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THE BACTERIOCIDAL AND BACTERIOSTATIC ACTIVITIES OF NORMAL BOVINE SERUM WITH AND WITHOUT EXTRANEOUS COMPLEMENT ON *EXCHERICHA COLI*

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ABSTRACT

The invitro Bacteriocidal and bacteriostatic activities of normal bovine serum with and without added guinea-pig complement tested against nine serotypes of Escherichia coli was investigated. Bovine serum without added guinea-pig complement tested within 24 hours of collection inhibited the growth of all nine serotypes of *E coli*. Bovine serum with added guinea-pig complement also inhibited the growth of all *E coli* tested. However slightly greater inhibition was recorded in the later trial (with added complement). In both cases growth inhibition was dependent on the index of serum dilution. Significantly greater survival of the organisms was recorded at higher dilutions (1: 16, 1: 32). Survival percentage in both trials was lowest at the 1.2. dilutions for all the organisms. However, survival percentage was higher for 02a (42.1%) 0101 (38.7%) and 011 (33.3%) respectively. There was significant difference (p= 0.05) between the mean of *E. coli* tested in serum with and without complement.

KEYWORDS: Invitro, Bacteriocidal, Bacteriostatic, complement, serotype.

INTRODUCTION

The natural occurrence of anti-body like substances in serum of various organisms has received extended investigations. Brock et al (1994) defined serum as the liquid portion of the blood with clotting proteins and cells removed. The activities of various sera including human and bovine results in growth inhibition and in extreme cases killing of microbial cells. These phenomena are referred to as bacteriostatic and bacteriocidal respectively.

Various investigators have demonstrated the specific complement-fixing and agglutinating substances in normal serum. Collins (1969), observed that such agglutination tendency in bovine serum was due to combined action of anti-body and the iron-building protein, lactoferin. The building takes place on the surface of ribosomes resulting in inhibition of protein synthesis and subsequently growths (Brock et al, 1994).

Escherichia coli and some members of paracolon groups are components of normal intestinal flora and give rise to disease only under exceptional conditions (Stainer et al, 1985). Collins (1969) reported that bovine colostrums is not bacteriostatic to *E. coli* unless the p^H of the mixture is adjusted to 7.2 to 7.4 by addition of $NaHCO_3$. This fact is also reported by Balows et al (1991). Addition of exogenous substances such as $NaHCO_3$ is supposed to significantly enhance the bacteriostatic potentials complement. The objectives of this investigation were to test the bacteriocidal and bacteriostatic activities of serum (bovine serum) with and without added Guinea-pig complement and without alterations in the pH of the mixture.

MATERIALS AND METHODS

Nine strains of *E. coli*: 0146, 02a, 09, 011, 0101, 0137, 062, 0137 and 0109, were originally obtained from the stock collections of Dr. J. P. Glantz of the Department of Veterinary Sciences, the Pennsylvania State University, USA. Cultures were maintained in trypticase soy agar slants at 37oC.

Serotypes	Strains
0146:k:H21	1206a
02a:ki:H14	1187
09:KO:H12	Beth G. W
011:K58:NM	Stroke W
0101:K:H19	OX 35
0137:K79:H41	RVC1787
062:K:H:30	F-10524-41
037:K:H:10	H510C
0109:K:H:19	H709C

Preparatory to the test: A single colony on the agar slant was picked and transferred into a 10ml trypticase soy broth and incubated overnight at 37oC, and then chilled in a deep freezer for 10 minutes to bring about 10oC and thus check further growth. Ten-fold dilutions of the culture were prepared in 0.85 percent NaCl solution by adding 1.0ml of the broth culture to 9.0ml sterile saline. The tube was shaken vigorously and a sterile pipette was used to transfer 1.0ml of the broth culture to 9.0ml sterile saline. This process was repeated until tenth dilution was made

The number of viable organisms in the 10^{-7} and 10^{-8} dilution was determined by pipetting 0.1ml of each of these dilutions into Petri-dishes to which 15ml of trypticase soy agar at 450C was added. The contents of the plate were mixed by swirling, allowed to solidify, incubated overnight at 37oC, and the colonies on each plate were counted.

In the interval before counting (about 18 hours), the bacteria dilutions were held at 4oC ice-bath, to check further growth. When the bacterial numbers in the suspension had been determined, the suspension was diluted to contain approximately 4,000 colony-forming units (CFU) per ml.

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Bovine Colostrum

Colostrum was obtained from normal Holstein cows in the mastitis Research Herd maintained by the Department of Veterinary Sciences of the Pennsylvania State University (USA). Blood was obtained from five cows by jugular venipuncture and allowed to clot overnight at room temperature. Serum was separated by centrifugation at 5°C, aspirated and pooled. Serum was sterilized by filtration through 0.45um membrane filter (Millipore, USA) and stored at -20°C.

(i) **Serum dilutions:** Tubes containing 0.5ml of the normal bovine serum in the following dilutions with saline were prepared; 1:2, 1:4, 1:8, 1:16, 1:32. Each series of dilutions included control tubes.

(ii) Approximately 2,000CFU of E. coli in 0.5ml saline were added to each serum dilution and control tube.

(iii) **Complement** Aliquots of 0.3ml of undiluted complement were prepared and added per tube. Varying amounts of diluent were added per tube to bring the volume to 2.0ml. The rack containing the tubes was removed from the ice-water and place at 37oC water bath for one hour. After incubation, 0.5ml of trypticase soy broth was added to each tube to terminate the bactericidal process Pour plates were prepared with 0.1ml in each tube and incubated overnight at 37°C and colonies counted.

RESULTS

The percentage of the different strains of *E. coli* surviving after one hour incubation in various dilutions of pooled bovine serum with added guinea-pig complement expressed as a percentage of a control incubation in saline, are presented in table 1. While the percentage of different strains of *E. coli* surviving after one hour incubation in various dilution of normal bovines serum without compliment is shown in tables 2. Survival was lowest in the 1:2 serum dilutions. Survival tended to increase with increasing serum dilutions. The data presented in Tables 1 and 2 suggests that some strains are more resistant than others to the bactericidal and bacteriostatic actions of normal bovine serum with and without added guinea-pig complement. The tendency towards increased survival in greater serum dilutions is also evident in these data. The addition of exogenous complement did not appear to enhance the bactericidal and bacteriostatic properties of the serum (Fig 1).

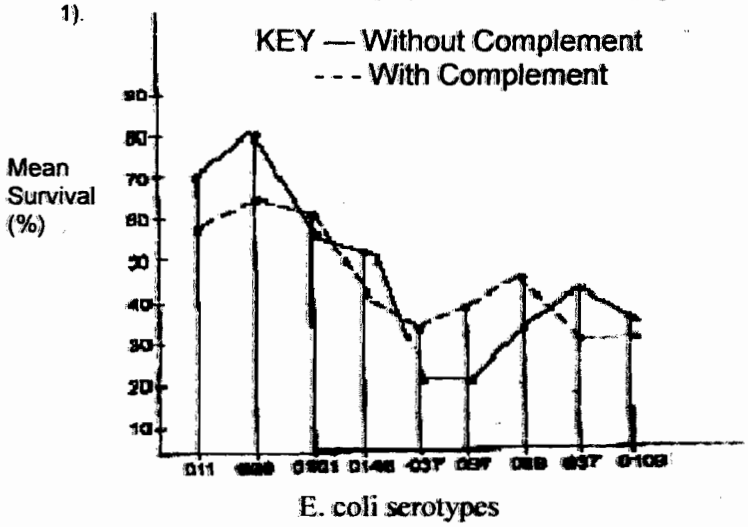

Fig. 1: Mean Percentage survival of E.coli Serotypes in all dilutions with and without added guinea-pig complement.

Table 1: Survival of nine strains of E. coli after one hour incubation in increasing dilutions of normal bovine serum with added guinea pig complement.

	Percentage Survival in Serum Dilutions of:				
E. coli 1:2	1:4	1:8	1:16	1:32	
011	39	41	56	83	75
02a	42	62	45	58	94
0101	30	52	57	66	64
0146	33	24	41	47	55
0137	0	31	37	43	50
062	17	23	41	52	68
09	29	38	46	67	41
037	0	23	34	41	53
0109	25	36	27	40	41

1 Percentage survival = $\frac{\text{CFU after incubation in serum}}{\text{CFU after control incubation saline}} X100$

Table 2: Survival of nine strains of E coli after one hour incubation in increasing dilutions of normal bovine serum without added guinea pig complement.

	Percentage Survival[1] in Serum Dilutions				
E. Coli 1:2	1:4	1:8	1:16	1:32	
011	50	70	61	60	96
02a	67	76	74	81	75
0101	64	68	43	23	66
0146	32	49	60	54	51
0137	6	11	18	32	37
062	0	0	16	50	42
09	16	23	46	32	40
037	33	36	39	46	32
0109	16	42	14	37	43

$$\text{Percentage survival} = \frac{\text{CFU after incubation in serum}}{\text{CFU after control incubation saline}} X100$$

DISCUSSION

In a preliminary screening of nine serotypes of *E coli*, variations among strains was found in their resistance to the bacteriocidal and bacteriostatic effect of normal bovine serum. This would be expected since individuals of the same species may express some degree of differences in phenotypic expression or physiological adaptation. In such case, the individual or cell will express altered environment without any permanent chemical changes in the genotype or genetic make-up (Levy et al, 1983).

The bacteriocidal and bacteriostatic activities of the serum plus complement was seen to be a ratio expressed as percentage of bacteria surviving in the various serum dilutions to the number surviving in saline. Addition of complement did not appear to improve the bacteriocidal and bacteriostatic properties of the serum as greater survival was observed in some *E. coli* strains in trails without Guinea pig complement.

Growth was inhibited in all cases with or without added Guinea pig complement. Also adjustment of pH was not a factor required for bacteriocidal and bacteriostatic activities of the bovine serum. It is believed that bovine serum has properties which inhibit protein synthesis and which therefore inhibit growth irrespective of the pH level. The effect of p^H adjustment of any degree to enhance serum activity as earlier reported needs to be studied in future research.

Percentage survival increased with increasing dilution. This is because the lower the concentration of a solution the les is the surface available for interaction between the components in the solution. As dilution decreases the combined action of antibody and iron binding protein, lactoferrine, is lowered thus allowing for greater survival of *E. coli*.

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