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Attempts to Induce a Passive Immunity to *Eimeria tenella* in Young Fowls (*Gallus domesticus*)

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Summary. Whole serum or γ globulin derived from fowls, either susceptible or immune to coccidiosis (*Eimeria tenella*), was injected into fully susceptible fowls.

The serum proteins were given by intravenous or intraperitoneal injection and subsequent attempts were made to infect these fowls by giving either a moderate dose of sporulated oocysts *per os* or a suspension of viable merozoites *per rectum*.

In the agar-gel double diffusion test, the serum from the donor birds, resistant to *E. tenella*, formed only weak lines of precipitate when reacting against an antigen prepared from the second schizont stage of the life cycle. However, the immune serum was considered satisfactory because the donor birds on challenge were immune.

Although relatively large amounts of γ globulin were injected into the susceptible fowls (up to 0·88 g. per kg. body weight) and subsequently only mild infections were given, no passively acquired resistance was shown either from the results of the oocyst counts on the faeces or by a macrosopic or microscopic examination of the caeca.

These results are discussed in relation to earlier studies; they show that passively acquired serum antibody at these dose levels did not provide protection.

INTRODUCTION

Tyzzer (1929) was unable to transfer a passive immunity to coccidiosis by giving susceptible fowls injections of blood or serum from convalescent birds by intraperitoneal, intra-rectal or subcutaneous routes. Bachman (1930) was also unable to immunize rabbits against *E. perforans* with serum containing specific precipitins and Becker, Hall and Hager (1932) and Becker and Hall (1933) using whole blood failed to immunize rats passively to *E. miyairii* and *E. separata*.

Pierce, Long and Horton-Smith (1962) and Rose and Long (1962) have shown that fowls can acquire an active immunity as the result of infection with graded doses of oocysts. The birds become completely resistant to infection with *E. tenella* and during the period of immunization precipitins can usually be detected, although their presence was not essential for a solid immunity. Also, previous work has shown that immunity to *E. tenella* actively acquired by one caecum of the young fowl is transferred to the

other, non-infected, caecum (Burns and Challey, 1959; Horton-Smith, Beattie and Long, 1961).

In view of these results it seemed appropriate to make some further attempts to immunize fowls passively by the injection of serum proteins derived from highly resistant fowls. The fowls were challenged with oocysts given *per os* (the number of oocysts was intentionally kept low to avoid an overwhelming infection) or, alternatively, by the intra-rectal injection of second generation merozoites. The subsequent infections were assessed first by the lesions produced and secondly by the total oocyst output when compared with fowls receiving serum from fully susceptible fowls and others which were given no serum injections.

In an effort to increase the amount of antibody passively transferred, in one experiment γ globulin, fractionated from the serum of resistant fowls, was injected into birds which were subsequently challenged with merozoites *per rectum*.

MATERIALS AND METHODS

HOUSING AND MANAGEMENT OF FOWLS

Male and female Rhode Island Red × Light Sussex fowls housed on wire mesh floors were used. The ration was fed *ad libitum* and has already been described (Horton-Smith and Long, 1956).

INFECTIVE MATERIAL

The oocysts used for challenge infections were isolated and maintained as previously described (Horton-Smith and Long, 1959). Merozoites were obtained from second generation schizonts released by grinding up caecal tissue from susceptible birds 5 days after infection with oocysts given orally. The merozoites liberated from the schizonts were washed in phosphate-buffered saline pH 7·2–7·6 and kept at 37°. They were inoculated within 2 hours of preparation. Challenge infections with oocysts were given *per os* and merozoites *per rectum*.

OOCYST COUNTS

The total oocyst production of each group was determined from counts on the total output in the faeces over each 24 hour period (Long and Rowell, 1958).

BLOOD SAMPLES AND SERUM INOCULATIONS

Blood was obtained from either the wing vein or by cardiac puncture, and left at room temperature during the night after which the serum was removed and stored at −20°. Fowls receiving serum were inoculated intraperitoneally or into the wing vein.

ELECTROPHORESIS

Certain sera and fractions of γ globulin were examined electrophoretically in borate buffer pH 8·6 using the Perkin-Elmer version of the classical Tiselius apparatus. Details of the method together with the planimetric analysis and calculation of mobilities have already been described (Pierce *et al.*, 1962).

PROTEIN ESTIMATIONS

Serum protein concentrations were calculated from micro-kjeldahl determination on duplicate samples assuming a protein/nitrogen ratio of 6·25. No allowance was made for non-protein nitrogen.

GLOBULIN FRACTIONATION

The γ globulin fractions used in experiment 4 were prepared from sera derived from fowls susceptible and resistant to *E. tenella*. The sera were dialysed against a solution of 18 g. Na_2SO_4 per 100 ml. H_2O at room temperature during the night. The precipitate was dissolved and redialysed for 24 hours against a solution of Na_2SO_4 at 15 g. per 100 ml. This precipitate was dissolved in phosphate-buffered saline pH 8·0 to approximately a quarter of the original volume of serum and extensively dialysed against 0·85 per cent saline.

SEROLOGICAL TESTS

Sera were examined for precipitins in agar gel by the double diffusion technique of Ouchterlony (1949, 1953). Details of the technique used and the preparations of the schizont antigen have been described (Pierce *et al.*, 1962).

THE PRODUCTION OF NORMAL AND IMMUNE SERUM

Fowls may be actively immunized by giving them respectively 500, 5000 and 50,000 oocysts of *E. tenella per os* at 3, 4 and 5 weeks of age (Pierce *et al.*, 1962). Birds treated in this way were challenged with 100,000 or 200,000 oocysts to prove their immunity, bled 1–2 weeks later for the preparation of the antisera and then challenged again to confirm their immunity. Other fowls, from which the normal serum was derived, were kept free from *E. tenella* infections and their susceptibility shown by a subsequent challenge with 100,000 or 200,000 oocysts; these birds were always found to be highly susceptible.

EXPERIMENTAL PROCEDURE

Fowls 2–3 weeks old received one to three serum or globulin inoculations according to the experiment. The birds were selected at random, weighed and divided into various groups. The order in which the different groups were infected was noted to determine whether this affected the severity of the subsequent infection; this could have significance when infecting with merozoite suspensions which are more susceptible to their *in vitro* environment.

In the text 'immune sera' and 'immune γ globulin' refer to serum proteins derived from fowls fully resistant to *E. tenella* and 'normal serum' and 'normal γ globulin' to serum proteins from fowls susceptible to *E. tenella*.

RESULTS

EXPERIMENT 1

Twenty-four chickens aged $2\frac{1}{2}$ weeks, with a mean body weight of 138 g., were divided into three groups of eight chickens. Group 1 received a single dose of immune serum; group 2, a single dose of normal serum and group 3 were not injected with serum. Three birds from each of groups 1 and 2 received 2 ml. serum intravenously and the remaining five birds in each group 4 ml. serum intraperitoneally.

Although the donor fowls from which this immune serum was derived were shown to

be completely resistant, their pooled serum only gave a single faint band of precipitate when tested against the schizont antigen by agar-gel diffusion (Fig. 1).

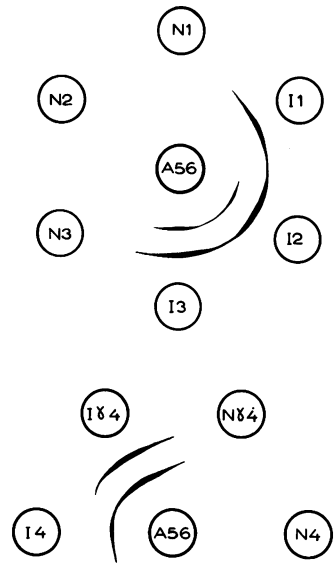

FIG. 1. Line drawings of precipitin bands formed in agar gel by sera or γ-globulin fractions used for experiments 1–4 against schizont antigen.
N=normal serum; I= immune serum; Nγ= normal γ globulin; Iγ=immune globulin; 1–4 refers to experiments 1–4; A56=schizont antigen.

One day after receiving the immune or normal serum all the fowls were infected orally with 5000 oocysts. On the 5th day after the challenge infection two birds from group 3 died, and on the 7th day another died, as a result of acute caecal coccidiosis. Two birds from group 1, one from group 2 and four from group 3 were killed on the 5th day; all were severely infected with *E. tenella*. However, although birds from groups 1 and 2 showed macroscopical lesions of equal intensity, they were somewhat less severe than those of group 3. Histologically, there was no detectable difference in the intensity of parasitism between the caeca from fowls which had received immune or normal serum although fowls from groups 1 and 2 showed many immature schizonts compared with mature schizonts found in the caeca of chickens from group 3.

The total oocyst output of the remaining fowls from 6–14 days after infection is shown in Table 1.

TABLE 1

EXPERIMENT 1. OOCYST PRODUCTION OF FOWLS FROM 6 TO 14 DAYS AFTER CHALLENGE WITH 5000 OOCYSTS

	Group 1			*Group* 2		
Serum injected	Immune*			Normal*		
Route	I.V.	I.V.	I.P.	I.V.	I.V.	I.P.
No. fowls	2	2	2	2	2	2
Volume/bird (ml.)	2	2	4	2	2	4
Total oocyst production ($\times 10^6$)	74	102	91	83	27	119
Total oocyst production/group ($\times 10^6$)		267			229	

I.V.=intravenous; I.P.=intraperitoneal.
* Definition see 'Experimental Procedure'.

EXPERIMENT 2

The immune serum used for this experiment was derived from fowls 7 weeks old and the results of the electrophoretic examination of the normal and immune sera are shown in Table 3 and Fig. 2a.

The electrophoretic pattern showed a major γ-globulin component with a mobility of 2·24 and 2·27 cm.^{2} per V. per second $\times 10^{-5}$ for normal serum and immune serum respectively. A shoulder on the leading edge of this component, particularly apparent in the ascending electrophoretic patterns for both sera, has been included at analysis with the γ globulin (see Fig. 2a).

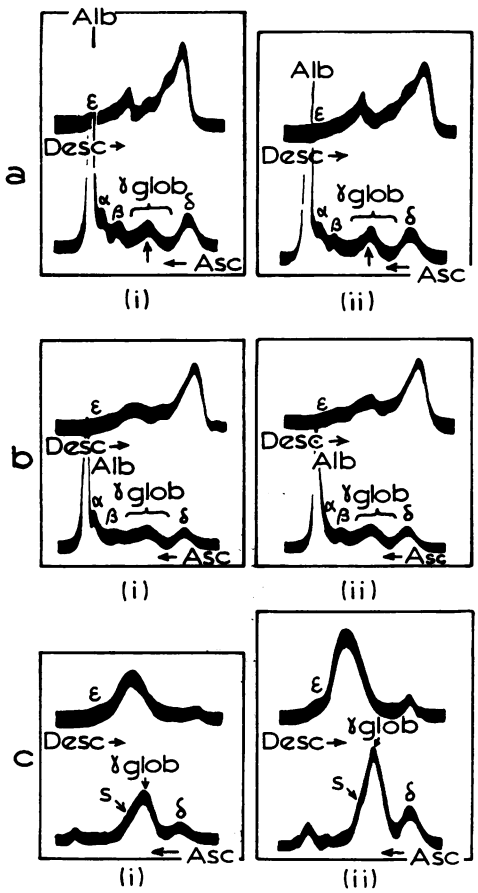

FIG. 2. Electrophoretic patterns at pH 8·6 in borate buffer for serum and γ globulin injected into fowls (see also Table 3).
(a) Experiment 2, (b) Experiment 3,
(i) normal serum; (ii) immune serum.
(c) Experiment 4; γ-globulin fractions precipitated at 15 g. Na_2SO_4 per 100 ml. serum
(i) normal γ globulin; (ii) immune γ globulin (definition, see 'Experimental Procedure').

The heterogeneity of the protein included in the γ globulin at analysis, is shown (a and b); some of this protein may be responsible for the shoulder 's' on the leading edge of the γ-globulin component shown at c (i) and (ii).

The immune serum showed two weak precipitin bands in agar (Fig. 1). As the precipitins have already been shown to be associated with the γ globulins (Pierce *et al.*, 1962) the amount of this component injected is shown also in Table 2. The details of the treatment and allocation of the thirty-four 3-week-old fowls used in this experiment are shown in Table 2.

TABLE 2

EXPERIMENT 2. THE DISTRIBUTION OF FOWLS IN THE DIFFERENT GROUPS AND THE SUMMARY OF THE SERUM INJECTIONS, OOCYST INFECTIONS AND OOCYST PRODUCTION

	Group 1				*Group* 2			*Group* 3	
Serum injected	Immune				Normal			None	
Route	I.V.	I.V.	I.P.	I.P.	I.V.	I.P.	I.P.		
No. fowls	4*	4*	4	4	4	2*	4	4*	4
Volume/bird (ml.)	6†				6†				
Serum protein/bird (g.)	0·20				0·19				
γ globulin/bird (g.)	0·051				0·039				
γ globulin/kg. body wt. (g.)	0·31	0·31	0·31	0·31	0·23	0·25	0·25		
Infection and oocyst production									
Oocyst dose/bird ($\times 10^2$)	50	5	50	5	5	50	5	50	5
Oocyst production/bird 6–14 days of infection ($\times 10^6$)	–	61	–	76	81	–	31	–	84

* Killed and autopsied for histological study 6 days after infection and therefore not available for oocyst production studies.

† Injected 9 and 4 days before oral infection with oocysts.

The chickens of groups 1 and 2 received serum 9 and 4 days before oral infection with oocysts. All the chickens from groups 1, 2 and 3, when they were killed and examined 5 days after a challenge dose of 5000 oocysts, showed haemorrhage and lesions typical of the infection, and of equal magnitude. The oocyst production was determined for four birds in each group which received only 500 oocysts (Table 2).

No evidence was found to suggest that the inoculation of immune serum either intraperitoneally or intravenously had a significant effect on oocyst production. In one subgroup of birds, that received normal serum, the oocyst output was much lower than in the other groups (see Table 2). One bird from this group was found at post mortem to have a retained caecal core which may account for the low oocyst production, i.e. the discharge of oocysts from the caecal lumen into the faeces was prevented by the core.

EXPERIMENT 3

Previous work has shown that fowls, resistant to challenge with sporozoites, are also resistant to merozoites (Horton-Smith, Long, Pierce and Rose, 1963). Also, severe caecal damage and haemorrhage as well as caecal cores can be avoided by giving a low dose which produces only a mild infection in fully susceptible birds. Therefore, any slight effect of the inoculated serum on susceptibility should be detectable.

The immune serum showed two faint precipitin lines when reacted with schizont antigen (Fig. 1). The experiment comprised fifteen 2-week-old chickens with a mean body weight of 102·5 g. The normal and immune serum pools used for this experiment

were examined electrophoretically; the results are shown in Table 3 and Fig. 2b. These sera were inoculated into the different groups of fowls 5 and 3 days before infection with 13×10^6 second generation merozoites inoculated *per rectum*, and, as the result of this type of infection, oocysts will appear in the faeces 24–72 hours later.

TABLE 3

THE ELECTROPHORETIC ANALYSES AT pH 8·6 IN BORATE BUFFER OF THE SERA USED IN EXPERIMENTS 2 AND 3 AND OF THE γ-GLOBULIN FRACTIONS PRECIPITATED FROM FOWL SERUM AT 15 g. Na_2SO_2/100 ml. AND USED IN EXPERIMENT 4

	Serum		*Protein component*				*Total protein* (*g.*/100 *ml. serum*)
			Albumin	*Globulin*			
				α	β	γ	
Expt. 2	Normal	(%)	50·5	17·9	11·0	20·6	
		(g./100 ml.)	1·60	0·57	0·35	0·65	3·16
	Immune	(%)	49·7	16·1	8·8	25·4	
		(g./100 ml.)	1·66	0·54	0·29	0·85	3·35
Expt. 3	Normal	(%)	50·1	16·8	8·9	24·2	
		(g./100 ml.)	1·80	0·6	0·32	0·87	3·59
	Immune	(%)	51·9	13·3	7·6	27·2	
		(g./100 ml.)	1·83	0·47	0·27	0·96	3·52

	Serum		*γ-globulin fraction*		*Total protein* (*g.*/100 *ml. serum*)
			γ-globulin	*Contaminating protein* (*mostly albumin*)	
Expt. 4	Normal	(%)	89	11	
		(g./100 ml.)	3·4	0·42	3·82
	Immune	(%)	87	13	
		(g./100 ml.)	3·5	0·52	4·02

The results, assessed entirely on the oocyst production, suggest that the immune serum did not modify the challenge infection (Table 4). One group which had received normal serum showed a reduced oocyst output (see Table 4). This may be due to the discharge

TABLE 4

EXPERIMENT 3. THE DISTRIBUTION OF FOWLS IN THE DIFFERENT GROUPS AND THE SUMMARY OF SERUM INJECTIONS, MEROZOITE INFECTIONS AND OOCYST PRODUCTION

	Group 1		*Group* 2		*Group* 3
Serum injected	Immune		Normal		None
Route	I.V.	I.P.	I.V.	I.P.	
No. fowls	3	3	3	3	3
Volume/bird (ml.)	6*		6*		
Serum protein/bird (g.)	0·21		0·22		
γ globulin/bird (g.)	0·057		0·053		
γ globulin/kg. body wt. (g.)	0·54	0·58	0·49	0·52	
Infection and oocyst production					
Merozoite dose/bird ($\times 10^6$)	13		13		13
Oocyst production/bird 1–4 days of infection ($\times 10^6$)	0·516	0·698	0·133	1·031	0·997

* Injected 5 and 3 days before rectal infection with merozoites.

of part of the infective material through the cloaca shortly after challenge and is a disadvantage in rectal infection.

EXPERIMENT 4

The experiment comprised fifteen 2-week-old fowls with a mean body weight of 146 g. The amount of γ globulin given was approximately equivalent to that contained in 14 ml. serum. The protein was injected either intravenously or intraperitoneally 5 days and 1 day before infection and also at the time of infection when the birds were challenged by rectal inoculation of merozoites. The details of the treatment, allocation of the birds in the different groups, the amount of γ globulin inoculated and the oocyst production are given in Table 5. The electrophoretic results are shown in Table 3 and in Fig. 2c.

TABLE 5

EXPERIMENT 4. THE DISTRIBUTION OF FOWLS IN THE DIFFERENT GROUPS AND THE SUMMARY OF SERUM INJECTIONS, MEROZOITE INFECTIONS AND OOCYST PRODUCTION

	Group 1		*Group* 2		*Group* 3
γ globulin injected	Immune		Normal		None
Route	I.V.	I.P.	I.V.	I.P.	
No. fowls	3	3	3	3	3
Volume γ-globulin/bird (ml.)	3·5*		3·5*		
γ globulin/bird (g.)	0·12		0·12		
γ globulin/kg. body wt. (g.)	0·84	0·88	0·87	0·84	
Infection and oocyst production					
Oocyst production/bird 1–4 days of infection ($\times 10^6$)	1·56	1·30	0·42	0·53	0·49

* Injected 5 and 1 day before rectal infection with merozoites.

The mobility of the main component in the γ-globulin fraction, from both immune and normal birds, was closely similar to those shown in experiment 2; 2·38 for the normal serum and 2·24 cm.^{2} per V. per second $\times 10^{-5}$ for the immune serum. In the ascending pattern, a shoulder on the leading edge of the major component may be related to the slightly faster and smaller component shown in the patterns for whole sera in Fig. 2 (a and b) which, in those analyses, was included in the γ-globulin complex. The major contaminating protein, when the globulin was precipitated by 15 g. Na_2SO_4 per 100 ml., was serum albumin. However between 87 and 89 per cent of the protein showed the rather fast mobility characteristic of chicken γ globulin. The immune γ globulin fraction reacted with the schizont antigen to give two lines of precipitate in agar gel, although the serum from which the fraction was derived gave only one faint band (Fig. 1). The immune γ-globulin fraction did not moderate the infection; Table 5 shows that the oocyst output was highest in the group of birds which received the immune globulin.

DISCUSSION

Although the natural infection of a susceptible host gives rise to an acquired immunity, serum derived from the actively immune host when injected into a susceptible fowl has failed to confer a passive immunity although large amounts of serum or serum γ globulin,

relative to body weight, were given. The serum proteins have been injected by different routes and at varying times relative to infection; small numbers of oocysts or merozoites have been used to avoid infections which might overwhelm any passively acquired resistance and the immune status of the groups of birds has been assessed by quantitative methods based on oocyst counts in addition to histological examinations.

The results of the unsuccessful attempts to transfer immunity passively by serum support those of Tyzzer (1929), Bachman (1930), Becker *et al.* (1932) and Becker and Hall (1933) in similar studies on coccidiosis infections in fowls, rabbits and rats respectively.

Relevant to these findings, Long and Rose (1962) failed to show a natural transfer of a passive immunity to *E. tenella* from fowls to their offspring, although in other infections, such as Newcastle disease (Brandley, Moses and Jungherr, 1946; Levine and Fabricant, 1950), infectious bronchitis (Jungherr and Terrell, 1948), *Salmonella pullorum*, *S. typhimurium* (Buxton, 1952) and duck hepatitis (Hanson and Alberts, 1960), a passive immunity was shown to be acquired by the newly hatched chick from maternal antibodies absorbed from the yolk.

At present it is not certain at exactly which stage in the life cycle of the parasite the immunity is effective, but actively immunized birds resist oocyst infection and challenge by the intra-rectal injection of second generation merozoites (Horton-Smith *et al.*, 1963). The latter type of infection is mild with little tissue damage in susceptible birds, but was not modified in the present experiments by the passive transference of immune serum.

No evidence has been found to suggest that the lines of precipitate, developed in agar gel by sera from infected birds, are associated with resistance to infection. In fact, birds which have well-developed precipitin lines, as the result of the injection of dead schizont antigen, are fully susceptible to challenge (Horton-Smith *et al.*, 1963). These fowls showed all the lines of precipitate which were present when the sera of naturally infected birds were tested with schizont antigen in agar gel. In addition, the sera of birds resistant to a challenge infection as the result of previous infection frequently failed to show demonstrable precipitins (Pierce *et al.*, 1962).

It was for these reasons that, although the γ globulin has been shown to contain the precipitins, it was not exclusively used in the present experiments. However, neither the injection into susceptible fowls of γ globulin nor other occult humoral factors, which may be distributed among other serum protein, modified the challenge infection produced by various stages of the life cycle of *Eimeria tenella*.

Koshland (1953) showed that the injection of cholera vaccine in adjuvant stimulated antibodies in the serum of the guinea-pig, which were not detected at the surface of the gut mucosa (copro-antibodies). However, when the serum antibodies were injected intraperitoneally into other guinea-pigs, they were detected in the faeces. Therefore, antibodies within the vascular system may give different results on a local infection compared to those given intraperitoneally. These considerations prompted the use of both intravenous and intraperitoneal routes for the injection of the sera. However, serum injected by neither route protected the recipient fowls.

It is interesting to compare the results obtained with *E. tenella* infection in the caecal tissues of fowls with those of Vasington, Laffer, Holst and De Volt (1960) working with Newcastle disease. Fowls with a mean body weight of approximately 560 g. were given an intramuscular injection of 2 ml. of γ globulin containing approximately 22 mg. protein per ml. 1 week before infection. This quantity of protein, mostly γ globulin,

with a mobility within the range observed in the present experiments, was equivalent to approximately 78·6 mg. protein per kg. body weight. There was a 5 per cent mortality in the fowls compared with 30 per cent in those which received no globulin. In the present experiments between 840 and 880 mg. γ globulin per kg. body weight was given with no observable effect on the course of infection developing from a light challenge with *E. tenella*.

Although the immune sera used for these experiments did not precipitate strongly with the schizont antigen, demonstrable precipitins do not appear to be an essential factor for resistance in actively immunized birds (Pierce *et al.*, 1962; Rose and Long, 1962). It was for this reason that the immune status of the fowls donating the immune serum was confirmed by challenging them with a very large infective dose of oocysts immediately after they were bled. Donors for normal serum were similarly challenged and were uniformly susceptible to *E. tenella* infection. Although Burns and Challey (1959) and Horton-Smith *et al.* (1961) have produced evidence that *E. tenella* infection of one caecum protected the other from a challenge dose given at a later date, the present results show that the immunity cannot be conferred passively to susceptible birds by using the stated dosage levels of serum from fully resistant birds.

The availability of humoral antibody in the control of essentially local infection has not been widely considered. Frequently, circulating antibodies (detected by an *in vitro* test) are assumed to distribute themselves through the tissues so that pathogenic organisms at a mucosal surface would experience effective concentrations. This is not true for *Trichomonas foetus* infection at the mucosal surface of the bovine reproductive tract (Pierce, 1959). Although *Eimeria tenella* is invasive it is usually confined to the epithelial and sub-epithelial tissues so that some consideration of the permeability of the caecal mucosa to antibody given by injection and of the concentration of antibody at the site of infection is relevant.

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