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Control of diarrhea in young pigs using therapeutic antibodies

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Introduction

The economic losses caused by intestinal and feed-borne pathogens are very large (Wells et al., 1998). It has been estimated that in the US alone, medical and productivity losses from bacterial, human-borne illness have been at $2.9 to 6.7 billion each year. The pathogens that are mainly responsible include the following:

- *Salmonella* species, up to $6 billion
- *Staphylococcus aureus,* $1.2 billion
- *Campylobacter* species, $1.0 billion
- *Escherichia coli,* more than $0.2 billion

To compete, US and Canadian industries must convince consumers in Canada, the US, and elsewhere that our products are of high quality and safe.

The cost of controlling pathogens in domestic livestock is also large, as world-wide expenditures on medicines, feed additives, biologicals, and pharmaceuticals are more than $11 billion per year (Wild, 1999). In addition, losses caused by mortalities, decreased productivity, and condemnation of products are equally high. A large part of the costs of controlling livestock pathogens is the cost involved in intestinal disease. Diarrheal diseases, especially those caused by *E. coli*, result in considerable economic losses to the livestock producer, particularly swine producers. It has been well recognized that diarrhea disease caused by entrotoxigenic *E. coli* (ETEC) is by far the most common enteric disease encountered in neonatal pigs (Morris and Sojka, 1985; Yokoyama et al., 1992; Alexander, 1994; Hampson, 1994). It is also known that colonization of the small intestine of the pig by ETEC adhering to the epithelium accounts for most gastrointestinal disorders in both neonatal and post-weaning piglets (Yokoyama et al., 1992; Alexander, 1994; Hampson, 1994). In a recent survey of pre-weaning disease, diarrhea had the highest morbidity and represented 11% of the pre-weaning mortality, with ETEC being the primary and sole infectious cause (Alexander, 1994). A second disease, referred to as post-weaning *E. coli* diarrhea (PWD), starts 3-10 days after weaning. This disease is a major cause of economic losses to the pig industry from both mortality and reduced growth rates and is the most common cause of post-weaning mortality on many farms, killing 1.5-2% of pigs weaned (Hampson, 1994).

The strains of ETEC that are associated with intestinal colonization are those that express the F4 or K88, F5 or K99, F6 or 987P, F18, and F41 fimbrial adhesins. These adhesins are located in the rod-like pili (fimbriae) that extend from the *E. coli* and are bound to specific receptors on the intestinal wall. Therefore, they provide a highly specific means of anchoring the *E. coli* to the host, a requisite for an infectious agent. Among the different ETEC, those expressing the K88+ fimbrial antigen are the most prevalent form of *E. coli* infection found worldwide wherever pigs are raised in high numbers (Rapacz and Hasler-Rapacz, 1986). It has been estimated that K88 ETEC is responsible for 50% of the 10 million piglet deaths each year (Waters and Sellwood, 1982).

A summary of the occurrence of ETEC strains in pre- and post-weaned pigs is given in **Table 1**. Overall it may be concluded that *E. coli* K88 is predominately found in pre-weaned pigs, with *E. coli* 987P being the next most common *E. coli* strain. K99, F41, and F18 strains are usually not detected in nursing pigs. K88 and F18 ETEC most commonly are responsible for diarrhea in post-weaned pigs while 987P, K99, and F41 ETEC are rarely involved. Rotavirus is another organism that commonly causes diarrhea in piglets, other young animals, and human infants (Ozpinar et al., 1996).

The problems associated with diarrhea in neonatal and post-weaning pigs will only become more serious in the future. A trend towards large, intensive herds and early weaning (at 14-21 days rather than 21-28 days of age) are important causes. In addition, the increased incidence of antibiotic resistance in microorganisms and the pressure by regulatory agents to ban or greatly reduce the use of antibiotics in feeds will further compound the problem. Alternative treatments to antibiotics are therefore required. One alternative is the use of specific immunoglobulins (antibodies)from the yolk of the chicken to control intestinal diseases such as *E. coli, Salmonella* spp. and Rotavirus. This review presents an overview of a means of controlling enteric disease in piglets using therapeutic antibodies, with emphasis on ETEC strains of *E. coli.*

Table 1: Occurrence of *E. coli* strains in pre- and post-weaning pigs.[1]

Fimbrial antigens	Comments[2]
Pre-weaning pigs	
K88 (F4)	Usually the most common strain (7% in US, 70% in Poland, 12% in Japan)
987P (F6)	Next most common strain (30% in US, 14% in Poland, 2% in UK). Receptors for the 987P fimbriae decrease with age of piglet.
F18	Usually not found in nursing pigs.
K99 (F5) and F41	K99 and F41 usually occur together and are mainly associated with ETEC of calves and sheep. They are occasionally found on ETEC (less 1%) from piglets but in some surveys their occurrence has been high (up to 44%).
Post-weaning pigs	
K88 (F4)	ETEC having this fimbriae are common as age of animal does not greatly influence presence and activities of intestinal receptors. Occurrence may be as high as 50%.
F18	Strains in Australia and Sweden have shown prevalence rates as high at 62 and 30%. This relatively new strain of *E. coli* invariably strikes during post-weaning resulting in mortalities as high as 30-40% to as low as 1-2%. Usually occurs 10 days after weaning.
987P (F6), K99 (F5) and F41	These strains of *E. coli* are usually not detected in post-weaned pigs.

1Data were from Osek and Truszczynski, 1992; Alexander, 1994; Hampton, 1994 and Yokoyama et al., 1997. These data are from one survey and are subjected to change from location to location and year to year.
2Many of the strains of ETEC are resistant to several antibiotics.

Antibiotics as feed additives and antibiotic resistance

Antibiotics have been referred to as miracle drugs and rightly so, killing the bacteria that cause many of humans' worst infectious diseases. However, today we are on the verge of a medical disaster, as many seemingly small infections could turn lethal for lack of effective drugs (Culotta, 1994). This gruesome prediction stems from the remarkable ability of bacteria to develop resistance to almost any antibiotic medical research has used. In addition, when one species learns a new resistant strategy, it has the ability to transfer the resistance gene to other unrelated species, an ability that accelerates the spread of antibiotic resistance. Currently, certain pathogenic microorganisms have developed multiple resistances to several antibiotics, including vancomycin, in many cases the drug of last resort. It is only a matter of time before multi-antibiotic resistant bacteria become widespread. Physicians and veterinarians will have no effective treatment against these superbugs. Equally worrisome is the relative dearth of new antibiotics being produced by the pharmaceutical industry, particularly those antibiotics with novel modes of action that would make it more difficult for bacteria to circumvent. This emerging problem has serious implications for the use of antibiotics in animal feed (**Table 2**) and for the successful treatment of diseases in humans. Recently, disturbing new findings have provided a key link in the chain of evidence connecting antibiotics used on livestock to outbreaks of disease caused by antibiotic resistant human pathogens.

In addition, the development of microorganisms that are resistant to antibiotics also is a problem for domestic livestock. Studies by Matthew et al. (1998) have demonstrated that many isolates of *E. coli* are resistant to several antibiotics and that this resistance is greater in farms with high compared to low antibiotic use (**Table 3**). Many other common species of microorganisms, including salmonella, have also developed antibiotic resistance.

Table 2: Effects of the use of growth promoters for different species.[1]

Species	Increase in weight (%)	Reduction of feed input (%)
Fattening chicken	3.6	- 3.4
Laying hens (laying performance)	2.8	- 2.7
Turkey hens	3.1	- 2.2
Pig	8.1	- 4.8
Fattening pigs	3.2	- 2.0

1Data from Schwartz, 1998.

Table 3: Percentage of resistant *E. coli* isolates from pigs of varying ages under high and low antibiotic use.[1]

Item	Day 63[2]	
	HU[3]	LU
Apramycin [4]	30	6
Carbadox	47	21
Gentamicin	86	73
Neomycin	65	34
Tetracycline	99	100

1Data from Mathew et al. (1998).
2Age of pig.
3HU = farms with high antibiotic use; LU = farms with low antibiotic use.
4Data derived from a standardized disk susceptibility test.

In order to address these issues, the European Union has issued new rules limiting the use of several livestock antibiotics, while the US Food and Drug Administration (FDA) has proposed similar regulations (Schwartz, 1998). Canada will presumably have to follow such regulations, as they would not be able to be export products from animals treated with antibiotics.

It may be concluded:

- Microorganisms are becoming resistant to antibiotics.
- Resistance is often common to many different antibiotics.
- Resistance is readily transferred among different microorganisms, including those of animal origin to human pathogens.
- Antibiotics, as a animal growth stimulant, will probably be banned in the foreseeable future.
- New antibiotics may not be used in animals.
- Alternate treatment to control pathogens must be utilized.

Producing pigs without antibiotics

Several alternative strategies can be used to replace antibiotics as growth promoters for pigs and for the control of disease. An important overall factor is to maintain clean, sanitary facilities and, particularly, to avoid transferring pigs from one producer to another. This latter precaution is often not possible. Several different dietary strategies can be utilized to reduce the pathogenic load, including adding the following to the pig diets (Close, 2000):

- Organic and inorganic acids
- Oligosaccharides, such as mannose, or functo-oligosaccharides
- Enzymes
- Herbs/flavors
- Materials such as copper or zinc
- Probiotic yeast and non-starch polysaccharides

Various combinations of these treatments have been shown to produce limited growth promotion and protection against pathogens. However, they have not been shown to be highly effective at controlling intestinal pathogens such as enteropathogenic *E. coli* or *Salmonella*.

Alternative strategies to control intestinal pathogens, such as *E. coli*, in domestic livestock include the use of vaccines for active immunity and antibodies for passive immunity. Vaccination of the sow against specific strains of *E. coli* will result in the production of immunoglobulins in the colostrum that will provide passive immunity to the nursing piglet against the injected pathogens. This protection, however, is transient and all protection is lost after weaning. Immunization of the piglets is also not practical, as colibacillosis will develop in the piglet before

they are able to develop immunity; as a result, immunization will not protect such pigs against the pathological effects of ETEC (Alexander, 1994; Hampson, 1994; Isaacson, 1994).

One highly attractive and effective alternative approach for the control of pathogens that infect the intestinal tract is to use therapeutic antibodies for passive immunity. These antibodies can be produced in any animal and can be administered orally to another animal to control a specific disease. The advantage of using antibodies is that they will provide a long-term and sustainable means of controlling pathogens. Such a treatment is highly effective, would not result in the development of resistant strains of microorganisms, would spare the use of antibiotics, and could be relatively inexpensive to use. Antibodies can be obtained from several sources, including the colostrum of cow's milk, blood of animals, transgenic plants or microorganisms, and, finally, the yolk of the laying hen. Antibodies from the colostrum of the lactating animal, particularly dairy cattle, is impractical as it is only produced over a short period of time. Antibodies as obtained from spray-dried plasma proteins are probably highly effective, but currently no information has been published on the ability of this product to counteract different intestinal pathogens, including *E. coli*. There undoubtedly are large variations in the ability of plasma proteins from different sources to counteract specific pathogens, as the ability of plasma protein to neutralize the effect of specific organisms is dependent on the immunization and disease history of the pigs from which the blood is collected. Current technology for monoclonal antibody production is prohibitively expensive. They, nevertheless, have been shown to be highly effective at controlling certain diseases. The ultimate goal is to provide a library of antibodies with nearly endless specificity and without dependence on animals or their cells for their synthesis. These latter procedures, which will involve the production of antibodies in microorganism or plants, are only being initiated and currently cannot be used for large-scale antibody production.

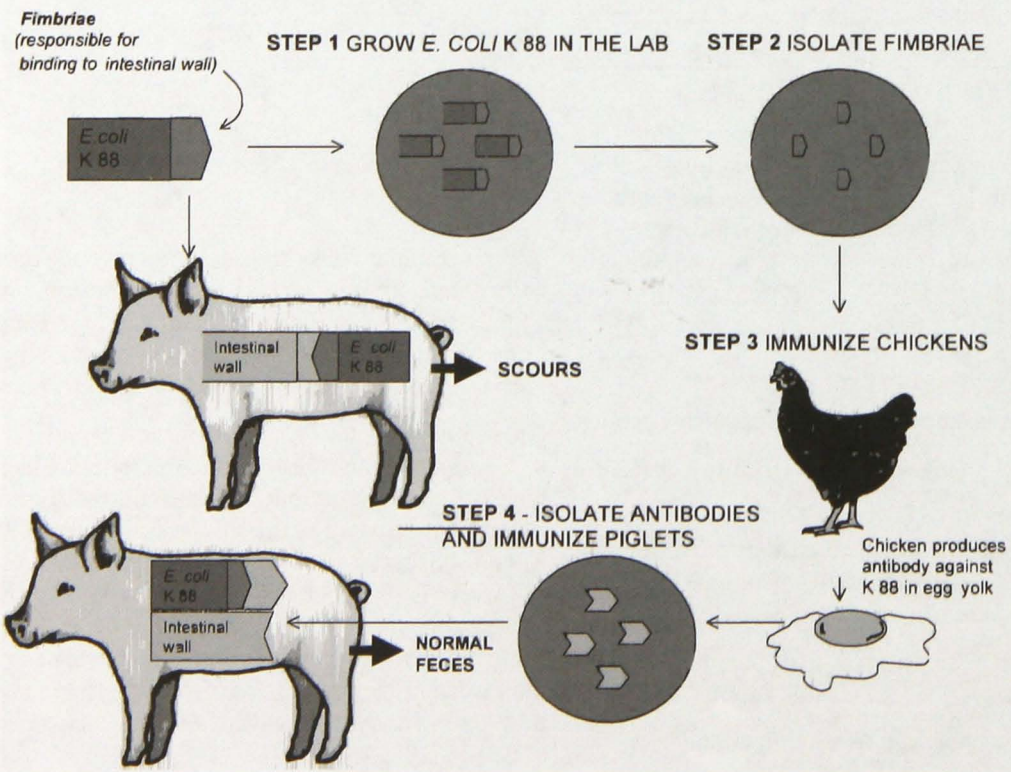
Among all of the different possibilities, the use of chicken egg-yolk antibodies to control intestinal pathogens appears to be one of the best alternatives.

Production and use of egg-yolk antibodies to control intestinal diseases

The chicken is an excellent source of immunoglobulins (antibodies). Large quantities of antibodies (IgY) are secreted into the yolk of the egg (up to 200 mg/egg) which can be harvested non-invasively (Gassman et al., 1990; Marquardt, 2000).

The method of producing and using chicken egg-yolk antibodies is illustrated in **Figure 1**. The procedure involves the following:

Figure 1: **A New Way to Prevent Scours Using Antibodies**



- Isolating the infectious agent from fecal swabs of pigs suffering from diarrhea (scours)
- Identifying the organism (*E. coli* K88, in this example)
- Culturing the organism in the appropriate media
- Isolating the virulence factor in pure form from the organism
- Injecting the factor into chickens

The chicken produces antibodies (immunoglobulins) against the antigen (factor) which are secreted into the yolk of the egg. The whole egg or its yolk is dried and the dried product is incorporated into the diet of pigs suffering from an *E. coli* K88 infection. The antibody reacts with the *E. coli,* preventing it from binding to the intestinal wall of the pig; as result, its pathogenicity is neutralized. The pig rapidly recovers from the *E. coli* K88 infection.

Egg-yolk antibodies have been shown to control intestinal infections of *E. coli* K88, K99, 987P, and F18 in young pigs (Yokoyama et al., 1992; Imberechts et al., 1997; Marquardt et al., 1999) and rotavirus in newborn calves (Ozpinar et al., 1996). Egg-yolk antibodies were also able to reduce mortalities in mice challenged with *Salmonella* spp. (Yokoyama et al., 1998) and mastitis organisms in the udder of lactating cows (Coleman, 2000).

Yokoyama et al. (1992, 1998) evaluated chicken egg-yolk immunoglobulins as a treatment for experimentally induced ETEC *E. coli* in colostrum-deprived piglets. The piglets were orally challenged with high doses of K88 or 987P *E. coli* four hours after birth. They were then orally administered the antibody three times a day for three consecutive days after the occurrence of diarrhea. Treatment of pigs with the antibody reduced mortalities to essentially zero and reduced fecal counts to near zero (**Table 4**). In contrast, non-antibody treated pigs had high mortalities (>80%), high incidence of diarrhea, high fecal scores, and shedded a considerable number of *E. coli* in the feces 1, 3, and 5 days after treatment.

A similar study was carried out by Marquardt et al. (1999) with *E. coli* K88, except the piglets were 21-days old when challenged with *E. coli* K88 (**Figure 2**). These data also demonstrated that the antibody reduced the incidence of scours, the fecal score, mortalities, and *E. coli* in feces to zero. In contrast, 72 h after treatment, the non-antibody treated pigs continued to have severe scours, 30% of the pigs died, and they continued to excrete *E. coli*. In another study with a newly identified and highly toxic form of *E. coli* F18, it was shown that the F18 antibodies diminished the incidence of diarrhea and death in animals infected with F18-positive *E. coli* (Imberechts et al., 1997).

Table 4: Clinical response of newborn piglets after challenge with ETEC K88+, K99+ and 987P+ strains and treatment with egg antibody powder.[1]

Strain	Antibody treatment (titre)	No. piglets with diarrhea/total (FC score)[2] on day			Total No. dead (%)	Percent fecal swabs positive on day:		
		1	3	5		1	3	5
K88	02500	7/7 (3.0)	4/4 (2.8)	1/1 (2.0)	6/7 (86)	962	50	80
		3/7 (1.3)	0/7 (0.1)	0/7 (0.1)	0/7 (0)	3	1	0
K99	02500	4/4 (3.0)	0/0	0/0	4/4 (100)	808	-	-
		3/4 (1.5)	0/4 (0.5)	0/4 (0.0)	0/4 (0)	1	25	1
987P	02500	5/5 (3.0)	1/1 (3.0)	1/1 (3.0)	4/5 (80)	922	40	40
		4/5 (2.0)	0/5 (0.0)	0/5 (0.0	0/5 (0)	1	0	0

1Data from Yokoyama et al., (1992).
2The fecal score was: 0, normal; 1, soft feces; 2, mild diarrhea; and 3, severe diarrhea.

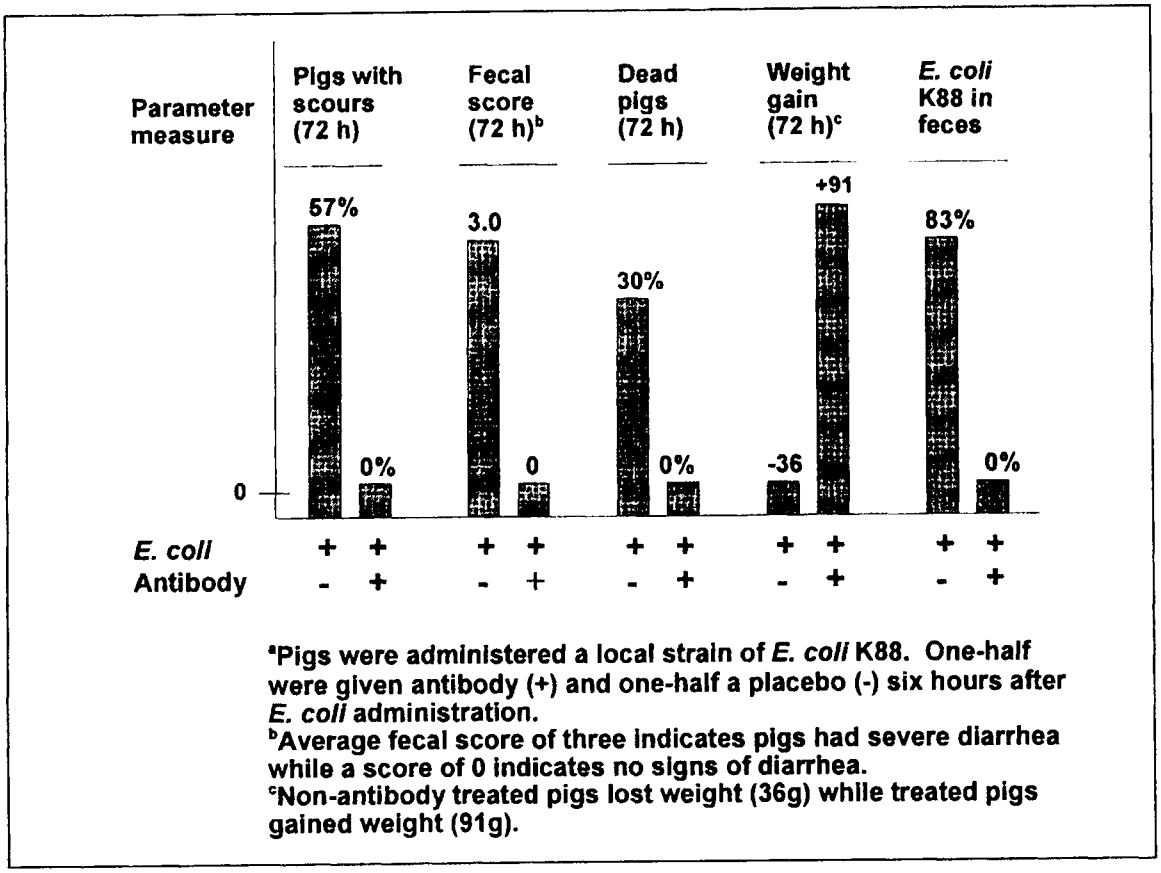


Fig 2. EFfect of Egg-Yolk Anitbody on Clinical Response in Pigs Challanged with *E. coli* K88[a]

Rotavirus from calves, pigs, mice, foals, infant humans, lambs, chickens, and turkey are antigenically related. Therefore, the appropriate anti-rotavirus antibodies will react with the virus present in any of these species of animals. Rotavirus is a major pathogen of infectious gastroenteritis, not only in children and infants, but also in domestic animals. In humans alone it has been estimated the rotavirus infections results in several millions of deaths. Animals are also seriously affected by this virus. Ozpinar et al. (1996), in studies with calves, reported that mortalities from rotavirus infection was approximately 9% for untreated animals and essentially zero for calves that received specific egg-yolk anti-rotavirus antibodies. Also, the duration and weight losses were reduced in the antibody-treated calves. Studies with pigs have not been reported, but it may be assumed that similar positive results would also be obtained by use of this antibody.

Salmonella species such as *S. enteritidis* and *S. typhimurium* are non-host serotypes which can cause disease syndromes like gastroenteritis and systemic infectious in a wide range of animal species, including humans. Therefore, it is important to control this disease, not only to reduce productive losses in domestic livestock, but to also prevent its transmission into the human food chain. Future legislation in the US will require that all food products, especially pork, poultry, and beef products, not only be completely free of salmonella, but also that animal feed be free of this organism. Studies by Yokoyama et al. (1998) have investigated the efficacy of egg-yolk antibodies specific for outer membrane protein (OMP), lipopolysaccharide (LPS), or flagella (Fla) for controlling *S. typhimurium* or *S. enteritidis*. They treated mice orally with the appropriate placebo or egg-yolk antibody following a challenge with *S. enteritidis*. Antibody treatment resulted in survival rates of 80, 47 and 60% using OMP-, LPS-, and Fla-specific antibodies, respectively, in contrast to only 20% in control mice. In the *S. typhimurium* trial, the survival rate were 40, 30 and 20% using OMP-, LPS-, or Fla-specific antibodies, respectively, in contrast to 0% in control mice. These preliminary results suggest that antibodies against specific *Salmonella* proteins can control salmonellosis when orally administrated to mice. These very encouraging results also suggest that similar benefits can be obtained with domestic livestock, in particular pigs.

The studies described above have demonstrated that egg-yolk antibodies are highly effective at controlling certain intestinal diseases in domestic livestock, including *E. coli*

K88, K99, 987P, and F18, and that they probably will also be effective against other diseases including Rotavirus and *Salmonella* spp. A considerable amount of additional research, however, must be carried out to identify the best antigens to use and appropriate treatment protocols.

Factors affecting stability and efficacy of egg-yolk antibodies

There are many factors that can affect efficacy of egg-yolk antibodies, particularly important are the following:

- pH
- Temperature
- Duration of treatment period
- Form or moisture content of the antibody being treated

Other factors include the following:

- Titer of product (amount of specific antibody in eggs)
- Amount of product added to the diet or administrated by gavage
- Disease status
- Number of colony-forming units in the animal
- Frequency and location of administration of the antibody
- Storage conditions of the antibody and its exposure to factors that will inactivate it.

Egg-yolk immunoglobulins, being glycoproteins, are sensitive to the same denaturing conditions as most proteins. Studies by Otani et al. (1991) demonstrated that egg-yolk antibodies are readily denatured in phosphate buffer (pH 7.2) at high temperatures and that this inactivation is time dependent (**Figure 3**). For example, 25 and 75% of the original activity was lost when the antibody was incubated for 10 and 30 min, respectively, at 70°C, pH 7.2. Likewise, the stability of egg-yolk antibody is greatly influenced by the pH of the media. As shown in **Figure 4**, very little activity was lost between pH 9.0 and 4.0, after which there was a precipitous drop in activity with decreasing pH. At pH 3.0 and 40°C, only 10% of the original activity remained. The inactivation at low pH values is further enhanced by the presence of the digestive enzyme pepsin (**Figure 5**). The stomach of animals have a low pH and pepsin, conditions which would favors inactivation of IgY. Presumably the presence of feed, especially the buffering effect of proteins, would increase the pH of the stomach over that without feed. Also, other proteins which are present in much greater concentrations

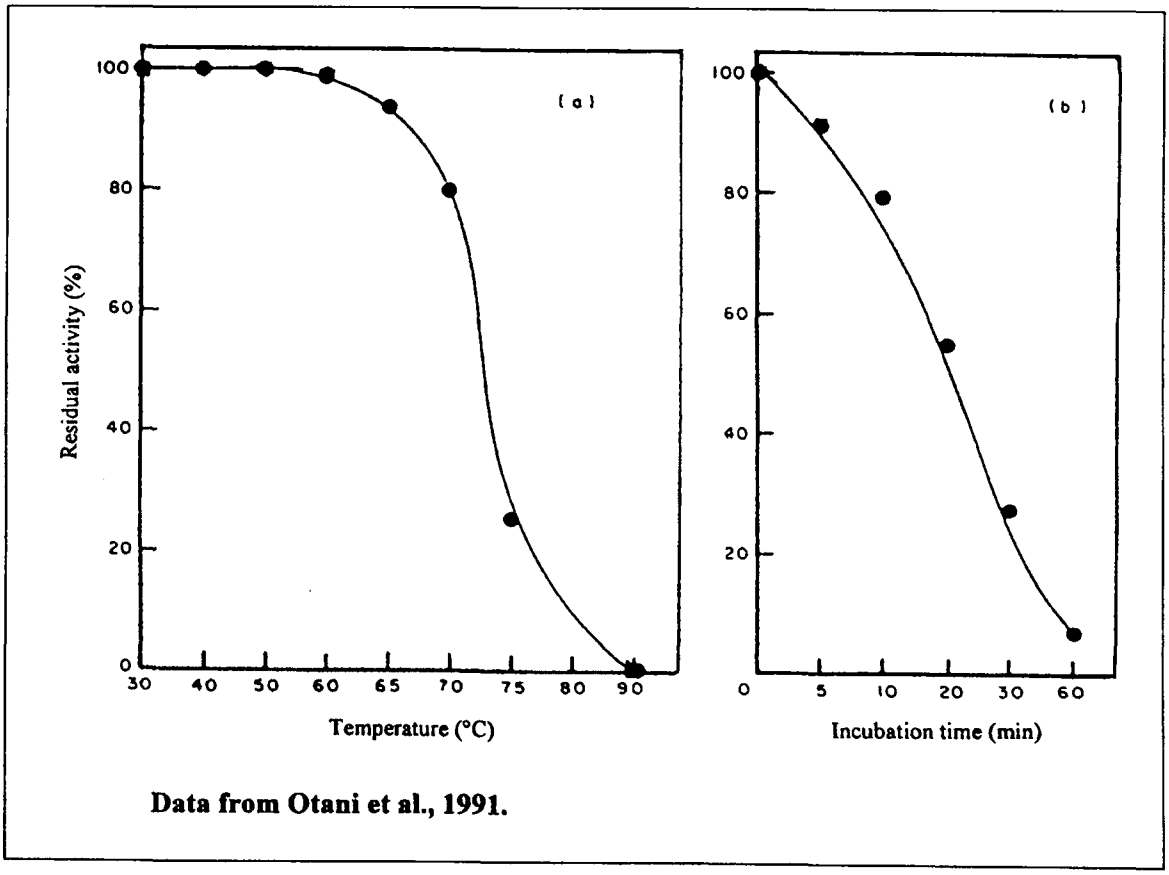


Fig 3. Heat stability of hen egg yolk IgY antibodies specific to αs-casein (a) incubated for 10 min at different temperatures; and (b) incubated at 70°C for different times.

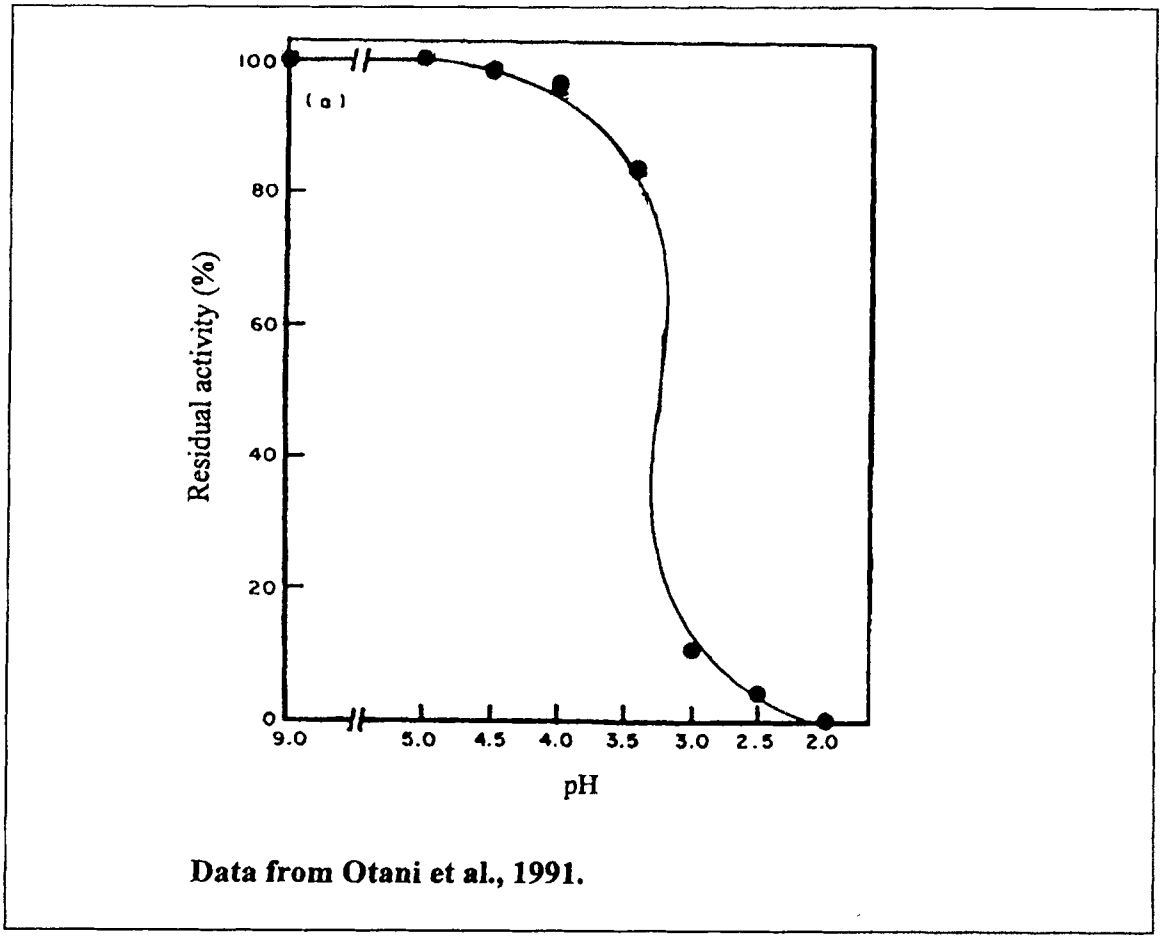
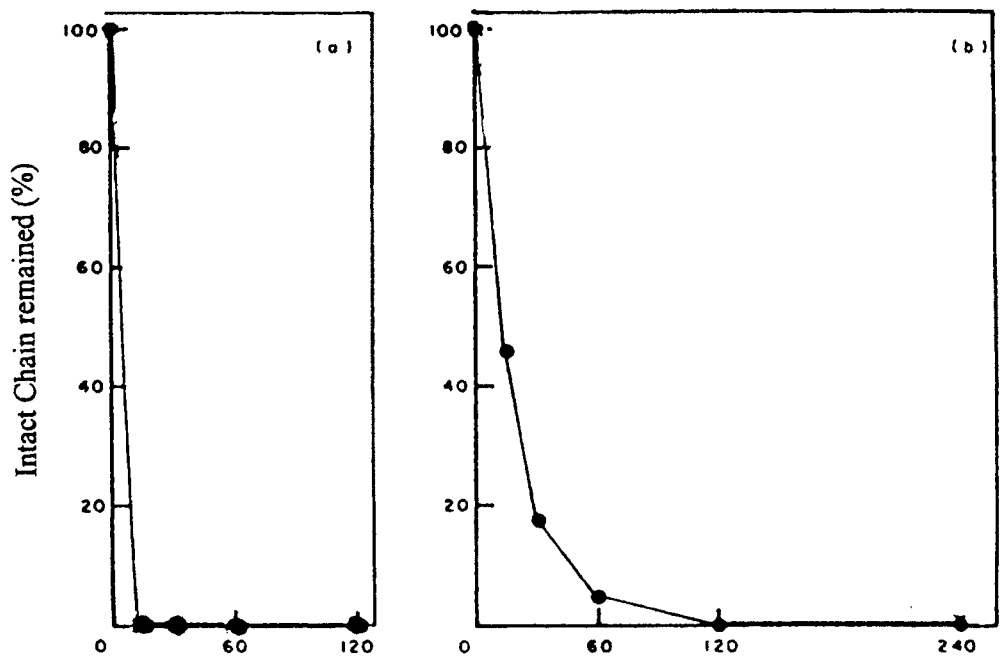


Fig 4. pH stability of hen egg yolk IgY antibodies specific to αs-casein. The antibodies solution was adjusted from pH 9.0 to 2.0, incubated for 1h at 40°C, and the residual antibody activity determined.

relative to that of IgY would competitively reduce the rate of cleavage of IgY by pepsin. The nature and extent of this effect has not been reported.

Most of these thermal stability studies with chicken egg-yolk antibodies have been carried out in aqueous solutions. The authors are not aware of heat stability studies on dried egg-yolk antibodies or antibodies that are present in the feed before or after steam pelleting. Presumably the stability of egg-yolk antibodies would be similar to that for enzymes and that this would be greatly influenced by temperature, duration of heat treatment and cooling period, and the moisture content of the diet. It is well known that a change in moisture content from 95 to 90 or 85% dry matter can dramatically decrease the stability of proteins when heated to high temperatures. Preliminary studies in our laboratories have demonstrated that low temperature steam pelleting (70°C) did not reduce antibodies' titer. Additional research must be carried out on this problem.

Another factor affecting the efficacy of IgY is the rate of passage through the digestive tract and its ability to retain its activity in the different sections. Yokoyama et al. (1993, **Table 5**), in an excellent study, monitored antibody titers at 0, 2, 6, and 24 h after an oral dose of anti-K88 antibodies in pigs 10 hours and 3, 6, 21, and 28 days after birth. The results demonstrated that IgY was absorbed into the blood in pigs that were 10 h of age but not by pigs that were three days of age. Therefore, avian IgY, like mammalian IgG, is readily absorbed within 24 h by the newly born pig. The half-life of chicken IgY was 1.85 days, which is considerably shorter than that of the homologous mammalian IgG (t2 = 12 to 14 days). The therapeutic benefit of chicken IgY in the serum of pigs would, therefore, be much less than that of the corresponding pig immunoglobulins. Also, the young pig (less than three days of age) may probably develop an allergic reaction to IgY, a foreign protein, if it was subsequently administered intravenously. These data would therefore suggest that egg-yolk antibodies should be administered with cau-



Data from Otani et al., 1991.

Fig 5. Digestion of hen egg yolk IgY antibodies with pepsin at 37°C pH 2.0 and pH 4.0.

tion to the very young pig (less than two to three days of age).

In contrast to the very young pig, the survival of antibody appears to be low at 28 d of age, probably because of the development of the digestive system of the pig, particularly after weaning. There is, however, very little or no information about the relative efficacy of egg-yolk antibodies in older animals. If this is a problem, it may be solved by stabilizing the antibody chemically or by encapsulating it in a substance that will by-pass the severe proteolytic effects of the stomach. Shimizu et al. (1993) have demonstrated that the encapsulation of IgY in liposomes (a mixture of cholesterol and lecithin) greatly reduces the effect of low pH, especially in the presence of pepsin (**Table 6**).

Finally, the results of Yokoyama et al. (1993) demonstrated that antibodies pass through the digestive system within a relatively short period of time (slightly more then 24 h), (**Table 5** and **Figure 6**). In addition, they reach all sections of the gastrointestinal tract within less than 10 h. As a result, young pigs should rapidly respond to antibody treatment, but the effect of treatment period of a single dose may be only 24 h, unless it is sufficiently high to neutralize all of the pathogenic organisms. Therefore, two or three doses should be administered per day, and this may have to extend over a two to three day interval. No study, however, has identified the best therapeutic treatment protocol for control of intestinal pathogens such as different strains of *E. coli*.

The data presented in this section indicates that a considerable amount of additional information must be obtained before the very great potential of therapeutic antibodies can be realized. This includes gathering further information on the following

- The stability of IgY when subjected to feed processing
- The frequency and duration of a therapeutic treatment required to ensure that reinfection does not occur
- The ability of egg IgY to induce allergic response in the very young pig because of the porosity of its di-

Table 5: Anti-K88 *E. coli* titers (IgY) in the gastrointestinal tract of pigs following oral administration of the antibody.[1]

Age after birth	Hours after given IgY	Anti-K88 fimbriae titer					
		Serum	Stomach	Duodenum	Jejunum	Ileum	Large intest.
10 h	0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	2	13	14	15	20	5	1
	6	4	3	2	2	22	37
	24	56	1	1	1	2	40
3 d	0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	2	< 0.1	28	18	19	16	1
	6	< 0.1	1	2	9	20	19
	24	< 0.1	0.4	< 0.1	1	1	32
6 d	0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	2	< 0.1	15	32	18	0.5	< 0.1
	6	< 0.1	1	2	11	35	22
	24	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
21 d	0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	2	< 0.1	15	22	33	1	< 0.1
	6	< 0.1	2	0.1	0.7	6	4
	24	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
28 d	0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	2	< 0.1	4	3	5	0.4	< 0.1
	6	< 0.1	1	< 0.1	< 0.1	2	< 0.1
	24	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1

1Data from Yokoyama et al. (1993).

gestive system

- The stability and efficacy of egg-yolk antibodies in older pigs (greater than 28 days of age)
- Possible means of enhancing egg-yolk antibodies' effects by chemical or physical modifications

Factors to consider when using egg-yolk antibodies

Egg-yolk antibodies can be administrated to young pigs in two forms: (1) as a preventive or prophylactic treatment, and (2) as a therapeutic treatment after infection occurs. It has been our experience that the addition to the diet of as little as 0.5 kg of egg-yolk powder per tonne of feed that has a high titer of the appropriate antibody can completely prevent *E. coli* infections in pigs from 3 to 28 days of age. The cost of preventative treatment would be only $0.16/pig if it is assumed that the cost/kg of antibody is $80/kg and that a normal feeding period for the antibody is from 18 to 28 days of age—a period in which they would consume approximately 4 kg of feed. This is equivalent to 2 g egg-yolk powder/pig or that in 0.25 eggs. Similar preventive treatments with antibiotics may not be feasible, as the organism may be resistant to the selected antibiotics or they may be banned for use in livestock. Another alternative is to use spray-dried pig plasma (SDPP) (Maxwell, 1992). A large part of its beneficial effects may be attributed to its content of different anti-*E. coli* immunoglobulins. Its cost, however, is much higher than that of egg-yolk antibodies. For example, if it is assumed that the cost of SDPP is $5.00/kg and that the equivalent value of protein in this product is $1.00/kg, then the net cost attributable to the antibodies would be $4.00/kg. Also, it is assumed that the incorporation rate of SDPP into the diet is a conservative 5% and, as indicated above, pigs from 18 to 28 days will consume 4 kg feed. Therefore, the cost of the therapeutic portion of this treatment, using the above assumptions, would be $0.20/kg feed or $0.80/piglet; this would be five-fold more ex-

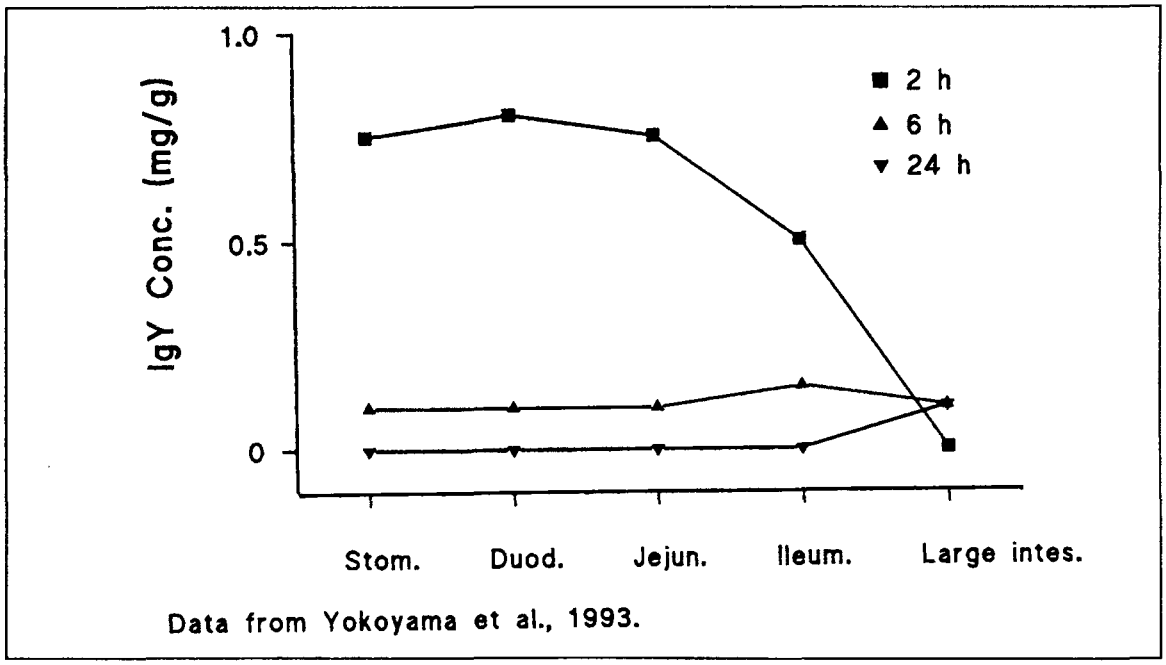


Figure 6. Egg-yolk concentration in the gastrointestinal tract as measured by ELISA. Antibody was administered 21 days after birth and ingesta samples were collected 2, 6 and 24 hours after antibody administration.

Table 6: Effect of liposomal encapsulation on the stability of IgY against peptic hydrolysis under acidic conditions.[1]

IgY	Pepsin	Relative antibody titer of IgY after incubation with and without pepsin	
		pH 2.8	pH 1.8
Unencapsulated	+	0.0	0.0
	−	0.6	0.03
Encapsulated	+	0.7	0.4
	−	0.8	0.4

1The titer is relative to that for native IgG which is taken as 1.0. IgY was incubated at 37_C. Data from Shimiza et al. (1993).

pensive than the use of egg-yolk antibodies. In addition, the titer of specific antibodies in SDPP varies considerably from source to source and time of year, whereas that in egg-yolks can be standardized. These data strongly suggest the egg-yolk antibodies may prove to be a highly economical alternative to that of SDPP, which currently is used by nearly all pig producers in North America. The reason for the widespread use of SDPP is that its addition to the diet of young pigs has been shown to increase feed intake and therefore weight gains by as much as from 5 to 40%, especially in pigs from units with a high disease status. However, no information is available on the relative benefits of using a mixture of egg-yolk antibodies plus a high quality protein compared with that of using SDPP, either alone or in contribution with egg-yolk antibodies. An egg-yolk supplement containing a highly digestible protein may produce performance values equal or superior to that obtained with SDPP but at a much lower cost.

In addition to using egg-yolk antibodies as a prophylactic, they can also be used as a therapeutic agent in pigs that have developed severe diarrhea. It has been shown, on the basis of our experience, that the administration as little as 1 to 3 g of egg-yolk antibody powder as a gavage

twice a day for two or three days is sufficient to completely overcome the pathogenic effects of *E. coli* K88. The total amount of antibody required would be approximately eight grams or $0.64/pig. This is equivalent to the amount of egg-yolk present in one egg. Another possible alternative, as discussed above, would be to incorporate the egg-yolk antibody into the diet of the sow for passive immunity. These data indicate that the use of egg-yolk antibodies not only are cost effective but are highly effective in the control of specific diseases. Other advantages of using egg-yolk antibodies have been outlined in a previous presentation (Marquardt, 2000).

Procedure for selecting the appropriate egg-yolk antibodies

General observations

- Most antibodies are specific for only one organism. That is, anti-*E. coli* K88 antibodies will only neutralize *E. coli* K88 and not other strains of *E. coli* such as *E. coli* K99, 987P, F18, etc.
- In general only one or, at most, two strains of *E. coli* are responsible for most cases of diarrhea in pigs. Also, the same organism tends to persist over time. Therefore, only one antibody may be required.
- The *E. coli* strains of greatest concern in piglets are those bearing the K88 and F18 fimbria. K88 is also of concern in calves.
- The cost of treatment is directly proportional to number of antibodies used. It is, therefore, highly desirable to identify the causative organism and administer only one antibody.

Identification of causative organism

This can be achieved by using several different approaches. They are the following:

- Assay blood of non-immunized pigs for specific antibodies. This will provide an overview of the disease status of the herd over time. Enzyme immunosorbent assays (ELISAs) have been developed that are able to detect different pathogens. Our laboratory routinely assays for six different *E. coli* antibodies.
- Determine the specific strain(s) of pathogens in fecal swabs using highly specific and sensitive DNA-based assays (PCR reaction). Different laboratories in Canada, including our laboratory, are capable of using this technology to identify specific strains of *E. coli*.
- Identify and quantify the number of species of pathogens in fecal swabs using ELISAs.

Treatment

- Prophylactic or therapeutic treatment can be initiated once the causative organism has been identified.

A failure of the treatment may be attributed to the following causes:

- The proper dose of antibody was not used.
- The antibody was either not mixed properly, heat inactivated, etc. As a result, the antibody may not be present in the diet at the proper concentrations. These problems can be avoided by assaying for antibody titer.
- The causative organism was either misdiagnosed or a second pathogen may have been involved. Under such conditions, a systematic analysis needs to be carried out to identify the causative organism.

It has been our observations that *E. coli* diarrhea can be readily controlled using the appropriate antibody. This treatment is not only highly effective, but it is environmentally friendly, as no residues are produced and is sustainable, as the antibodies can be designed for any antigen.

Summary and conclusions

Advantage of egg-yolk antibodies

- They can be 100% effective for *E. coli* strains F3 (K88), F4 (K99), F5 (987P), F18 (F107), and F41.
- Their use as a preventive treatment should promote substantial improvements in weight gain (10 to 50%, hypothetically).
- Only a small amount of antibody is required per pig; therefore, the treatment is highly cost-effective.
- Collection of eggs is noninvasive.
- The treatment is safe. Live organisms are not used.
- The procedure is environmentally friendly.
- No toxic residues are produced.
- Egg-yolk and its antibodies are natural.
- Potential applications are enormous, as antibodies undoubtedly will be used to control other intestinal pathogens such as *Salmonella*, etc.
- Egg-yolk antibodies will probably be used to control human pathogens.

Disadvantages of egg-yolk antibodies

- Use of egg-yolk antibodies to control intestinal pathogens is in its infancy. Therefore, numerous studies must be carried out to define their limitations and to optimize their use.

Conclusions

- Egg-yolk antibodies can be 100% effective for the control of diarrhea diseases in young animals, especially those caused by *E. coli.*
- Treatment will be inexpensive, sustainable, environmentally friendly, effective, and will probably provide the best alternative to antibiotics.
- Treatment can be used to control many intestinal pathogens.
- This is the treatment of the future.

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