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► **To cite this version:**

F.J. Bourne, T.J. Newby, P. Evans, K. Morgan. THE IMMUNE REQUIREMENTS OF THE NEWBORN PIG AND CALF. *Annales de Recherches Vétérinaires*, 1978, 9 (2), pp.239-244. hal-00900996

HAL Id: hal-00900996

<https://hal.science/hal-00900996>

Submitted on 11 May 2020

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THE IMMUNE REQUIREMENTS OF THE NEWBORN PIG AND CALF

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As a result of placental structure and function the newborn pig and calf are born devoid of circulating immunoglobulins and rely on colostrum as a source of circulating antibody during the early part of their life.

The importance of mammary secretion in terms of immune protection of the neonate does however extend beyond this — not only are absorbed colostral immunoglobulins re-secreted onto mucosal surfaces, but the continued ingestion of milk provides a constant source of immunoglobulin for the mucous surface of the gut, affording it protection until the young animal is capable of providing its own.

In terms of immunoglobulin content, the site of formation of immunoglobulins and the response to antigenic stimulation that leads to immunoglobulin formation, both colostrum and milk are ideally fitted to their immunological roles.

The main immunoglobulin of colostrum is IgG, which comprises 80 % of the total, but a 30-fold decrease in IgG concentration occurs during the first week of lactation and immunoglobulin A, which is a minor component of colostrum, predominates in milk. IgM is a minor component of both colostrum and milk (Curtis and Bourne, 1971). It can be seen that a marked change occurs in the immunoglobulin profile of mammary secretions during the early part of lactation; from an IgG to an IgA predominance (table 1).

Comparing the immunoglobulin content of colostrum with serum, and milk with intestinal juice, similarities are apparent which indicate their suitability in performing their very different immune functions.

The complete transition from colostrum to milk takes place over the first week of lactation.

Site of formation of colostrum and milk immunoglobulin

There is evidence from work on a number of mammalian species that IgA is locally produced in the mammary gland. The contribution of locally produced immunoglobulins to pig mammary secretions has been studied by Bourne and Curtis (1973). This work shows that all colostrum IgG is derived from serum and although 60 % of colostral IgA is locally produced this makes up only a small part of the total immunoglobulin content, so consequently colostrum, in the immunological sense, can be regarded as a serum transudate.

The source and site of synthesis of milk immunoglobulins is very different — more than 90 % of IgA and IgM are formed locally in mammary tissue, and milk, immunologically, can be regarded as a true secretion.

Immunohistological studies of plasma cell

Table 1.—Immunoglobulin levels (mg/ml) in serum, colostrum, milk and intestinal juice in pigs.

Biological fluids	Classes of Ig			
	IgG	IgA	IgM	IgA/IgG
Serum	24.3	2.4	2.9	0.1
Colostrum (o h)	61.8	9.7	3.2	0.16
Milk	1.4	3.0	0.9	2.1
Intestinal juice (relative levels)	0.2	2.6	Trace	13.0

(Bourne, 1973)

development and distribution in the mammary gland support the above observations (Brown, Bourne & Denny, 1975). IgA plasma cells predominate at all times but increase in number, along with IgG and IgM producing cells, reaching a maximum at peak lactation. All classes of immunoglobulin occur in the luminal contents of the glands but only IgA and IgM are detected in epithelial cells — these being the cuboidal cells lining ducts, rather than the alveoli themselves. It is possible that this indicates an intracellular secretory route for IgA and IgM as has been observed in the gut (Brown and Bourne, 1976).

Immunoglobulin forming cells are present in the mammary glands of gilts three weeks before farrowing. It is unlikely that these would have developed in response to local antigenic challenge via the teat canal. Recent experimental evidence would suggest that they originate from the gut as stimulated lymphocytes.

Antigenic stimulation of the mammary gland

The influence of vaccination of the dam on immune protection of the neonatal pig will be determined by the effect this has on the antibody content of colostrum and milk. It is necessary therefore to appreciate not only the site of formation of colostrum and milk antibody but also the influence of the route of vaccination of the dam on their production.

A comparison of various routes of vaccination in the pig (Bourne *et al.*, 1975) and in other species (Bienenstock, 1974) indicates that irrespective of route, antibody

activity is found in all immunoglobulin classes in colostrum, milk and serum although quantitative differences do occur. A summary of our own studies is shown in Table 2.

Intramammary vaccination generally leads to a local production of immunoglobulin in both stimulated and non-stimulated glands. Antibody levels in both colostrum and milk are higher than following intramuscular vaccination (Bourne *et al.*, 1975; Chidlow and Porter, 1977) although IgA may not necessarily be the major antibody produced (Bohl *et al.*, 1974); this may be due to a variability in the site of antigen deposition when glands are locally stimulated by routes other than the teat canal, although a quantitative predominance of IgA antibody to an intramammary injected antigen would not be expected in colostrum where IgG is so quantitatively significant.

Studies have recently been published on the successful use of oral vaccination in stimulating a mammary gland response and the work of Cebra (1977) has indicated how a gut-mammary gland link occurs. He suggests that IgA precursor cells are initially stimulated in Peyer's patch tissue from where they migrate, while in the lymphoblast stage, via lymphatics and blood stream to secretory sites; here they mature and synthesise and secrete IgA.

From this work a concept has arisen that all IgA synthesising cells derive from precursors that were originally stimulated in the Peyer's patch (Rowley, 1977) although one should not exclude the contribution that other mucous membrane associated lymphoid tissues (e.g. BALT) might make.

The above concept is supported by the finding that specific IgA antibody secreting

Table 2.—Intramammary vaccination in the sow with a variety of antigens using Freund's Complete Adjuvant.

	Sow No	Antigen	% Specific antibodies		
			G	A	M
Colostrum	1	<i>E. coli</i> K.88	90.4	3.7	5.9
	2	K.88	80.1	13.2	6.7
	3	K.88	91.1	4.5	4.4
	4	Ferritin	74.2	19.4	6.4
	5	Ferritin	70.9	22.4	6.7
Milk	1	<i>E. coli</i> K.88	40.0	22.2	37.8
	2	K.88	39.6	32.1	28.3
	3	K.88	42.6	19.2	38.2
	4	Ferritin	22.9	65.6	11.5
	5	Ferritin	19.6	50.0	30.4
	6	DNP-BGG	23.5	71.8	4.7
	7	DNP-BGG	65.6	4.3	30.1
	8	DNP-BGG	43.2	39.2	17.6

cells are found in human colostrum following colonisation of the mother's intestine with the corresponding serotype of *E. coli* (Goldblum *et al.*, 1975). Similarly live transmissible gastroenteritis (T.G.E.) virus when orally dosed to sows results in the appearance of IgA antibodies to TGE in colostrum and milk (Bohl and Saif, 1975).

Our own studies with oral vaccination also indicate a functional relationship between the gut and mammary gland IgA systems. We are only able to demonstrate IgA antibody, or an increase in IgA antibody, in milk, following oral vaccination, if a concurrent production of antibody occurs in the intestinal tract. We are able to achieve this only when vaccinating with live organisms; dead or non-replicating antigens have not been shown to stimulate a local IgA response in the gut or in the mammary gland.

In initial experiments sows were fed 10^{12} heat killed *E. coli* (08, K.88 positive); 15 doses were fed over a 29 day period after the first week of lactation. No increase in antibody could be detected in serum or milk. Two days after the final dose the sows were slaughtered — intestinal secretions contained no detectable antibody activity.

Following this lactating sows were dosed

with 2×10^{11} live *E. coli* — 20 doses being given over a 30-day period. Low levels of antibody were present in milk prior to dosing; seven days after antigen exposure antibody levels increased and reached a peak 17 days after the start of dosing, remaining at this level until slaughter. Milk antibody was almost exclusively of the IgA class with very low levels of IgM antibody.

Serum antibody had also increased in titre by day 8 and peaked at day 17 — thereafter falling slightly; serum antibody was predominantly IgM and IgG. At slaughter 48 hours after the final oral dosing anti-08 IgA antibody was detected in gut secretions — antibody to this antigen is normally undetectable in gut secretions.

Further studies with *E. coli* antigen have indicated that stimulation of the gut IgA system is dependent upon colonisation of the intestinal tract — sows fed 2×10^{11} live *E. coli* for two days did not always shed organisms in the faeces; in such sows there was no detectable antibody in the gut or mammary gland. Other sows fed the same number of organisms but for 4 days shed organisms in the faeces for 5 days after cessation of dosing — we regard this as an indication of colonisation of the intestine by

E. coli. In these sows IgA antibody appeared in milk, peaking 13 days after the start of dosing and persisting at this level until the end of lactation — 6 weeks after the final dose was given.

Similar results were obtained with young pigs (40-50 kg) fed varying levels of live *E. coli* ranging from 2×10^8 organisms to 2×10^{11} organisms per day. These pigs were dosed for 6 days and slaughtered 48 hours after the final exposure.

In groups fed 2×10^8 and 2×10^9 bacteria per day gut colonisation, as indicated by absence of shedding of *E. Coli*, did not occur and no IgA antibody in gut secretions was demonstrated at slaughter. Groups fed respectively 2×10^{10} and 2×10^{11} bacteria shed organisms in their faeces until slaughter. IgA antibody was detectable in gut secretions of animals in these groups.

These findings plus preliminary results from mouse studies suggest that antigenic load is an important factor in stimulating a gut immune response; colonisation of the intestinal tract with *E. coli* is able to provide the necessary antigenic load to stimulate the IgA system.

Whereas we have so far been unable to stimulate a gut IgA response following oral vaccination with a non-replicating antigen in the pig, we are able to achieve this in the mouse using relatively very much higher levels of antigen.

Antibody absorption by the newborn pig

The intestinal tract of the newborn pig is capable of absorbing colostral immunoglobulin molecules during the first day or so of life and appears to be non-selective — immunoglobulins A (9.5S and 11.6S), G and M all being absorbed.

Peak serum levels are obtained at 24 h of age but there is a great deal of variation between litters and within litters (Curtis and Bourne, 1971).

At 24 h the mean values for IgG concentrations in litters range from 18.7 to 39.0 mg/ml; levels then decrease gradually to a mean minimum value of 6.3 mg/ml at 36-40 d. Immunoglobulin A levels vary from 2.1 to 8.5 mg/ml and decrease rapidly after 24 h to their lowest values at 17-22 days of age. IgM levels varied from 1.1 to 2.0 mg/ml at 24 h and then decreased rapidly to their

Table 3.—IgA/IgG ratios in nasal secretions and serum.

Age (weeks)	IgA/IgG ratios	
	Nasal wash	Serum
1st week (0-7 days)	1.22 ± 0.14	0.07 ± 0.02
2nd week (7-14 days)	0.76 ± 0.21	0.08 ± 0.04

The much higher relative amount of IgA in nasal washings compared to serum is apparent (Morgan, unpublished data).

lowest level (0.6 mg/ml) at 8-14 days of age.

In addition to supplying circulating antibody IgA molecules are selectively redistributed from serum to respiratory tract secretions. After suckling immunohistological techniques have demonstrated IgG, IgA and IgM in the lamina propria of nasal and tracheobronchial mucosa; in addition serous gland cells contained IgA, prior to the appearance of immunoglobulin producing plasma cells, indicating that colostrum derived IgA is selectively transported into respiratory tract secretions from the serum (Bradley, Bourne and Brown, 1976). The great difference in IgA:IgG ratios in serum and nasal secretions confirms that selective secretion of IgA is occurring (Table 3).

Whether the IgA in respiratory secretion is purely 11S in type is not known although the finding that the serum half life of the secretory 11.6S IgA molecules is only 24 hours suggests that this molecule may be more readily selectively transferred from serum than the 9.5S IgA molecule.

Studies in the pig with human colostrum 11S IgA confirm the existence of a secretory route for the 11S molecule. 11S IgA first appeared in serum 2 hours after feeding and in respiratory tract secretions 4 hours after feeding. Peak serum concentration occurred 10 hours after feeding and the serum half life was 15.5 hours.

Immunity in the calf

The immune system of the bovine would appear to be deficient in IgA when compared to other mammalian species — the only exception being respiratory secretions in the

Table 4.—Ratio of IgG₁/IgA in nasal and tracheal secretions in the calf.

Secretions	Week					
	1	2	3	4	5	6
Nasal washing	25.0 ± 12.6	15.7 ± 5.7	10.2 ± 3.8	8.6 ± 2.2	6.1 ± 2.7	4.4 ± 2.7
Tracheal washing	23.7 ± 4.7	22.9 ± 15.7	12.0 ± 3.1	9.9 ± 5.0	4.4 ± 2.8	2.4 ± 1.3

adult; even so IgG₁ producing plasma cells outnumber IgA cells in respiratory tract tissue (Bradley, 1974; Morgan *et al.*, 1977).

The major immunoglobulin of bovine colostrum and milk is IgG₁, which is almost entirely derived from serum (Pierce and Feinstein, 1965). Of the small amounts of IgA, IgG₂ and IgM that are present in colostrum, about 50 % of these are locally produced (Newby and Bourne, 1977) but unlike the situation in the pig and many other mammalian species, local production of immunoglobulin in the mammary gland virtually ceases during lactation.

The functional relationship that exists between the immune system of the intestinal tract and the mammary gland in the pig at all stages of lactation occurs in the bovine only during the period of colostrum formation, the normal lactating gland receiving no stimulation from the gut. That this is so is indicated by the local production of immunoglobulin following local presentation of antigen. Following local stimulation most antibody is found in the IgG₁ class with smaller amounts in IgA, IgM and IgG₂ classes of immunoglobulin. Further evidence that the lactating bovine mammary gland is normally non-stimulated derives from the observation that following local stimulation 90 % of secreted immunoglobulin possesses antibody activity specific to the presented antigen. This massive commitment of the immune system to one antigen suggests that there is little antigenic stimulation from elsewhere (Newby and Bourne, 1977). Histological evidence of plasma cell occurrence and distribution lends further support to this contention since plasma cells are identified in the mammary gland during the latter part of the gestation period and during the immediate post-partum period but disappear during lactation (Campbell *et al.*, 1950).

The possibility of using oral vaccination in

the bovine to stimulate colostrum antibody levels has not been investigated although it would be likely to be effective in stimulating a local production of immunoglobulins that could contribute to colostrum antibody; any influence on milk antibody would be through serum.

The predominance of IgG₁ in intestinal juice and in mammary secretions suggests that in this species it is important in local immunity — a function normally assigned to IgA alone. That this may be so is also indicated by features other than its quantitative predominance. It has been established in the pig that IgA and IgM both possess an intracellular secretory route from the lamina propria to the intestinal lumen that does not exist for IgG. A similar intracellular secretory route occurs in the bovine for IgG₁ but not for IgG₂. An accumulation of IgA but not IgG at the apex of crypt epithelial cells occurs in the pig; similarly IgG₁ but not IgG₂ accumulates at this site in the bovine intestinal tract. A characteristic of mammalian IgA is its ability to resist proteolytic degradation; bovine IgG₁ possesses a similar resistance to proteolysis (Newby and Bourne, 1976).

While there is evidence for an intracellular secretory route for IgA in the respiratory tract of the young calf (Bradley, 1974) and for a redistribution of circulating secretory IgA of colostrum origin to the mucous surface of the gut (Porter *et al.*, 1972) IgG₁ greatly predominates in the respiratory tract of the young calf during the first few weeks of life and is the major immunoglobulin throughout life in the intestinal tract.

The predominance of IgG₁ in respiratory tract secretions is shown in Table 4; at 6 weeks of age IgG₁:IgA ratios decline but do not compare with an IgA:IgG₁ ratio of 13:1 found in the adult (Duncan *et al.*, 1972).

Similarly a high proportion of IgG₁ in intes-

tinal juice is serum derived, the proportion being dependent upon serum levels of circulatory IgG₁ (Newby and Bourne, 1976) and it does seem likely that in the first few weeks of life colostral derived IgG₁ will contribute significantly not only to respiratory tract protection but also to protection of the gut.

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