40	41
037	7,740	33	32	53
0109	12,00	29	43	41

Mastitis index = $\frac{\text{Number of mouse mammary glands infected}}{\text{Number of mouse mammary glands inoculated}}$ x 100

E coli are arranged in decreasing order of virulence (up-down)

DISCUSSION

The infusion of graded doses of E coli into indicating mouse mammary glands resulted in acute mastitis of variable degrees of severity. The response from each mouse was probably a reflection of both virulence of the organism and resistance of the host. Differences between strains must have played a major role in the types of response since identical doses of different serotypes gave raise to variable inflammatory responses.

CONCLUSION

Coliform organisms are abundant and cam cause severe mastitis. Inoculation of E coli of varying virulence into glands systems of mice has been used to show that mastitis similar to that in the cow can be produced in the mouse and that strains of E coli differ in their virulence as assessed by the histological lesions in the infected glands. Establishment of bacterial mastitis in mice similar to what obtains in cattle is of great significance in mastitis research. None availability of an inexperience laboratory animal model for mastitis research has impeded progress in the campaign against mammary gland disease. It is obvious that the bacteria mouse mastitis model will greatly enhance progress in the fight against udder infections.

ACKNOWLEDGEMENT

I am grateful to the Pennsylvania Sate University USA for providing the funds and facilities for this study. I deeply appreciate the cooperation of Pros R. E. Eberthat and A. L. Bortree of the Dept. of Vertinary Science.

REFERENCES

Andinarayana, N. and Sigh, S. B., 1968. Caprine mastitis due to Klebsiela Peneumoniae Indian Vet. J 45:373.

Aenfelt, E., and Krause, D., 1955 Bact. Coli mastitis in the cow. Dtsch. Tierarztl. Wschr. 62.211.

Anderson, J.C., 1974a. Effect of treat damage on incidence of experimental mastitis in the mouse. Res. Vet. Sci. 13390.

Anderson, J.C., 1972. Experimental staphylococcal mastitis in the mouse. Effect of inoculating different strains into separate glands of same mouse. J. Comp. Path 84103.

Anderson, J.C. and Mason, A. J., 1974b. Effect of cell-free staphylococcal toxin on the mammary gland of the mouse. Res. Vet. Sci. 1623.

Anderson, J.C.,1974c.Experimental staphylococcal mastitis in the mouse. Effects of extracellular products and whole bacterial cells from a high virulence and low virulence strain of staphylococcus aureus.

Bushnell, R. B., 1974. Where are we on coliform mastitis National mastitis Council Inc. annual meeting p. 69.