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STUDIES ON THE ROLE OF COLOSTRIDIAL ORGANISMS AND OTHER BACTERIA IN CALF DIARRHOEA WITH SPECIAL REFERENCE TO THEIR SUSCEPTIBILITY TO SOME ANTI-BACTERIAL AGENTS.

(With 8 Tables & One Fig.)

By

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دراسات على الدور الذى تلعبه ميكروبات الكلوستريديا والميكروبات الاخرى فى اسهال العجول مع دراسة حساسية هذه الميكروبات لبعض المضادات الحيويه

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اشتملت هذه الدراسه على دراسة أنواع البكتريا الهوائيه واللاهوائيه لعينات مأخوذه من عجول سليمه ظاهريًا وعجول مصابه بالاسهال وذلك لمعرفة أهم أنواع البكتريا الهوائيه واللاهوائيه المسببه للاسهال فى العجول كما تم اجراء اختبار الحساسيه للميكروبات المعزوله لبعض المضادات الحيويه وكذلك مركبات السلفا ولقد أوضحت النتائج ما يلى :-

١ - تم عزل البكتريا المعويه بنسبة ٩٦٪ من العجول المصابه بالاسهال بينما تم عزلها بنسبة ٤٣٪ من العجول السليمه ظاهريًا .

٢ - فى العجول المصابه بالاسهال تم عزل ميكروبات عائلة الانتروبكترياس بنسبة ١٧٪ حيث وجد أن ميكروب الاشيرشاكولاى أعلى ميكروب تم عزله من هذه العائله بنسبة ٦١٪ وتم عزل أيضًا عائلة الكلوستريديم بنسبة ٢٤٪ حيث وجد أن ميكروب الكلوستريديم برفرينجيز أعلى نسبة عزل من هذه العائله بنسبة ٤٩٪ بينما وجدت العدوى الخليطه بنسبة ٥٥٪ .

كما أوضحت التجارب أيضًا أن أعلى نسبه للاصابه بعائلة الانتروبكترياس بنسبة ٩٦٪ كانت فى الاشهر الاولى من الشتاء بينما أقل نسبه للاصابه كانت فصل الصيف بنسبة ٤٤٪ بينما كانت أعلى نسبه للاصابه بعائلة الكلوستريديم كانت فى الاشهر الاخيره من العام خريفًا بنسبة ٨٨٪

بينما أقل نسبه للاصابه كانت فى الربيع بنسبة ٧٦ ٪ .

٣ - فى العجول السليمه ظاهريًا تم عزل ميكروبات عائلة الانتروبكترياس بنسبة ٣ ٪ حيث وجد أن ميكروب الاشيرشا كولاى أعلى ميكروب تم عزله من هذه العائله بنسبة ٦٠ ٪ وتم عزل أيضًا عائلة الكلوستريديم بنسبة ٢٣ ٪ وقد وجد أن ميكروب الكلوستريديم برفرينجيز أعلى نسبة عزل من هذه العائله بنسبة ٤٦ ٪ بينما عجدت العدعى الخليطه بنسبة ١٧ ٪.

٤ - وقد تم تصنيف الميكروبات المعزوله لعائلة الانتروبكترياس حيث تم التعرف على ميكروب الاشيرشاكولاى بنسبة ٦١ ٪ ، الكلبسيلا اوكس توكا بنسبة ٨٠ ٪ ، الكبسلا أوزونى بنسبة ٥٥ ر ٥ ٪ ، السالمونيلا تيفيوريم بنسبة ٧٧ ر ٢٪ ، السالمونيلا اوندرستبورت بنسبة ٣٨ ر ١٪ ، والستروبكتر داى فرسس بنسبة ٣٨ ر ١٪ وهذه الميكروبات تم عزلها من العجول المصابه بالاسهال أما العجول السليمه ظاهريًا تم عزل الميكروبات الاشيرشاكولاى بنسبة ٦٠ ٪ والكلبسيلا اركس توكا بنسبة ٢٠٪ والكبسيلا أوزونى بنسبة ٢٠٪ بينما الميكروبات الاخرى لم يتم عزلها من العجول السليمه ظاهريًا .

٥ - أما بالنسبه لعائلة الكلوستريديم المعزوله كانت أهمها ميكروب الكلوستريديم برفرينجيز أنواع أ ، ب ، جـ ، د حيث تم عزلها من العجول المصابه بالاسهال بنسبة ٢٥ ٪ ، ٥٤ ر ١١ ٪ ، ٧٦ ر ٥٪ ، ٤٦ ر ٣٨ ٪ على التوالى ولم يتم عزل النوع جـ وقد تم عزل أنواع أخرى من ميكروبات الكلوستريديم المختلفه وقد تم التعرف عليها .

٦ - تم اختيار حساسية الميكروبات المعزوله لبعض المضادات الحيويه ومركبات السلفاولقد أوضحت النتائج أن الميكروبات كانت أكثر حساسيه للسيتوكستين وحامض النااليد كسيك والنتروفور أنتوين بينما كانت الميكروبات أكثر مقاومه لباقى المضادات الحيويه المستخدمه ومن هذه الدراسه نجد أن مجموعة الانتيروبكترياس مع ميكروبات الكلوستريديا تلعب دورًا هامًا فى احداث الاسهال بالعجول وهذا يتطلب سرعة العلاج بالمضادات الحيويه المختاره بعد اجراء اختبار الحساسيه هوائيًا حتى لا نستهلك أدويه ليس لها لزوم وقد تؤدى الى نتيجه عكسيه كما ننصح باستخدام اللقاحات لتحصين الامهات أثناء الحمل لتكوين الاجسام المضاده فى دمها فتفرزها فى لبن السرسوب لترضعه العجول والولده وبذلك نضمن وقاية تلك العجول حتى يتم نضوج الجهاز المناعى لها وبالتالى تقل نسبة الاصابه والنفوق بسبب الاسهال وتزيد الثروه الحيوانيه.

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SUMMARY

Recovery of enteric bacteria from diarrhoeic calves showed high incidence (96.00%). This recovery was more than that recovered from apparently normal ones (43.00%). Among enteric bacteria, combination of members of Family *Enterobacteriaceae* and *Genus Clostridium* in the examined calves was higher in frequency than single isolates of each of *Clostridium* and *F. Enterobacteriaceae* respectively. Isolated members of F. Enterobacteriaceae were *E.col* in a highest rate followed by *K. oxytoca, K.ozaenae, S.typhimurium, S.onderstepoort* and *C.diversus*. Members of *G. Clostridium* that were recovered were mostly *Cl.perfringens* included types D, A, B and C respectively followed by *Cl.sporogens, Cl.tertium, Cl.sordellii, Cl.histolyticum, Cl.bifermentans, Cl.barati, Cl.cadaveris, Cl.cochlearium, Cl.fallax, Cl.spehenoides* and *C.paraputrificum*. There were seasonal variations in the isolation of enteric bacteria from diarrhoeic calves where *F.enterobacteriacease* recorded the highest incidence at winter but closteridial infection was high at autumn. Susceptibility of isolated bacteria showed that mixed *E.coli* strains were highly sensitive to *Cefalothin, Nalidixic acid* and *Nitrofurantoin.* Mixed *Enterobacteriaceae* isolates were susceptible to Nalidixic ancid and Cefalothin; and mixed *Cl.perfrigens* isolates were highly sensitive to Cefoxitin, Cefalothin and Nitrofurantoin. On the other hand, mixed *E.coli* and *Cl.perfringens strains, mixed Enterobacteriaceae* and *Clostridial* isolates and mixed cloistral isolates recorded complete resistance which give us an idea to apply the vaccination program for the control of bacterial causes of calf diarrhoea without depending completely on the use of these medicaments.

INTRODUCTION

Diarrhoea is a clinical entity which causes serious economic losses as it may lead to calf mortality, weight loss or even late growth. It is a complex syndrome resulting from interaction between four components. These components are the infective agents including bacteria, virus or protozoa; environmental factors; nutritional factors and host.

Diarrhoea can be attributed to infection with a single agent (in very young or stresed animals) or more commonly to multiple agents. Its severity depends partially on non-infective contributing factors and on the nature of involved organisms *(TZIPORI, 1981)*.

Enteric bacteria as an infective agent were found to be numerous. They may be one or more of members of Family Enterobacteriaceae (facultative anaerobic bacteria), Genus Clostridium (strict aerobic bacteria) and or Genus Campylobacter (CO_2- bacteria).

Field treatment of diarrhoea includes the use of antibiotics and or sulphonamides beside electrolyte therapy and intestinal coasters. Mis-use of such chemotherapeutic agents in the treatment of their use as food additives resulted in the production of bacterial strains having a wide range of antibiotic resistance.

Accordingly, this work was designed to investigate the role of bacteria in calf diarrhoea with the study of their sensitivity to different chemotherapeutic agents to select the effective ones.

MATERIAL and METHODS

Faecal samples were colected from diarrhoeic and apparently normal calves. they were taken from ICAW (International Company for Animal Wealth, named previously Tonsi-farm); Dina farm and sporadic cases located at Giza, Beheira and Kalioubia Governorates respectively. Distribution of samples is deminstrated in Table (1). However, collection of samples from diarrhoeic calves was done equally per seasons of year (25 samples per season). In addition to that, the age of examined calves ranged from one week up to six months.

Table 1: Number of faecal samples examined from different origins according to the health status of calves.

Origin	Health status of calves		Total
	Diarrhoeic	Apparently normal	
ICAW	65	55	120
Dina farm	20	30	50
Sporadic cases	15	15	30
Total	100	100	200

Each sample was divided into two portions, the first portion was examined for Enterobacteriaceae on the basis of *KNOEMAN et al (1983).* It was streaked directly on the surface of Mac-Conkey's agar and XLD agar. Inoculated plates were incubated aerobically at 37°C for 24-48 hours. At the same time, Salmonella was detected by enriching faecal samples on selenite F broth at 37°C for 18 hours, then subcultured on MacConkey's agar and XLD agar plates. Suspected colonies for Salmonella and other Enterobacteriaceae were purified and preserved on semi-solid medium for further identification.

The second portion was examined for clostridial organisms. It was inoculated into two tubes of freshly prepared cooked meat broth. One of these tubes (T_1) was incubated anaerobically at 37°C for 24 hours. Then, it was subcultured on the surface of neomycin sheep blood agar for detecting *Clostridium perfringens.* Meanwhile, the other tube (T_2) was heated at 80°C for 10 minutes to kill all vegetative bacterial cells and incubated anaerobically. Then, it was grown on sheep blood agar for the diagnosis of other clostridial organisms. Inoculated plates were incubated anaerobically at 37°C for 24 hours. Suspected colonies for clostridia were purified and preserved for further identification.

Biochemical identification of enteric bacteria included members of *F.enterobacteriaceae* and *G.clostridium* was applied on the basis of *CRUICKSHANK et al. (1975)* and *KONEMAN et al. (1983).*

Serological typing of Salmonela isolates was done with the help of Kauffmann-White scheme as described by *EDWARDS and EWING (1972), KAUFFMANN (1973) and BERGY'S MANUAL (1984).*

Toxigenicity of *Cl.perfringens* isolates was detected by both lecithinase test *(SMITH and HOLDEMAN, 1968)* and their pathognicity to guinea pigs *(WILLIS, 1977).* Meanwhile, the toxigenicity of *Cl.perfringens* strains were determined by dermnecrotic test in guinea pigs *(BULLEN, 1952* and *OAKLY and WARRACK, 1953).*

Sensitivity of isolated enteric bacteria to antibiotics and sulfonamides was studied for a mixture of cultures. Mixed cultures included mixture of *Escherichia coli* (6 strains), mixture of Enterobacteriaceae (6 strains of *E.coli* and one strain from each of *Klebsiella oxytoca, K.ozaenae, Salmonela typhimurium, S.onderstepoort* and *Citrobacter diversus),* mixture of *Cl.perfringens* (8 strains, two from each type A,B,C and D), mixture of clostridia (8 strains of *Cl.perfringens* and one strain from each of *Clostridium sprogens, Cl.tertium, Cl.sordellii, Cl.histolticum, Cl.bifermentans, Cl.barati, Cl.cadaveris, Cl.cochlearium, Cl.fallax and Cl.sphenoides),* mixture of *E.coli* (6 strains) and *Cl.perfringens* (8 strains),

mixture of *E.coli* (6 strains) and *Cl.perfringens* (8 strains), and mixture of *Enterobacteriaceae* and *Closteridia* (as mentioned before).

Each bacterium was cultivated in liquid medium (nutrient broth for Enterobacteriaceae or cooked meat broth for clostridia) and incubated either aerobically or anaerobically at 37°C for 24 hours. The mixture was prepared by standardization of one ml from the cultivated broth of each strain, then transfered into sterile MacCarteny bottle, shaked well and thorough mixed using Pasteur pipettes.

Zone diameter of growth inhibition was determined using disc difusion method as described by *CRUICKSHANK* et al. *(1975) and RUSSELL and QUESNEL (1983).* The inoculated plates were incubated either aerobically for Enterobacteriaceae or anaerobically for Clostridia and mixtures of Enterobacteriaceae and Clostridia.

RESULTS

Are presented in table 8 and Fig 1.

DISCUSSION

Calf scour is a complex syndrome because it has been diffecult to determine the role of many different infective agents which have been isolated from the faeces and tissues of affected ones *(ACRES* et al., *1975).* However, predisposing factors in such cases play an important role in the establishment of calf scour *(AMROUSI* et al., *1971).*

Table (2) shows that the enteric bacteria were regarde as one of the main causes of diarrhoea in calves. Difference in the recovery of enteric bcteria between diarrhoeic (96.00%) and apparently normal ones (43.00%) was due to the enhanced growth of facultative pathogens in diarrhoeic calves and their intermittent excretion in the faeces of healthy ones. Similar results were obtained by *SHERWOOD* et al. *(1983), BARRANDEGUY* et al. *(1988) and GONZALEZ MORTEO* et al. *(1990).*

Rate of diarrhoeic calves showed mixed Enterobacteriaceae and clostridial infection was high (55.00%) in comparison to the cases exhibited single Enterobacteriaceae or clostridial infection (17.00% or 24.00%, respectively). Also, mixed infections produced different types of combinations. These observations were explained by *TZIPORI (1981)* who showed that the infection with several enteropathogens, occurring in several combinations was more common than with a single agent. *YALCIN,*

et al. (1969) recorded mixed cultures of *E.coli* and clostridial micro-organisms from 27.50% diarrhoeic and septicaemic dead calves. *MUZYCHIN (1970)* isolated *Cl.perfringens* type A and *E.coli* together from calves with enterotoxaemia and *RAMISSI* et al. *(1979)* reviewed various combinations of 2,3 or 4 types of *Cl.perfingens and E.coli* from faeces of diarrhoeic young calves and other animals.

Single isolates of members of *F. enterobacteriaceae* that recovered from both diarrhoeic and apparently normal calves as demonstrated in Table (3) were *Escherichia coli, Klebsiella oxytoca and Klebsiella ozaenae.* Moreover, *Salmonella typhinurium, Salmonella onderstepoort* and *Citrobacter diversus* were isolated only from diarrhoeic calves. In addition to that, the mixed isolates were recorded only from diarrhoeic ones. *E.coli* was recovered from diarrhoeic calves by *VALENTE* et al. *(1982), SHERWOOD* et al. *(1983), POHL* et al. *(1984), TRIPATHI and SONI (1984), MOHAMMED* et al. *(1985), AL-DABBAS and WILLINGER (1986), CHANTER* et al. *(1986), MOXLEY and FRANCIS (1986), NIGRELLI* et al. *(1989), OTOI* et al. *(1990), PANWAR* et al. *(1990) and VARTANYAN* et al. *(1990).* Klebsiella was isoalted from the faeces of diarrhoeic calves by *AMROUSI* et al. *(1971) and VARTANYAN* et al. *(1990).* Salmonella was detected from the faeces of diarrhoeic calves by *AMROUSI* et al. *(1971), JOHNSTON and JONE (1983), MARTEL* et al. *(1980), TAOUDI* et al. *(1983) and ZRELLI* et al. *(1990).* Finally, Citrobacter was reviewed from diarrhoeic calves by *ACRES* et al. *(1975) and VARTANYAN* et al. *(1990).*

Presence of anaerobic micro-organisms in a properly collected clinical specimen should not be ignored. However, they may not be the primary organisms but they may have marked influence on the severity of lesion *(OSBALDISTON and STOWE, 1971).* Species of genus clostridium were regarded as one of anaerobes which able to produce diarrhoea in calves such as *Clostridium perfringens* type A *(AL-MASHAT and TYLOR, 1983).*

As shown in Table (4), highest incidence of clostridial micro-organisms from diarrhoeic and apparently normal calves (59.5%) was due to the highest recovery of *Cl.perfringens* from both cases of calves (40.50%). At the same time, the principal habitat of *Cl.perfringens* was the intestinal contents of man and animals *(SMITH and HOLDEMAN, 1968) and WILLIS (1977).* That is to say, *Cl.perfringens* was the main clostridial isolate.

Cl.perfringens types D, A and B were isolated respectively from diarrhoeic and apparently normal calves (Table 5). This result was supported by *NIILO and AVERY (1963), and MORAILLON and YALCIN (1966)* who isolated *Cl.perfringens* types A and D from cases of bovine enterotoxaemia. Also, *ANGELOV and KARADZHOV (1979)* isolated *Cl.perfringens* type D from

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Table (2) :

Incidence of the isolated enteric bacteria from diarrhoeic and apparently normal calves related to the total number of examined samples.

Isolated bacteria	Diarrhoeic calves *		Apparently normal ** calves		Total No. of positive samples	Incidence ***
	No. of positive samples	Percentage of positive	No. of positive samples	Percentage of positive		Percentage of positive
Enterobacteriaceae	17	17.00%	3	3.00%	20	10.00%
Clostridia	24	24.00%	23	23.00%	47	23.50%
Enterobacteriaceae and Clostridia	55	55.00%	17	17.00%	72	36.00%
Total	96	96.00%	43	43.00%	139	69.50%

* The total number of examined diarrhoeic samples was 100

** The total number of examined apparently normal samples was 100

*** The total number of examined samples was 200

Table (3) :

The single and mixed isolates of Enterobacteriaceae isolated form diarrhoeic and apparently normal calves.

Isolated species of Enterobacteriaceae	Diarrhoeic calves		Apparently normal calves		Total	
	No.	%	No.	%	No.	%
1. Single :						
E.coli	44	61.11	12	60.00	56	60.86
K.oxytoca	6	8.33	4	20.00	10	10.86
K.ozaenae	4	5.55	4	20.00	8	8.69
S.typhimurium	2	2.77	0	0.00	2	2.17
S.onderstepoort	1	1.38	0	0.00	1	1.08
Cl.diversus	1	1.38	0	0.00	1	1.08
Total	58	80.55	20	100.00	78	84.78
2. Mixed :						
E.coli& K.oxytoca	5	6.94	0	0.00	5	5.43
E.coli& K.ozaenae	3	4.16	0	0.00	3	3.26
E.coli, K.oxytoca& S.typhimurium	2	2.77	0	0.00	2	2.17
E.coli, K.ozaenae& S.typhimurium	2	2.77	0	0.00	2	2.17
E.coli, K.ozaenae& Cl.diversus	2	2.77	0	0.00	2	2.17
Total	14	19.44	0	0.00	14	15.21
* Over all total	72	100.00	20	100.00	92	100.00

* % Calculated according to over all total.

Table (4) :

Incidence of C. perfringens and other Clostridia related to the total number of examined samples.

Isolated Clostridia	Diarrhoeic calves		Apparently normal Calves		Total	
	No.	* %	No.	* %	No.	* %
C.perfringens	49	49.00	32	32.0	81	40.5
Other Clostridia	27	27.00	5	5.00	32	16.0
C.perfringens & other Colstridia	3	3.00	3	3.00	6	3.00
Total	79	79.00	40	40.0	119	59.5

* % Calculated according to No. of collected samples (100 diarrhoeic, 100 apparently normal and 200 total)

Table (5) :

Typing of Cl.perfringens isolates.

Types of Cl.perfringens	Diarrhoeic calves		Apparently normal calves		Total	
	No.	%	No.	%	No.	%
Type A	13	25.00	6	17.14	19	21.83
Type B	6	11.54	2	5.71	8	9.19
Type C	3	5.76	0	0.00	3	3.44
Type D	20	38.46	14	40.00	34	39.08
* Mixed types	3	5.76	4	11.42	7	8.04
Non - toxic	7	13.46	9	25.71	16	18.39
** Total	52	100.00	35	100.00	87	100.00

* All mixed types were types A&D except one apparently normal case showed types B&D.

** % Calculated according to total No. of clostridial isolates in each item (Diarrhoeic, apparently normal and total).

Table (6) :

Biovars of other Clostridia isolated from diarrhoeic and apparently normal calves either single or mixed with C.perfringens.

Isolated species of Colstridia	Diarrhoeic calves		Apparently normal calves		Total	
	No.	%	No.	%	No.	%
1. Single :						
C.sporogenes	8	26.66	1	12.50	9	23.66
C.tertium	3	10.00	1	12.50	4	10.52
C.sordellii	4	13.33	0	0.00	4	10.52
C.histolyticum	3	10.00	0	0.00	3	7.89
C.bifermentans	2	6.66	1	12.50	3	7.89
C.barati	2	6.66	0	0.00	2	5.26
C.cadaveris	2	6.66	0	0.00	2	5.26
C.cochlearium	1	3.33	1	12.50	2	5.26
C.fallax	1	3.33	0	0.00	1	2.63
C.sphenoides	1	3.33	0	0.00	1	2.63
C.paraputrificum	0	0.00	1	12.50	1	2.63
Total	27	90.00	5	62.50	32	84.21
2. Mixed with C.perfringens						
C.tertium & type D	1	3.33	0	0.00	1	2.63
C.cochlearium & type D	1	3.33	0	0.00	1	2.63
C.histolyticum & types A and D	1	3.33	0	0.00	1	2.63
C.sporogenes & types B and D	0	0.00	1	12.50	1	2.63
C.cochlearium & types A and D	0	0.00	1	12.50	1	2.63
C.bifermentans & types A and D	0	0.00	1	12.50	1	2.63
Total	3	10.00	3	37.50	6	15.78
* Over all total	30	100.00	8	100.00	38	100.00

* % Calculated according to over all total.

Table (7) :

Rate of isolation of Enterobacteriaceae and Clostridium from diarrhoeic calves every season.

Season	Examined samples *	Enterobacteriaceae		Clostridium	
		No. of positive	Percentage of positive	No. of positive	Percentage of positive
Winter	25	24	96.00%	21	84.00%
Spring	25	21	84.00%	19	76.00%
Summer	25	11	44.00%	17	68.00%
Autumn	25	16	64.00%	22	88.00%
Total	100	72	72.00%	79	79%

* % Calculated according to examined samples.

Table (8) :

Sensitvity of mixed Enterobacteriaceae and/or clostridialisolates to antibiotics and sulfonamides.

Antibacterial substance	Mixed E.coli	Mixed Enterobac-teriaceae	Mixed Cl. perfringens	Mixed Clostridia	Mixed E.coli and Cl. perfringens	Mixed Enterobac-teriaceae and Clostridia
Neomycin	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)
Kanamycin	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)
Tetracycline	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)
Streptomycin	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)
Sulfonamide	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)
Gentamicin	R (0)	R (0)	R (0)	R (7mm)	R (0)	R (0)
Penicillin G	R (0)	R (0)	R (0)	R (9mm)	R (0)	R (0)
Polymyxin - B	I (10mm)	R (8 mm)	R (0)	R (0)	R (0)	R (0)
Ampicillin	R (0)	R (0)	R (5mm)	R (7mm)	R (0)	R (0)
Chloramphenicol	R (0)	R (0)	R (8mm)	R (9mm)	R (0)	R (0)
Erythromycin	R (0)	R (0)	I (14mm)	R (6mm)	R (0)	R (0)
Cefalithin	R (0)	R (0)	S (18mm)	R (10mm)	R (0)	R (0)
Nalidixic acid	S (19mm)	S (19mm)	R (6mm)	R (0)	R (0)	R (0)
Cefoxitin	S (20mm)	S (18mm)	S (20mm)	R (8mm)	R (0)	R (8mm)
Nitrofurantoin	S (17mm)	R (11mm)	S (17mm)	R (12mm)	R (8mm)	R (12mm)

0 = No zone diameter of growth inhibtion.

(R): Resistant.

(I): Intermediately sensitive.

(S): Sensitive or Susceptible.

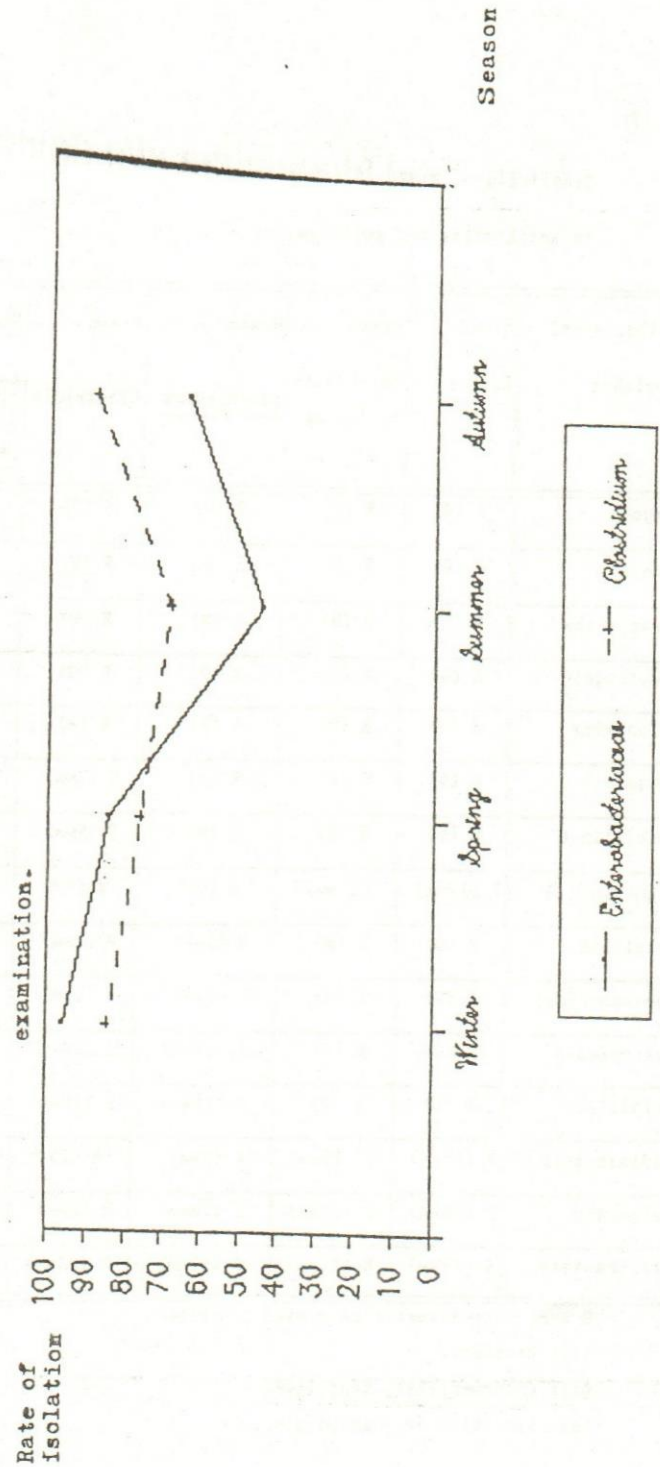

Figure (1) : Correlation between rates of isolation of enteric bacteria from diarrhoeic calves and season of examination.