Rotagen Combo Technical Datasheet

Background information

The key ingredient of Rotagen “Combo” is Globigen, which is derived from egg yolk of vaccinated chickens. Chickens from high health status flocks are vaccinated against specific antigens, and antibodies are produced which are harvested from the egg yolk. The egg yolk is processed, heat treated and spray-dried, killing any potential poultry pathogens. The yolk immunoglobulin (IgY) levels are determined by titre so a consistent Globigen quality is obtained by Vetpak. The Globigen is extended by Vetpak, adding dextrose, kaolin, and sodium bicarbonate to produce Rotagen “Combo”.

It is technically possible to produce IgY’s against almost any organism. This has been successfully done against a wide range of bacteria, viruses and protozoa. Vetpak have made for us Globigens that separately contain IgY’s against:

- Bovine Rotavirus 6 & 10 (A & B)
- Cryptosporidia
- *Salmonella typhimurium*
- *Escherichia coli K99*, and
- Bovine Coronovirus

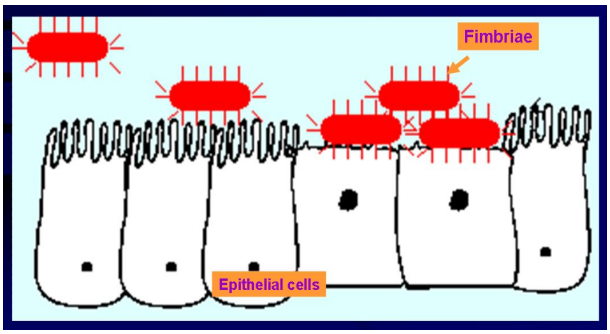
So, it is possible for Vetpak to mix any combination of the above IgY’s to produce a mix to meet a specific requirement depending on the organisms involved in a scour outbreak or to prevent against any organisms that have been diagnosed in the past.

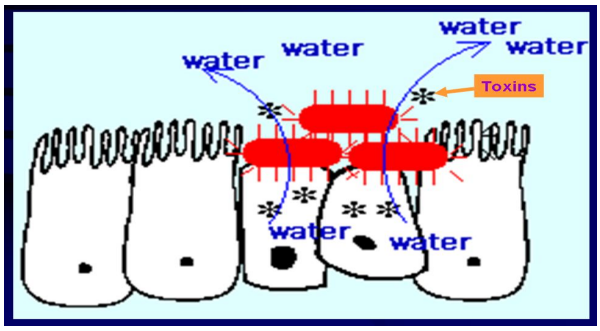
Overseas, Globigen is used very successfully in the pig industry. This is by far its greatest application. Globigen is also used successfully as a growth promotant in calves, where small quantities of IgY’s against a range of antigens are used. New Zealand is the only country so far where a product containing Globigen (Rotagen “Combo”) is a registered animal remedy, and where specific IgY’s are included against specific diseases. Globigen is being used in some countries to treat and prevent calf scours as an un-registered nutritional supplement.

How does Rotagen work?

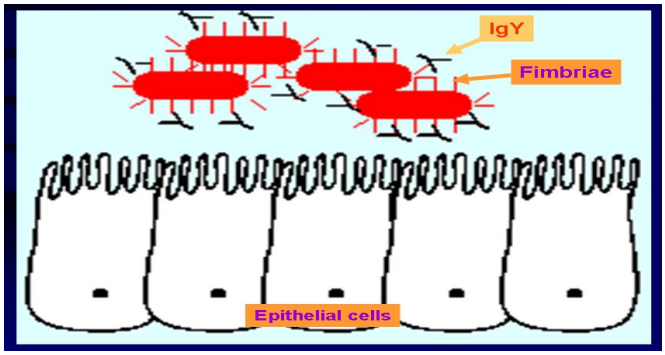
Bacteria

Enterotoxic E. coli strains adhere to epithelial cells by fimbrial adhesions and colonize here. Enterotoxins are released from the colonizing strain causing severe diarrhoea to develop.



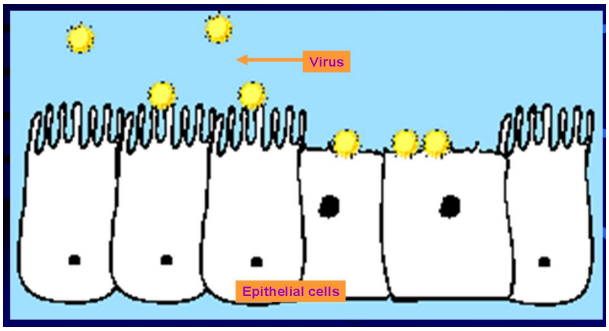


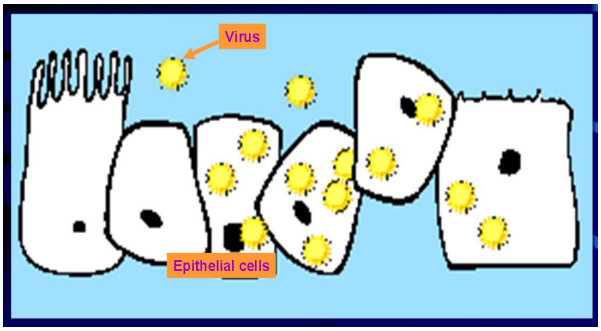
IgY can effectively block the initial stages of the infection process.



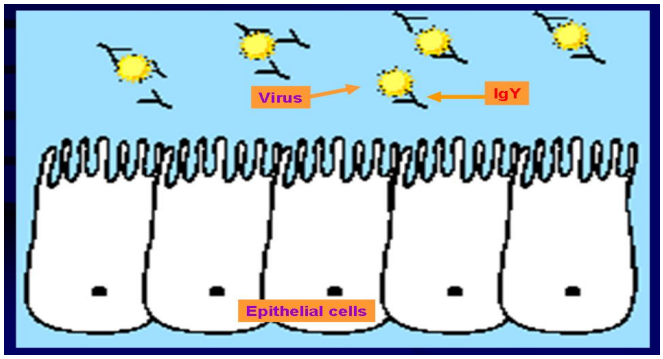
Viruses

The virus attaches to the cell surface receptors, the virus infects, permitting the virus to replicate in the epithelial cells.





IgY can effectively block the infective stages of the infection process.



Epidemiology of Scour outbreaks, managing them, and understanding the role of Rotagen

Epidemiology

Most scour outbreaks in New Zealand in neonatal calves involve Rotavirus. Evidence from New Zealand Animal Health Laboratories suggests that on numbers of diagnoses, the prevalence of pathogens is in the following order, highest to lowest: Rotavirus, Cryptosporidia, *E coli* K99, *Salmonella typhimurium* and Coronovirus. On infected properties Rotavirus is present in the gastro-intestinal tracts of the cows and is transmitted from the cow to new-born calves soon after birth by faecal contamination. Surveys have shown that 75% of properties are infected with Rotavirus. Once infected, the virus in neonatal calves multiplies and the calves can become shedders of virus for several days. The virus multiplies rapidly causing gross environmental contamination. As part of the control of Rotavirus it is important that the environmental contamination is reduced to an absolute minimum.

The role of colostrum

Colostrum is protective against a wide range of diseases and it is important that newborn calves get an adequate dose of colostrum as soon as possible after birth. Surveys have shown that at least 30% of calves receive inadequate colostrum. Farmers can ensure an adequate intake of colostrum by removing calves from the cows twice daily and stomach tubing them with saved colostrum. The best colostrum is colostrum from the first milking which should be kept separate from subsequent milkings, and used for tube feeding newly born calves. The calves are then fed colostrum saved from subsequent milkings for a few days.

Prevention using Rotagen "Combo"

Information should be used from previous years to choose an appropriate Rotagen "Combo" to prevent scours. It is important that all calves are treated at birth for four days before any build up of environmental contamination can occur. Because Rotagen "Combo" produces passive immunity, it is dependent on contact with the pathogen to render the virus inactive. This is why calves should be treated twice daily to provide that contact. Only small amounts of immunoglobulins are absorbed with a relatively short half life[i]. Therefore, any build up of environmental contamination is going to render the calves susceptible to re-infection after the prevention ceases. So, methods of reducing environmental contamination must be followed:

- Clean calf sheds well before calving and spray with a suitable bactericide and virucide. ***Vetpak Vetsan***™ is a safe effective product.
- Spray the calf shed out once a week or more often. ***Vetpak Vetsan***™ is a safe effective product and calves can also be sprayed with it, and Vetpak recommend this.
- Fill the calf shed pen by pen, moving on to the next pen when the current one is full.
- Avoid contact between young and older calves. Feed in pens if at all possible to prevent younger calves coming in contact with older calves.
- Avoid stress by avoiding overcrowding.
- Avoid stress by providing warm facilities but with adequate ventilation.

Treatment using Rotagen "Combo"

Most scour outbreaks in New Zealand in neonatal calves involve Rotavirus. Vetpak recommend that whenever an outbreak of scours occurs; start treating with Rotagen

Rotavirus 6 & 10 while faeces samples are tested until the results are at hand. Once the diagnosis is established, the correct Rotagen "Combo" combination can be administered. It is important to treat all calves to help reduce the environmental contamination. It is important that adequate re-hydration of dehydrated calves occurs. Veterinary advice on n adequate rehydration programme should be given. ***Vetpak Enerlect*** is a very effective electrolyte replacer containing more energy than most other electrolyte replacers. The best way to manage dehydrated calves is to keep them in their pen, mark them and treat them. Severely affected calves can be isolated in a corner of the pen which is partitioned off.

Rotagen "Combo" Prevention and treatment regimes

Using Rotavirus 6 & 10 immunoglobulins:

Prevention	Day 1 to 5	10gms daily for 5 days
Treatment	Day 1	20gms daily for 1 day
	Thereafter	10gms daily for 3 days.

Using Rotavirus 6 & 10 plus Cryptosporidia immunoglobulins

Prevention	Day 1 to 5	10gms daily for 5 days
Treatment	Day 1	10gms twice daily for 1 day
	Thereafter	5gms twice daily for 3 days.

Note: In severe infections the above treatment rates may need to be doubled.

Using Rotavirus 6 & 10 plus Coronovirus immunoglobulins

Prevention	Day 1 to 5	10gms daily for 5 days
Treatment	Day 1	20gms daily for 1 day
	Thereafter	10gms daily for 3 days.

Using Rotavirus 6 & 10 plus *E. coli* immunoglobulins

Prevention	Day 1 to 5	10gms daily for 5 days
Treatment	Day 1	20gms daily for 1 day
	Thereafter	10gms daily for 3 days.

Using Rotavirus 6 & 10 plus *Salmonella typhimurium* immunoglobulins

Prevention	Day 1 to 5	10gms daily for 5 days
Treatment	Day 1	20gms daily for 1 day
	Thereafter	10gms daily for 3 days.

Administration of Rotagen "Combo"

Rotagen "Combo" may be fed to calves from day one of life. At 14 days of age a calf should no longer need ***Rotagen "Combo"*** to combat Rotavirus infection, but in the case of a scours outbreak ***Rotagen "Combo"*** may continue to be used to maintain calf health.

Rotagen "Combo" can be mixed with milk or milk replacer. For an individual dose it can be mixed with a small quantity of warm water and then drenched or fed individually.

Rotagen "Combo" powder can be mixed daily with at least 20 ml of warm water at body temperature per calf dose.

- If calves are feeding and not dehydrated then the mixed ***Rotagen "Combo"*** can be added to the calf's milk e.g. to treat whole pens of calves. The best system for this is where the calf feeder has individual compartments for each calf. Otherwise, mix up sufficient for one day's dosing and use a drench gun and back pack, taking care to agitate the mixture regularly.
- Quarantine dosing of ***Rotagen "Combo"*** to the calves as they are brought into the shed is readily achieved by drenching calves individually with a drench gun using a back pack.
- If the calf is dehydrated then veterinary advice must be given to ensure that adequate rehydration occurs.
- If the calf is moribund, veterinary advice must be given, but take it off milk for one feed and tube feed ***Rotagen "Combo"*** with electrolytes.

Frequently asked questions

The Rotagen "Combo" that I am using does not appear to be working. Why is this?

There are a number of possible reasons for this.

- The most likely reason is that the incorrect combination of immunoglobulins is being used. Even though faeces samples have been taken and a diagnosis made, the diagnosis should always be reviewed. Multiple infections are common, and in the course of an outbreak the aetiology can change. Also, when the initial diagnosis was made, secondary infections can be masked, and become prevalent once treatment for one of the causes has commenced. Examples of this are secondary Salmonella typhimurium or Coronovirus infections following or concurrent with Bovine Rotavirus.
- There is a grossly contaminated environment and the calves are being continually re-infected. Measures need to be taken to reduce the environmental contamination as outlined above.
- When the level of contamination is severe, it is possible that there are insufficient immunoglobulins to inactivate the number of pathogens causing the problem. In this case, the dose rate of Rotagen "Combo" should be increased. The best way to do this is to keep the dose rate the same, but to increase the frequency of treatment. Vetpak have seen this in some severe Cryptosporidia outbreaks. Another option is for Vetpak to make a stronger product containing more immunoglobulins on prescription.

Calves that are treated with Rotagen "Combo" showed improvement but have started scouring again. What is the likely cause of this?

Once again there are a number of possible causes.

- The most likely reason is that there is gross environmental contamination. Measures to control this should be taken, as outlined above.
- The diagnosis should be reviewed by taking more faeces samples.
- If the correct combination of immunoglobulins is used, the dose rate can be increased by increasing the frequency of treatment.

I have a client who has had a problem in the past with calf scours; I know the cause, what can I do in the future to prevent this?

The most practical and cost effective approach is to use the correct Rotagen "Combo" as a preventative. From the start of calving, treat every calf as it comes into the shed. Keep spraying the calf shed with an effective sanitizer and make calf rearing the pleasure it should be.

I have vaccinated a herd against Bovine Rotavirus. I am still getting calf scours. How is this possible?

Vetpak have seen this in the past. For the vaccine to be effective, the timing of vaccination has to be correct. Also this method of protection depends on the calf getting an adequate feed of colostrum. Surveys have shown that 30% of calves do not get adequate colostrum. If the Rotavirus challenge is severe enough, calves will succumb. Remember that one scouring calf in a calf shed is shedding trillions of virus particles, leading to gross environmental contamination. The best solution is take faeces tests to determine the cause, and immediately start treating with Rotagen "Combo".

Review Articles

The following review articles are available:

- Oral passive immunization using chicken egg yolk immunoglobulins against bovine rotavirus and coronavirus infections[i].
- Chicken Egg Yolk Antibodies as Therapeutics in Enteric Infectious Disease: A Review[ii].
- Passive Immunity for Protection against Mucosal Infections and Vaccination for Dental Caries[iii]

Trials

There have been many trials conducted over several years demonstrating the protective effect of administering specific immunoglobulins to a variety of species. These specific antibodies are derived from the yolk of eggs from vaccinated chickens.

General

Egg yolk IgG's can be absorbed.

Chicken egg yolk can be absorbed and transferred[iv] as efficiently as colostral antibodies in the blood of neonatal pigs. The half life is shorter (1.85 days) as opposed to colostral antibodies. Similar to colostral antibodies, egg yolk IgG absorption from the intestine ceased at about 34 hours of age after a logarithmic decrease in absorption rate from birth.

Rotavirus

Passive protection against bovine rotavirus in calves by specific immunoglobulins from chicken egg yolk.

The efficacy of chicken egg yolk immunoglobulins from hens immunized with bovine rotavirus (BRV) serotype G6 or G10 (A & B) for protection against homologous BRV in calves was investigated. A significant protection by anti-BRV yIg having 6400 neutralizing antibody titre per dose was achieved in calves ($P<0.01$)[v]

Field evaluation of chicken egg yolk immunoglobulins specific for bovine rotavirus in neonatal calves

The oral efficacy of chicken egg yolk immunoglobulins (yIg) specific for bovine rotavirus (BRV) serotypes G6 and G10 in protecting neonatal calves was examined in a herd of cattle under field conditions. In one trial yIg treated calves tested under high relative humidity showed a significantly increased body weight ($P< 0.05$) and a decrease in number of calves shedding high titre of BRV (G6) in stools compared to controls ($P<0.01$)[vi]

Cats with Human Rotavirus[vii]

Chickens were immunized with Bovine rotavirus serotype 1 and the egg yolk immunoglobulins were orally administered to specific pathogen free cats. The cats were infected with human rotavirus. Treated cats remained clinically healthy, whereas diarrhoea occurred in the placebo fed cats. Virus antigens were detected in the faeces in all the diarrhoea cases in the placebo fed cats but were only sporadically detected in the immunoglobulin fed cats.

Mice with Bovine Rotavirus[viii]

Chicken egg yolk immunoglobulins against Bovine rotavirus strains 6 and 10 were used for oral passive immunization in suckling mice challenged with Bovine rotavirus. Significant protection resulted compared with controls fed yolk from mock-immunized hens. The titre of infectious Bovine rotavirus antigen decreased with increasing yolk immunoglobulin.

E. coli

Specific *Escherichia coli* egg yolk IgG's are protective in piglets.

Passive protection of neonatal piglets[ix] against fatal enteric colibacillosis was achieved with powder preparations of specific antibodies against K88, K99 and 987P fimbrial adhesions of enterotoxigenic *Escherichia coli*. The anti-K88, -K99, and -987P antibody preparations reacted specifically against the corresponding fimbrial antigens in an enzyme linked immunosorbent assay. Orally administered antibodies protected in a dose-dependent fashion against infection with each of the three homologous strains of *E. coli* in passive immunization trials with a colostrum-deprived piglet model of enterotoxigenic *E. coli* diarrhoea.

Specific *Escherichia coli* egg yolk IgG's are protective in calves.

The protective effects of egg yolk powder[x] prepared from hens vaccinated with antigens from K99- piliated enterotoxigenic *Escherichia coli* (ETEC) were evaluated in a colostrum-fed calf model of ETEC induced diarrhoea. Control calves had severe diarrhoea and dehydration and died within 72 hours of infection. Calves fed IgG containing milk with titres of 1:800, and 1:1600, had transient diarrhoea, 100% survival and good weight gain.

Rabbits with Escherichia coli[xi]

Egg yolk immunoglobulins produced by vaccinating hens with E. coli B16-4 were fed to rabbits for 4 days prior to challenge with the same strain. The rabbits were protected against diarrhoea compared to controls which showed severe diarrhoea within 72 hours. It was demonstrated that in vitro, immunoglobulins from egg yolks interfered with the binding of E. coli to purified small bowel mucins.

Reduced intestinal colonization with Escherichia coli in weaned pigs fed chicken egg antibody against the fimbriae

Newly weaned pigs were fed specific IgG's of *Escherichia coli* against fimbriae F18 in chicken egg powder at high and low levels. They were challenged with enterotoxigenic *E. coli* and compared to controls. Pigs fed IgG's were fully protected.[xii]

Pigs can be protected from clinical disease by feeding IgG's in egg yolk produced by vaccinating hens with F18+Escherischia coli.

Body weight gain, frequency and severity of diarrhoea, and recovery from challenge were measured in this trial[xiii]. Weaned pigs (4 weeks of age) were challenged with virulent $F18^+$ E. coli daily for 3 days, and treated with IgG's daily for 9 days from the first day of challenge. Data shows that pigs can be protected by oral antibody from loss of body condition following challenge. There was reduced frequency, severity and duration of diarrhoea and frequency of excretion of infective E coli in test pigs.

Salmonella

Prevention of fatal Salmonellosis in calves

Compared with controls, calf survival was significantly higher among calves given antibodies with titres of 500 ($P<0.05$) and 1000 ($P<0.01$) for *Salmonella typhimurium*, and 5000 ($P<0.01$) with Salmonella Dublin. Egg yolk IgG's specific for whole cell *S. typhimurium* and *S. dublin* are protective against fatal Salmonellosis when given in sufficiently high concentration, and may be clinically useful during a salmonella outbreak.[xiv]

Mice with Salmonella enteritides challenge[xv]

Test mice treated with SEF-14 antibodies (titre 128) had a survival rate of 77.8% compared to 32% in control mice. In vitro adhesion of Enteritidis to mouse intestinal epithelial cells was reduced by anti-fimbrial antibodies. An indirect immunofluorescence method demonstrated the localization of Enteritides along the villous margins of the small intestine of control mice whereas in test mice, adherent bacteria were not detected.

Mice with Salmonella enteritides and Salmonella typhimuriumxvi

Mice challenged orally with Salmonella enteritides and Salmonella typhimurium were orally treated with yolk antibody three times daily for three days. Mice challenged with Salmonella enteritides and treated with antibodies specific for outer membrane proteins, lipopolysaccharide or flagella resulted in a survival rate of 80%, 47% and 60% compared to 20% in controls. Mice challenged with Salmonella typhimurium and treated with antibodies specific for outer membrane proteins, lipopolysaccharide or flagella resulted in a survival rate of 40%, 30% and 20% compared to 0% in controls.

Coronovirus

Passive protection of calves using Bovine Coronovirus antibodies

The protective effect of egg yolk and colostrum powders was investigated in a challenge model with a virulent bovine coronavirus virus (BCV) strain. All calves were challenged 24 – 36 hours after birth. Treatment groups received egg yolk powder or cow colostrum containing BCV specific antibodies. Control calves had severe diarrhoea and died within 6 days after infection. Calves fed milk containing egg yolk or colostrum with neutralizing titres of 1:2560 or 1:10240 all survived and had positive weight gain unlike the other treatment groups[xvii].

Cryptosporidia

Passive protection of mice against Cryptosporidia parvum[xviii]

The effect of egg yolk antibody against C. parvum infection was examined. Immunoglobulins were prepared from eggs of chickens immunized with C. parvum oocyst antigens. In vitro, antibody-treated sporozoites showed reduced binding to Caco-2 cells, and lost vitality, which was not observed in controls. Mice administered immunoglobulins showed partial reduction in oocyst shedding after challenge.

Regulatory

Registered pursuant to the ACVM Act, 1997, No A9928. See www.nzfsa.govt/acvm for registration conditions.

---

[i] Oral passive immunization using chicken egg yolk immunoglobulins against bovine rotavirus and coronavirus infections. Masahiko Kuroki. Recent Res. Devel. Virol., 1 (1999): 95-106

[ii] Chicken Egg Yolk Antibodies as Therapeutics in Enteric Infectious Disease: A Review. Yoshinora Mine and Jennifer Kovacs-Nolan. JOURNAL OF MEDICAL FOOD. Vol 5, No 3, 2002

[iii] Passive Immunity for Protection against Mucosal Infections and Vaccination for Dental Caries. Shigeyuki Hamada. Mucosal Vaccines (Hiroshi Kiyono et al) Academic Press, Inc. 1996, pp 187-197

[iv] Detection of passage and absorption of chicken egg yolk immunoglobulins in the gastro-intestinal tract of pigs by use of enzyme-linked bioassay and fluorescent antibody testing. Hideaki Yokoyama, *et al*, AMERICAN JOURNAL OF VETERINARY RESEARCH Vol 54, No 6, Pages 867-872, 1993

[v] Passive protection against bovine rotavirus in calves by specific immunoglobulins from chicken egg yolk. M. Kuroki, *et al*. Arch Virol (1994) 138: 143-148

[vi] Field evaluation of chicken egg yolk immunoglobulins specific for rotavirus in neonatal calves. M. Kuroki, Arch Virol (1997) 142: 843-851

[vii] Prevention of Human Rotavirus Infection with Chicken Egg Yolk Immunoglobulins Containing Rotavirus Antibody in Cat. Chikane HIRAGA, *et al*. The Journal of the Japanese Association for Infectious diseases 1990, 64: 118-123

[viii] Passive protection against bovine rotavirus-induced diarrhoea in murine model by specific immunoglobulins from chicken egg yolk. Masahiko Kuroki, *et al*. Veterinary Microbiology, 37 (1993) 135-146.

[ix] Passive Protective Effect of Chicken Egg Yolk Immunoglobulins against Experimental Enterotoxigenic *Escherichia coli* Infection in Neonatal Piglets. Hideaki Yokoyama *et al*. INFECTION AND IMMUNITY, Mar. 1992, p 998-1007.

[x] Protection of neonatal calves against fatal enteric colibacillosis by administration of egg yolk powder from hens immunized with K99-piliated enterotoxigenic Escherichia coli. Yutaka Ikemori, *et al*, AMERICAN JOURNAL OF VETERINARY RESEARCH Vol 53, No 11, Pages 2005-2008, 1992.

[xi] Oral Ingestion of Egg Yolk Immunoglobulin from Hens Immunized with an Enterotoxigenic Escherichia coli Strain Prevents Diarrhoea in Rabbits Challenged with the Same Strain. C. O'Farrelly, *et al*, Infection and Immunity, July 1992, pp 2593-2597.

[xii] Reduced intestinal colonization with F18 positive enterotoxigenic *Escherichia coli* in weaned pigs fed chicken egg antibody against the fimbriae. Armando Zúñiga, *et al*, FEMS Immunology and Medical Microbiology (1997) 153-161.

[xiii] Effect of Oral Egg Antibody in Experimental F18+ Escherichia coli Infection in Weaned Pigs. Hideaki Yokoyama, *et al*. J. Vet. Med. Sci. 59 (10): 917-921, 1997

[xiv] Prevention of fatal Salmonellosis in neonatal calves, using orally administrated chicken egg yolk *Salmonella*-specific antibodies. Hideaki Yokoyama, *et al*, AMERICAN JOURNAL OF VETERINARY RESEARCH, Vol 59, No 4, April 1998 Pages 416-420.

[xv] Passive immunization against experimental Salmonellosis in mice by orally administered hen egg-yolk antibodies specific for 14-kDa fimbriae of *Salmonella enteritides*. R. C. Peralta, *et al*. J. Med. Microbiol. Vol 41 (1994) 29-35.

[xvi] Oral passive immunization against experimental salmonellosis in mice using chicken egg yolk antibodies specific for *Salmonella enteritidis* and *Salmonella typhimurium*. Hideaki Yokoyama, *et al*. Vaccine 1998, **16**: 388-393

[xvii] Passive protection of neonatal calves against bovine coronavirus-induced diarrhoea by administration of egg yolk or colostrum antibody powder. Yutaka Ohta, *et al*. Veterinary Microbiology 58 (1997) 105–111.

[xviii] Effect of egg yolk antibody on experimental *Cryptosporidium parvum* infection in *scid* mice. Chizu Kobayashi, *et al*. Vaccine 23 (2004) 232-235