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IMMUNE RESPONSE TO DEAD AND LIVE *ESCHERICHIA COLI* VACCINES AND COLOSTRAL TRANSFER OF IMMUNITY TO CALVES AND LAMBS

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ABSTRACT

CAMERON, C. M. & FULS, W. J. P. Immune response to dead and live *Escherichia coli* vaccines and colostral transfer of immunity to calves and lambs. *Onderstepoort J. vet. Res.*, 37, 157–164 (1970)

Administration of a single injection of killed polyvalent *Escherichia coli* (Migula, 1895) vaccine to ewes and cows resulted in a marked increase in the mouse protective properties of their sera. Antibodies were effectively transferred via the colostrum and the degree of passive immunity thus obtained was sufficient to protect lambs against colisepticaemia.

Live vaccine prepared from rough strains was able to protect mice against infection but it did not elicit a good antibody response in ewes in terms of OK titres and mouse protecting antibodies.

INTRODUCTION

Since the discovery and recognition of the phenomenon of infectious drug resistance among bacteria and the potential danger associated with the indiscriminate use of antibiotics (H. W. Smith 1958a & b; Watanabe, 1967; Royal, Robinson & MacDiarmid, 1968) the necessity for the development of an effective vaccine for the control of colibacillosis has again come to the fore.

Infections due to *Escherichia coli* (Migula, 1895) are of particular importance in pigs (Nielsen, Moon & Roe, 1968) and calves (Gay, 1965). The disease may manifest itself in a variety of clinical entities but enteritis, toxaemia, and septicaemia resulting in gross electrolyte and fluid imbalance are the most common sequelae (Gay, 1965). In lambs, encephalitis is a very prominent feature (Roberts, 1957; Charles, 1957; Terlecki & Shaw, 1959). Similar outbreaks have also been encountered in this country (H. J. W. Botes, formerly of this Institute, unpublished data, 1966).

Although poor feeding and husbandry practices are very important predisposing factors for the development of colibacillosis (Henning, 1956) the timely ingestion of colostrum and subsequent absorption of antibodies is critical for the prevention of infection (T. Smith & Little, 1922a; T. Smith, 1930).

Injection or feeding of serum from cows will enhance the resistance of calves to *E. coli* infections (T. Smith & Little, 1922b; T. Smith & Little, 1930; Kohler & Bohl, 1966b; Hein, 1967). This is, however, an expensive and tedious method and it is doubtful whether this procedure would be applicable in practice.

Oral immunization is a possible, but as yet little explored, method of control (Mochmann, Ocklitz, Hering, Schmidt & Richter, 1968). Parenteral immunization is therefore apparently the method of choice and should be directed at stimulating high levels of protective antibodies in the sera of pregnant females in order to obtain maximum transfer of maternal antibodies to the new-born.

The precise mechanism of immunity to *E. coli* infections is not known, but the work of numerous authors has clearly shown that the presence of K (capsular) antigens is closely associated with virulence (T. Smith & Bryant, 1927; T. Smith, 1928; Medearis, Camitta & Heath, 1968), and that antibodies directed against the K antigens play the most important role in conferring a solid immunity (T. Smith, 1928; Briggs, 1951; Briggs, Lovell, Aschaffenburg, Bartlett, Kon, Roy, Thomson & Walker, 1951). A good vaccine should therefore contain sufficient K antigens to stimulate a good antibody response.

E. coli readily gives rise to avirulent rough mutants with loss of K antigens (T. Smith & Bryant, 1927) and it has been shown that administration of a live vaccine prepared from similar mutants will produce an antibody response in cows. Calves which have suckled from these cows are much more resistant to *E. coli* infections than control animals (Botes, 1966, unpublished data).

One of the major obstacles to the development of an *E. coli* vaccine is the fact that although certain serotypes are more frequently isolated, a large number of serotypes is potentially pathogenic and may be involved in outbreaks of colibacillosis (Sojka, Lloyd & Sweeney, 1960; Cameron, 1963; Gay, 1965; Barnum, Glantz & Moon 1967; Nielsen, Moon & Roe, 1968). Consequently a vaccine with wide application should contain as many serotypes as possible. The serotypes used in this study were selected on the basis of their international occurrence and association with disease as well as their prevalence in this country.

The inclusion of a large number of serotypes in one vaccine presents not only technical but also immunological difficulties in that competition of antigens may occur. The former problem is particularly important in the case of a live vaccine which must be issued in lyophilized form. Allowance must be made for organisms which die during the process of lyophilization as well as for dilution just before administration. An exceptionally high concentration of bacteria is therefore required in order to obtain the desired number of live bacteria in the final vaccine. For this reason the live vaccine prepared by Botes (1966, unpublished data), which contained fifteen serotypes, was originally divided into three separate sets and administered successively at 10 day intervals.

The object of this investigation was to compare the immunizing properties of a live vaccine prepared from avirulent rough serotypes with those of a killed vaccine prepared from smooth strains which still contain their full complement of K antigens. Experiments were also undertaken to evaluate the effectiveness of transfer of passive immunity from ewes and cows to their offspring.

Botes (1964) found that the quality of inactivated paratyphoid vaccine deteriorated rapidly on storage and for this reason particular attention was paid to the keeping quality of dead *E. coli* vaccine.

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Materials and Methods

Bacterial strains

The fifteen serotypes used in this study were obtained from Prof. F. Orskov*. Serotype 078:K80(B) is particularly virulent and prevalent (Fey, 1957) and was therefore used as the challenge strain in all the immunity experiments.

Serotypes

The following serotypes were used throughout: Set I: 078:K80(B), 08, 015, 026 and 035; Set II: 020, 050, 054, 086 and 0137; Set III: 045, 059, 0101, 0107 and 0127.

Preparation of live vaccine

Avirulent rough mutants were selected by growing the organisms in broth containing homologous antiserum. Serial passages were made until the culture was homogeneously rough and readily agglutinated in 0.85 per cent NaCl and in normal bovine serum (Botes, 1966, unpublished data).

The organisms were cultured on nutrient agar at 37°C for 24 hours. The growth was collected by means of a curved glass rod, a dense suspension prepared in 0.85 per cent NaCl and the number of live organisms determined by means of plate counts. The suspensions were kept at 4°C overnight and the density adjusted with 0.85 per cent NaCl to contain 5×10^{10} bacteria per ml. Equal volumes of five strains were mixed to give three sets of vaccine each containing five serotypes (live pentavalent vaccine). An equal volume of buffer solution consisting of 5 per cent lactose and 1 per cent Difco peptone in 0.02M phosphate buffer pH 7.2 to 7.4, was then mixed with the suspensions. Two ml volumes of this suspension were distributed in bottles and lyophilized. The dried vaccine was made up to 10 ml with saline and the number of live bacteria determined.

By this method a total of 10^9 bacteria per strain per ml was obtained and the final live count was usually in the range of 10^9 live bacteria per ml, i.e. 2×10^8 live bacteria per ml for each strain. Two ml of each of the three sets of vaccine was administered to sheep at 10 day intervals (*vide infra*).

Preparation of formalized (dead) vaccine

The virulent, smooth counterparts of the same strains which were used for the preparation of live vaccine were used for the preparation of dead vaccine. Suspensions were similarly prepared and the density of each strain adjusted to contain 5×10^9 bacteria per ml. Equal volumes of five strains were mixed to give a final concentration of 10^9 bacteria of each strain per ml. The vaccine contained 0.8 per cent packed cells per ml and had an optical density of 0.42 as measured in an Eel colorimeter with a blue filter (dead pentavalent vaccine).

The organisms were killed with 0.3 per cent formalin and precipitated by the addition of 3 ml of a 10 per cent potassiumalum solution and 1.5 ml of a 7.4 per cent potassium hydroxide solution per 100 ml vaccine.

In later experiments it was desired to incorporate all fifteen strains in a single 5 ml dose (polyvalent vaccine). In order to obtain the same number of bacteria per 5 ml dose as was present in the dead pentavalent vaccine, the concentration of the suspension was adjusted to an optical density of 0.523.

Immunization of mice

Groups of ten adult male albino mice were used in all the experiments. The vaccine to be tested was either diluted to give the desired number of organisms per ml required for a particular experiment, or different doses were administered. In all instances a single subcutaneous injection was given. The vaccinated animals and unvaccinated controls were challenged 14 days later by intraperitoneal injection of 0.1 ml of a suspension *E. coli* 078:K80(B) with an optical density of 0.056. Such a suspension contained approximately 10^9 live bacteria per ml and the challenge dose was therefore approximately 10^8 bacteria per mouse. Deaths were recorded daily for three days and the percentage survival calculated.

Immunization of sheep

Groups of either six or eight young adult Merino wethers were used in the experiments designed to investigate the immune response to live pentavalent and dead pentavalent vaccine and the keeping quality of the killed vaccine, and to compare the immune response to three doses of dead pentavalent vaccine with the response to a single injection of dead polyvalent vaccine. The dose for the pentavalent vaccine for sheep was 2 ml of each of the three sets administered at 10 day intervals. When polyvalent vaccine was used, a single 5 ml dose was employed. The sheep were bled at the time of immunization, two weeks later and at further intervals depending on the experiments. The sera were stored at −20°C and the OK titres as well as the passive protection properties determined as described below.

Two groups of eight pregnant Dorper ewes were used in the experiment designed to determine the efficiency of passive transfer of immunity from ewes to their lambs. Each ewe received three consecutive subcutaneous 2 ml injections of the different sets of live and dead pentavalent vaccine at 10 day intervals. A control group received no vaccine. The ewes were bled at the time of the first injection and again within 24 hours of lambing. The sera were stored at −20°C and subsequently assayed for mouse protective properties and the OK titres determined. The lambs were challenged within 24 hours of birth by the intravenous injection of 2.0 ml of a suspension of *E. coli* 078:K80(B) with an optical density of 0.155, which contained approximately 3.5×10^9 bacteria per ml.

The lambs were observed for 14 days and autopsies carried out on those that died. Cultures were made of the intestinal contents, liver, spleen and brain to confirm that death was due to *E. coli* 078:K80(B).

Immunization of cows

Ten ml of polyvalent killed vaccine was administered subcutaneously to ten crossbred beef cows 3 to 4 weeks before calving. Sera were obtained from these cows at the time of injection as well as within 48 hours of calving. Their calves were also bled within 48 hours of birth. Sera were similarly obtained from a group of 10 control cows and their calves. The sera were stored at −20°C until they were assayed by means of passive mouse protection tests and the OK titres determined.

Determination of OK titres

Antigen was prepared by resuspending the bacteria from an overnight broth culture in 0.85 per cent NaCl containing 0.3 per cent formalin and adjusting the

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density to correspond to an optical density of 0.5. Serial twofold dilutions starting at 1 in 5 were made in 0.5 ml volumes in Dryer tubes and 0.5 ml antigen added to each tube. The tubes were incubated overnight in a waterbath at 50°C and the end point taken as the highest dilution of serum showing approximately 50 per cent agglutination.

Passive mouse protection tests

Ten adult male mice were used to assay each serum. The serum was injected intravenously in 0.2 ml quantities and the mice challenged 18 hours later with *E. coli* 078:K80(B) as for the active immunity experiments (*vide supra*).

Electrophoresis of calf sera

The sera of all the calves were analysed electrophoretically using the Beckman Microzone apparatus and cellulose acetate membranes according to the procedures laid down by the manufacturer. The membranes were subsequently stained and scanned on the Beckman model RB analytrol densitometer (Van Zyl, 1967).

Results

It was first desired to find out how many live organisms were required to immunize mice. In order to cover as wide a range as possible, lyophilized vaccine containing five strains was made up to five times the normal concentration and tenfold dilutions prepared in 0.85 per cent NaCl. The concentrated vaccine thus obtained contained 2×10^8 live bacteria of each strain per dose of 0.2 ml. From the results in Table 1 it can be seen that 10^7 bacteria, of which 2×10^6 were live, will give a demonstrable immunity and that doses higher than this will afford solid protection.

Table 1 Estimation of minimum dose of live lyophilized vaccine required to immunize mice to *E. coli* 078:K80(B). The vaccine contained five serotypes

Total number of bacteria per serotype per 0.2 ml mouse dose	Live bacteria per serotype per 0.2 ml mouse dose	Percentage protection
10^9	2×10^8	100
10^8	2×10^7	100
10^7	2×10^6	30
10^6	2×10^5	0
Controls	—	0

In Table 2 the results of an experiment are given in which the active immunizing activity of standard live and dead vaccines for mice are compared. In this particular experiment the potency of the two vaccines proved to be identical. Essentially similar results were also obtained with other batches of vaccine. Mice can therefore be quite easily immunized against infection with *E. coli* and it has been shown that this can be accomplished not only with whole bacteria, but also with washed bacteria, the wash water and culture filterates (Turgeon, Borduas & Frappier, 1962).

Table 2 Comparison of immunizing potency of live and dead pentavalent vaccines

Dose in ml	Total bacteria per serotype per mouse dose	Percentage protection	
		Live vaccine	Dead vaccine
0.2	2×10^8	100	100
0.1	1×10^8	100	100
0.05	5×10^7	90	90
Controls	—	10	10

A comparison of the immune response in sheep to live and dead vaccine in terms of OK titres and passive protection values are shown in Fig. 1 and 2. In both instances the response to the dead vaccine was obviously superior to that of the live vaccine.

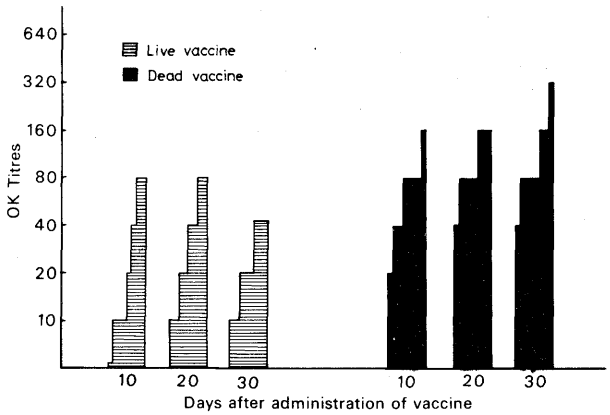

Fig. 1 Comparison of OK titres of sheep which had received a single dose of either live or dead pentavalent vaccine of serotype 078:K80(B)

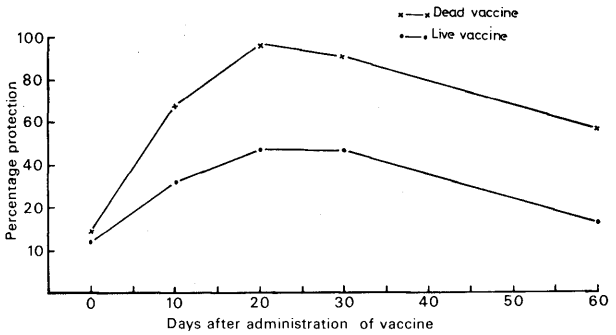

Fig. 2 Comparison of mouse protective values of the sera of groups of sheep which had received a single injection of either live or dead pentavalent vaccine

The results of an experiment showing the actual passive protection afforded to lambs are given in Table 3. There was little difference in the OK titres between the group that received live vaccine and the group that received dead vaccine but the sera from the latter group were markedly more potent in terms of their ability to protect mice passively against *E. coli* infections. A good immunity was transferred from the ewes in both experimental groups to their lambs and in this respect there was little difference between the vaccines.

From the results thus far obtained it was evident that a dead vaccine is as good if not better than a live vaccine. The next step was to determine whether a dead vaccine which contained fifteen strains in one dose would be as satisfactory as a dead vaccine administered in three doses each containing five strains.

Polyvalent and pentavalent vaccines were first tested for their ability to protect mice. Because of the differences in the concentration of the two vaccines an exact comparison could not be made. If these differences are, however, taken into consideration the vaccines performed equally well (Table 4).

TABLE 3 Immune response of ewes and passive immunity of lambs after immunization of ewes with live or dead *E. coli* vaccine

Vaccine type	Sheep No.	Interval between last injection and lambing in days	OK titre of serum		Percentage protection afforded by serum in passive protection tests		Interval between challenge of lamb and death in days
			Before immunization	At time of lambing	Before immunization	At time of lambing	
Live vaccine	1	9	20	320	40	100	Survived
Live vaccine	2	40	10	160	30	90	Survived
Live vaccine	3	27	20	320	0	40	3
Live vaccine	4	27	40	640	20	40	Survived
Live vaccine	5	27	20	640	10	50	Survived
Live vaccine	6	37	20	640	10	20	Survived
Live vaccine	7	62	20	640	10	20	Survived
Live vaccine	8	37	40	640	10	50	Survived
			Average 25	500	16.3	51.3	
Dead vaccine	9	1	40	640	30	90	Survived
Dead vaccine	10	8	20	640	10	100	Survived
Dead vaccine	11	24	10	320	10	90	12
Dead vaccine	12	17	40	640	30	40	Survived
Dead vaccine	13	34	40	640	30	90	Survived
Dead vaccine	14	32	20	320	0	80	Survived
Dead vaccine	15	37	10	320	10	90	Survived
Dead vaccine	16	44	10	640	40	70	3
			Average 24	520	20.0	81.3	
None	17	n.t.*	n.t.	0	n.t.	0	1
None	18	n.t.	n.t.	10	n.t.	10	Survived
None	19	n.t.	n.t.	10	n.t.	10	Survived
None	20	n.t.	n.t.	10	n.t.	0	9
None	21	n.t.	n.t.	20	n.t.	0	10
None	22	n.t.	n.t.	10	n.t.	30	9
None	23	n.t.	n.t.	20	n.t.	10	10
None	24	n.t.	n.t.	20	n.t.	0	Survived
None	25	n.t.	n.t.	20	n.t.	10	1
None	26	n.t.	n.t.	40	n.t.	10	2
			Average	17		8.0	

*n.t. = not tested

TABLE 4 Comparison of the immunizing potency of dead pentavalent and polyvalent vaccines for mice

Dose in ml	Pentavalent vaccine			Polyvalent vaccine			
	Total bacteria per serotype per dose	Percentage protection		Total bacteria per serotype per dose	Percentage protection		
		Exp. 1	Exp. 2		Exp. 1	Exp. 2	Exp. 3
0.4	n.t.*	n.t.	n.t.	3.2×10^8	n.t.	60	100
0.3	n.t.	n.t.	n.t.	1.6×10^8	n.t.	60	n.t.
0.2	2×10^8	100	90	8×10^7	80	0	60
0.1	1×10^8	100	80	4×10^7	20	n.t.	n.t.
0.05	5×10^7	90	80	2×10^7	30	n.t.	n.t.
Controls	—	10	0	—	0	0	0

*n.t. = not tested

These findings, based on the OK titres of five strains and passive protection to strain 078:K80(B), were confirmed in sheep (Fig. 3 and 4). In fact, the polyvalent vaccine gave a slightly better response than the pentavalent vaccine.

The keeping quality was more than adequate. Mice which were immunized with 8 months old pentavalent vaccine were just as well protected as mice which received fresh vaccine (Fig. 5). Similarly, sheep responded as well to 8 months old vaccine as to fresh vaccine (Fig. 6 and 7).

The polyvalent vaccine is also intended for use in bovines and the immune response in cows and the passive transfer of colostral antibodies to their calves were therefore investigated. The cows showed a good antibody response to a single injection of the vaccine and a high antibody titre was also transferred to the calves (Fig. 8). The increase in protective activity of the sera was also quite spectacular, particularly in the case of the calves (Fig. 9).

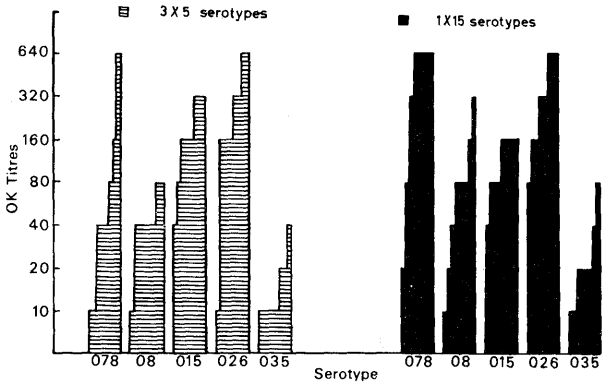

FIG. 3 Comparison of OK titres of sheep which had received either three 2 ml injections of dead pentavalent vaccine, each containing five different serotypes, or a single 5 ml injection of dead polyvalent vaccine containing 15 serotypes

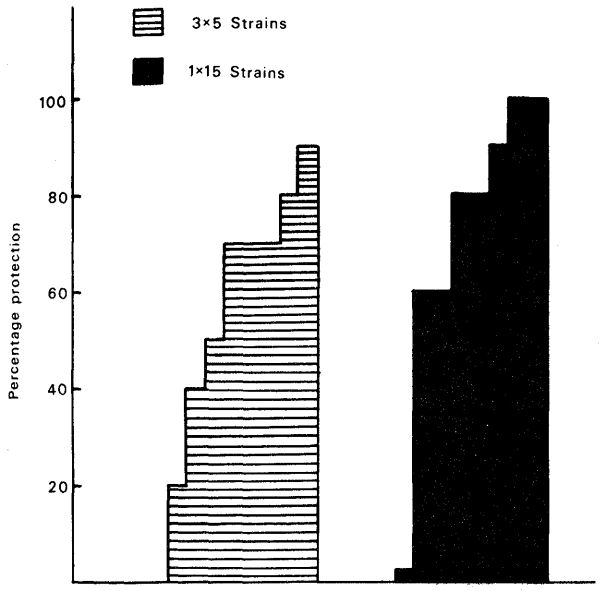

FIG. 4 Comparison of mouse protective values of sera of sheep which had received either three 2 ml injections of dead pentavalent vaccine, each containing five different serotypes, or a single 5 ml injection of dead polyvalent vaccine containing 15 serotypes

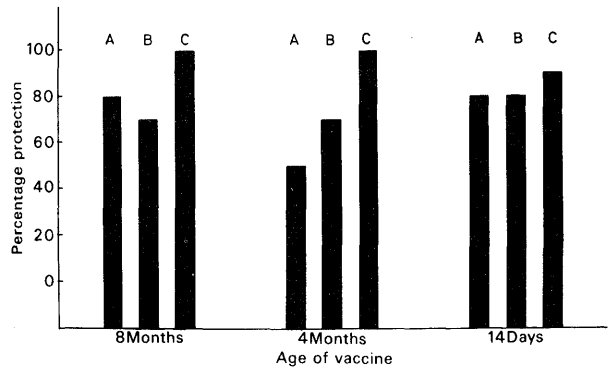

FIG. 5 Protection afforded to mice by active immunization with 8 months old, 4 months old and 14 day old pentavalent vaccine
A = 0.05 ml B = 0.1 ml C = 0.2 ml

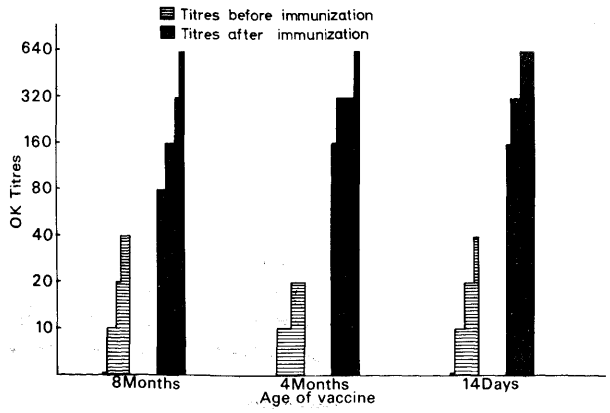

FIG. 6 Comparison of OK titres of sheep given 8 months old, 4 months old and 14 day old pentavalent vaccine respectively

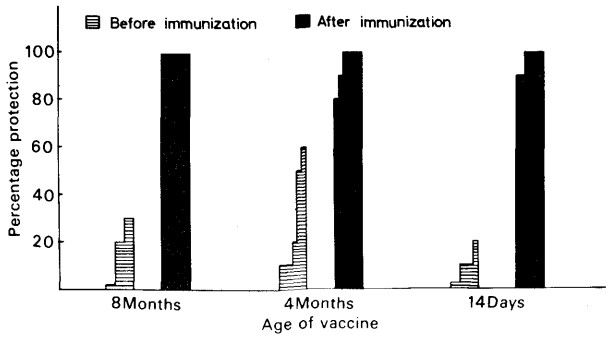

FIG. 7 Mouse protective values of sera from sheep which had received 8 months old, 4 months old and 14 day old pentavalent vaccine

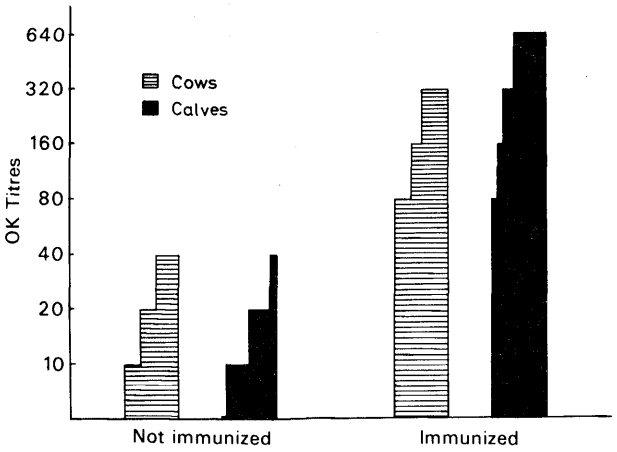

FIG. 8 OK titres of immunized cows at parturition and of their calves after ingestion of colostrum

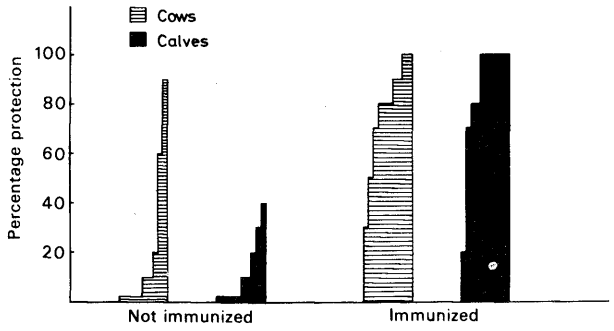

FIG. 9 Mouse protective values of sera of immunized and non-immunized cows at time of calving and of their calves after ingestion of colostrum

The post-colostral sera of all the calves were subjected to cellulose acetate electrophoresis. Some of the sera were unfortunately slightly haemolyzed and gave unsatisfactory results. It was nevertheless obvious that, as could be expected, there was no overt difference between the total globulin levels of calves which had suckled on immunized cows and calves from non-immunized cows. As shown in Fig. 10, there was, however, one calf which remained agammaglobulinaemic despite the ingestion of colostrum.

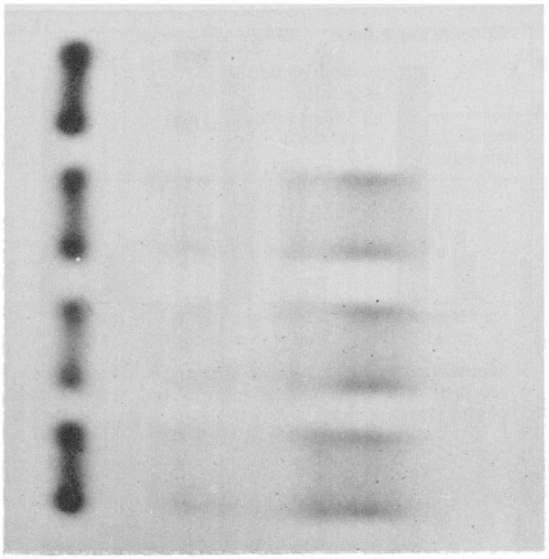

FIG. 10 Electrophoretograms of sera from four calves which had ingested colostrum from immunized cows

DISCUSSION

These investigations have shown that the administration of an alum precipitated polyvalent dead *E. coli* vaccine to cows results in a significant transfer of specific antibodies to their calves. Passive immunity transferred from ewes to their lambs was also found to be of sufficient magnitude to protect them against colisepticaemia. Our results are therefore in close agreement with the findings of Gay, McKay & Barnum (1964a) who used essentially the same type of vaccine in cows. Similar results have also been obtained in swine (Kohler & Bohl, 1966a; Kohler, Moore & Smith, 1968).

Insight into the mechanisms of immunity to *E. coli* is important when considering the preparation of a vaccine. According to Rowley (1954, 1955) immunity to *E. coli* is primarily dependent on the bactericidal effect of serum in the presence of complement, while Medearis & Kenny (1968) have stressed the importance of effective phagocytosis. As already indicated, antibodies directed against the K antigens play a key role in immunity (T. Smith, 1928; Briggs *et al.*, 1951; Gay *et al.*, 1964a). They probably act through the process of opsonization and, in association with the reticulo-endothelial system, constitute the primary defence mechanism (Smith & Halls, 1968). Furthermore, Arbuckle (1968) proved that O antibodies alone will not produce immunity and will not protect against infection with organisms containing K antigens (T. Smith, 1928). Particular K antigens are usually associated with certain O antigens, but in the light of the above knowledge it would be advantageous rather to select strains for inclusion into a vaccine on the basis of their K antigens than to rely exclusively on the O antigens. K antigen typing is, however, difficult and at present still somewhat confused and it is therefore preferable to select vaccine strains by means of immunity experiments.

Another question, which as yet remains unanswered, is whether increased serum antibody levels will also be effective in protecting against forms of colibacillosis where a systemic infection does not occur. In classical white scours of calves the infection is frequently restricted to the intestinal lumen without systemic involvement (Barnum *et al.*, 1967). Unless copro-antibodies are involved or considerable quantities of colostral antibodies remain associated with or attached to the intestinal mucosa, it is difficult to imagine how circulating serum antibodies could influence the course of infection in the intestine. It has nevertheless been found by Gay, McKay & Barnum (1964b), who have conducted numerous field trials, that immunization of cows markedly reduces the incidence of white scours in calves. Conversely, Top (1969) reports that immunization of cows did not reduce the incidence of calf diarrhoea but that the mortality rate was lowered. Similar poor results have been reported by Sellers, Smith & Pook (1962). They, however, used a heat-killed vaccine in which some of the K antigens might have been destroyed. Nonetheless, colostrum always has a very marked beneficial influence on the incidence of white scours (Briggs *et al.*, 1951).

In pigs *E. coli* infection frequently assumes a toxic nature, particularly in older animals (Stevens, 1963). This toxaemic form may either be due to endotoxin or enterotoxins (Nielsen *et al.*, 1968). Kohler & Bohl (1966b) have postulated that the protective effect of immune serum administered to gnotobiotic pigs is due to neutralization of endotoxin in the lumen of the intestine.

Kohler *et al.* (1968) found that pigs respond well to *E. coli* vaccines and a good antibody response has also been documented by Lemcke & Hurst (1961). In practice the application of vaccines has, however, generally given indifferent results (Lemcke & Hurst, 1961; Jones, Sellers & Smith, 1962; Ingram, 1968). This could possibly be due to an ineffective transfer of colostral antibodies to all the piglets if suckling commences before parturition is completed (Bourne, 1969). Another complicating factor is the finding that although calves and lambs do not selectively absorb different classes of colostral immunoglobulins, certain individuals remain agammaglobulinaemic after ingestion of colostrum. This has also been observed by other authors and may be one possible explanation why certain animals succumb to infection despite the use of vaccine (Halliday, 1965; Klaus, Bennett & Jones, 1969).

The practical value of *E. coli* vaccines therefore still remains a very open question.

SUMMARY

The immunizing potency of polyvalent vaccine prepared from rough avirulent serotypes was compared with polyvalent dead vaccine prepared from smooth virulent serotypes. A single injection of dead vaccine protected mice as effectively as the live vaccine and gave a superior immune response in terms of OK titres and mouse protective antibodies in sheep and cows. The antibodies were effectively transferred from immunized cows to their calves through the colostrum, and the vaccine offered adequate passive protection to lambs against colisepticaemia. The vaccine maintains its potency for at least eight months and the immune response was not jeopardized by the inclusion of as many as fifteen different serotypes.

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