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SERUM PROTEIN LEVELS IN PIGS  
FROM BIRTH TO MATURITY  
AND IN YOUNG PIGS WITH AND WITHOUT  
ENTERIC COLIBACILLOSIS\*

By

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SVENDSEN, J., M. R. WILSON and E. EWERT: *Serum protein levels in pigs from birth to maturity and in young pigs with and without enteric colibacillosis*. Acta vet. scand. 1972, 13, 528—538. — The changes with age in serum protein concentrations of 29 normal pigs from 3 litters were estimated. Adult serum protein concentrations were reached by 21—24 weeks of age. Significant litter to litter differences in protein concentrations were found in the various age groups.

Serum protein concentrations were determined in 327 pigs at 2 days and 3 weeks of age with and without *E. coli* associated diarrhoea. The gamma globulin concentrations were normally distributed in the 2-day-old pigs and there was no apparent correlation between serum gamma globulin concentrations and incidence of *E. coli* associated diarrhoea.

serum protein levels; pigs; enteric colibacillosis.

Pigs are virtually devoid of gamma globulins at birth; they receive their antibodies from the mammary secretions of the dam during the first 24—48 hrs. of life (*Moustgaard & Højgaard Olsen* 1953; *Lecce et al.* 1964; *Kim et al.* 1966).

*E. coli* associated diarrhoea (enteric colibacillosis) in young pigs can be controlled or prevented through a continuous oral intake of gamma globulins (and thus antibodies) acting locally

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in the intestinal tract (Owen *et al.* 1961; Kohler 1967; Rejnek *et al.* 1968; Wilson & Svendsen 1971).

However, the significance of the serum gamma globulins in relation to *E. coli* associated diarrhoea in pigs is poorly understood. Breitling (1965) reported a hypo- or agammaglobulinaemia present in 17 % of litters from 200 sows and found a higher incidence of various diseases in these litters; Meese (1967) suggested that a defect can occur in the gamma globulin absorption via the intestinal tract in young pigs, thus resulting in a hypo- or agammaglobulinaemia which could give rise to higher incidence of disease in suckling pigs. Similarly, Fey & Margadant (1961, 1962) found that approx. 90 % of colostrum fed calves, which died from colisepticaemia, had hypo- or agammaglobulinaemia. Martinsson (1970), however, compared the IgG levels found in normal pig sera, with the IgG levels found in sera from pigs with diarrhoea or from pigs which died from "septic enteritis"; he concluded that hypogammaglobulinaemia did not increase the susceptibility of pigs to diarrhoea or "septic enteritis".

The purpose of this investigation was to establish normal serum protein levels in swine in this geographical area, and, using the values obtained, to determine whether pigs which developed enteric colibacillosis had lower (or higher) serum gamma globulin concentrations than could be expected in a normal pig population of the same age. Normal values were established in sows, and in pigs up to 6 months of age. Correlation between the incidence of *E. coli* associated diarrhoea and serum gamma globulin concentrations at 2 days and 3 weeks of age was attempted.

## MATERIALS AND METHODS

### *Serum samples*

Four ml of blood was collected from the anterior vena cava of each pig and kept for 2 hrs. at ambient room temperature. Serum was removed after centrifugation of the clotted blood at  $3000 \times g$  for 10 min.; it was stored at 4°C until use. Pigs from 5 herds (1, 2, 3, 4, 5) were bled.

*Herd 1* consisted of approx. 120 sows. The progeny were crosses between the following breeds — Landrace, Yorkshire, English Black and Chester White. Over the past year *E. coli* associated diarrhoea had been diagnosed by post mortem and

bacteriological examinations in pigs up to 3—4 weeks of age. Ninety-four pigs from 11 litters were bled at 2 days and 3 weeks of age.

*Herd 2* contained 50 sows of the Landrace breed. Over the past 2 years *E. coli* associated diarrhoea had been confirmed by post mortem and bacteriological examinations. Forty-seven pigs from 4 litters were bled at 2 days and 3 weeks of age.

*Herd 3* consisted of approx. 90 sows. The progeny was cross-breeds between Duroc and Yorkshire. The herd had a history of *E. coli* associated diarrhoea in the young pigs, but none had been observed during the 6 months prior to bleeding. Fifty-one pigs from 7 litters were bled at 2 days and 3 weeks of age and 29 pigs from 3 litters were bled at 2 days, 3, 6, 9, 12, 15, 18, 21 and 24 weeks of age. The dams of the 3 litters were bled at approx. 2 years of age.

*Herd 4* was a large, specific pathogen free (SPF) herd of Yorkshires. Sixty-nine pigs from 9 litters were bled at 2 days and 3 weeks of age. No significant outbreaks of enteric diseases had been diagnosed on the premises.

*Herd 5.* There were 13 sows in the herd and the progeny was crossbreeds between Yorkshire and Duroc. No signs of enteric diseases had been observed; 37 pigs from 6 litters were bled at 2 days and 3 weeks of age.

#### *Total protein determination*

The Biuret method as modified by *Henry et al.* (1957) was used to determine the total protein concentration in the serum samples; all determinations were performed within 36 hrs. after sample collection.

#### *Electrophoretic fractionation*

Electrophoretic separations were performed on cellulose acetate membranes\* with 0.075 M barbital buffer at pH 8.6. All samples were electrophoresed at room temperature for 20 min. at 100 v. After electrophoresis the membranes were stained with Ponceau-S, rinsed with 5 % acetic acid and cleared with ethyl acetate-glacial acetic acid solutions. The separated protein fractions were quantitated using a Phoroscope Densitometer\*.

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\* Millipore Corp., Bedford, Mass., USA.

*E. coli associated diarrhoea*

The occurrence of diarrhoea was recorded by the owners of herds 1, 2 and 3. Whenever possible, rectal swabs were taken from pigs with diarrhoea for bacteriological examination. Rectal swabs were taken from all pigs with diarrhoea, which were bled at 2 days and 3 weeks of age.

*Statistical analysis*

The arithmetic mean was used to calculate mean and standard deviations. A pig was defined as hypogammaglobulinaemic, if the gamma globulin level was more than 1 standard deviation below the mean value of the entire population. A pig was defined as being hypergammaglobulinaemic, if the gamma globulin level was more than 1 standard deviation above the mean value of the entire population. Student's t-test was used to determine the significance of differences in the protein concentrations observed.

## RESULTS

*Protein concentrations in pigs up to 2 years of age*

Serum protein concentrations in 29 normal pigs and the dams of 3 litters from herd 3 are presented in Table 1.

The gamma globulin level fell precipitously in the period between 2 and 21 days and then rose slowly from 6 weeks of age to reach adult levels by 6 months. The alpha globulin concen-

Table 1. Protein concentrations in sera of pigs from 2 days of age to maturity. Standard deviations in brackets.

Age	Number of animals	Total protein g/100 ml	Albumin g/100 ml	X-protein g/100 ml	Alpha globulin g/100 ml	Beta globulin g/100 ml	Gamma globulin g/100 ml
2 days	29	5.1 (1.0)	1.0 (0.2)	0.2 (0.0)	1.0 (0.2)	1.2 (0.3)	1.9 (0.6)
3 weeks	29	4.9 (0.4)	2.9 (0.3)		0.9 (0.1)	1.9 (0.1)	0.1 (0.0)
6 weeks	29	4.9 (0.4)	3.1 (0.3)		0.9 (0.2)	0.9 (0.2)	0.1 (0.1)
9 weeks	29	5.6 (0.3)	3.3 (0.2)		1.2 (0.2)	0.9 (0.1)	0.3 (0.1)
12 weeks	29	6.6 (0.6)	3.4 (0.5)		1.3 (0.2)	1.1 (0.2)	0.7 (0.2)
15 weeks	29	7.0 (0.6)	3.4 (0.4)		1.4 (0.2)	1.2 (0.2)	1.0 (0.2)
18 weeks	29	6.3 (0.7)	2.9 (0.3)		1.1 (0.2)	1.2 (0.2)	1.1 (0.3)
21 weeks	29	6.9 (0.5)	3.2 (0.3)		1.1 (0.1)	1.5 (0.3)	1.2 (0.2)
24 weeks	8	7.3 (0.4)	3.1 (0.3)		1.3 (0.1)	1.6 (0.1)	1.4 (0.3)
2 years	3	7.7 (0.8)	3.1 (0.3)		1.3 (0.1)	1.9 (0.1)	1.4 (0.3)

trations varied slightly throughout the period of investigation, while the beta globulin concentration started to increase by 18–21 weeks of life. During the first 3 weeks the albumin concentration rose as the gamma globulin concentration fell, after 6 weeks of age the albumin concentration remained stable. The ratio of gamma globulin to albumin concentrations changed from 1:0.04 at 3 weeks to 1:0.45 over 6 months. There were considerable litter to litter differences in the serum protein concentrations, especially in the 6-week and 15-week serum samples. A small fraction, described here as X-protein, was observed between the albumin and alpha region after electrophoresis of 2-day-old pig sera.

#### *Protein concentrations in 2-day-old pigs*

The means and standard deviations of the protein concentrations in serum from 2-day-old pigs in each of the 5 herds studied are shown in Table 2. Significant herd to herd differences in serum protein concentrations were observed in the albumin, beta and gamma globulin concentrations.

Table 2. Protein concentrations in sera of 2-day-old pigs from 5 herds. Standard deviations in brackets.

Herd	Number of animals	Total protein g/100 ml	Albumin g/100 ml	X-protein g/100 ml	Alpha globulin g/100 ml	Beta globulin g/100 ml	Gamma globulin g/100 ml
1	94	5.8 (1.4)	1.1 (0.3)	0.2 (0.1)	0.9 (0.3)	1.4 (0.4)	2.2* (0.7)
2	47	6.6 (1.0)	1.3 (0.2)	0.3 (0.1)	1.1 (0.6)	1.8 (0.3)	2.2 (0.4)
3	80	5.6 (1.2)	1.2 (0.3)	0.2 (0.1)	1.0 (0.4)	1.3 (0.4)	1.9 (0.6)
4	69	5.6 (1.3)	1.4 (0.5)	0.2 (0.1)	1.1 (0.3)	1.3 (0.4)	1.6** (0.7)
5	37	5.7 (1.0)	1.2 (0.2)	0.2 (0.1)	1.1 (0.3)	1.3 (0.2)	1.9 (0.9)

\* gamma globulin concentrations in herds 1 and 2 significantly different from those in herds 3 and 4.

\*\* gamma globulin concentrations significantly different from those in herd 3.

The pigs bled at 2 days of age from the 5 herds were considered representatives of a population of 2-day-old pigs. The serum gamma globulin levels in the 2-day-old pigs appeared to be normally distributed about the mean. Fifty-one (15.6 %) of the pigs were hypogammaglobulinaemic. Five pigs (1.7 %) had a gamma globulin level below 2 standard deviations from

the mean. Eight pigs from herd 1, 2 pigs from herd 2, 11 pigs from herd 3, 19 pigs from herd 4 and 11 pigs from herd 5 were hypogammaglobulinaemic. The means and standard deviations for the serum protein concentrations in hypo- and hypergammaglobulinaemic pigs are shown in Table 3. The total serum protein of hypogammaglobulinaemic pigs was markedly lower (4.5 g/100 ml) than that of the entire population (5.8 g/100 ml). Whereas gamma globulin comprised 34.5 % of the total protein in the serum of all pigs studied, it represented only 20.4 % in the hypogammaglobulinaemic pigs. The percentage of alpha globulin was increased. The percentages of albumin, X-protein and beta globulin were the same as in the entire population.

Table 3. Protein concentrations in sera of 2-day-old pigs with hypo- or hypergammaglobulinaemia. Standard deviations in brackets.

	Number of animals	Total protein g/100 ml	Albumin g/100 ml	X-protein g/100 ml	Alpha globulin g/100 ml	Beta globulin g/100 ml	Gamma globulin g/100 ml
Hypogammaglobulinaemia	51	4.5 (1.0)	1.0 (0.4)	0.3 (0.1)	1.2 (0.5)	1.1 (0.3)	0.9 (0.0)
Hypergammaglobulinaemia	40	7.6 (1.2)	1.4 (0.4)	0.3 (0.2)	1.2 (0.6)	1.7 (0.5)	3.1 (0.4)

Forty-one pigs (12.5 %) were hypergammaglobulinaemic, having both elevated total protein concentrations (7.6 g/100 ml) and a higher proportion of that protein being gamma globulin (40.8 %). Eleven (3.4 %) had a gamma globulin concentration above 2 standard deviations from the mean. Sixteen pigs from herd 1, 3 pigs from herd 2, 9 pigs from herd 3, 5 pigs from herd 4 and 8 pigs from herd 5 were hypergammaglobulinaemic.

#### *Protein concentrations in 3-week-old pigs*

Table 4 shows the serum protein concentration in 3-week-old pigs. Notably, the gamma globulin concentration had fallen to approx. 10 % of the concentration in the serum of 2-day-old pigs. The total albumin concentration increased over the 3-week period by approx. the same amount as the gamma globulin concentration fell.

Table 4. Protein concentrations in sera of 3-week-old pigs.  
Standard deviations in brackets.

Herd	Number of animals	Total protein g/100 ml	Albumin g/100 ml	Alpha globulin g/100 ml	Beta globulin g/100 ml	Gamma globulin g/100 ml
1	80	5.4 (0.8)	2.7 (0.4)	1.2 (0.3)	1.2 (0.2)	0.3 (0.2)
2	47	5.1 (0.6)	2.9 (0.4)	0.9 (0.2)	1.0 (0.2)	0.3 (0.2)
3	86	5.2 (0.6)	2.8 (0.3)	1.0 (0.2)	1.2 (0.2)	0.2 (0.1)
4	60	5.2 (0.6)	2.8 (0.4)	1.3 (0.4)	1.0 (0.2)	0.2 (0.2)
5	35	5.4 (0.5)	2.9 (0.3)	1.3 (0.2)	1.0 (0.2)	0.2 (0.1)
Total	308	5.3 (0.7)	2.8 (0.4)	1.1 (0.3)	1.1 (0.3)	0.3 (0.2)

*Gamma globulin concentrations and E. coli associated diarrhoea*

Ninety of the pigs observed in herd 1, 13 of the pigs in herd 2 and 10 pigs in herd 3 suffered from diarrhoea at some time during their first 3 weeks of life. More than 50 % of the bacteria isolated from rectal swabs collected from herd 1 and 2 were haemolytic *E. coli* whilst these could not be demonstrated in swabs taken from pigs in herd 3.

The gamma globulin levels at 2 days of age in herds with enteric colibacillosis (herds 1 and 2) were significantly different from the gamma globulin levels in herds 3 and 4, but not significantly different from the levels in herd 5. The gamma globulin concentrations in herds 3 and 4, however, were significantly different from each other.

Forty-two pigs from herd 1 and 1 pig from herd 2 had *E. coli* associated diarrhoea when bled at 2 days of life. There was no significant difference between the serum gamma globulin concentrations of pigs with enteric colibacillosis and those without symptoms of this disease within the same herd at 2 days of age.

In herd 1, 37.5 % of the hypogammaglobulinaemic pigs, 50 % of the pigs with normal gamma globulin concentrations and 25 % of the pigs with hypergammaglobulinaemia had *E. coli* associated diarrhoea at 2 days of age.

Seven pigs from herd 1 had *E. coli* associated diarrhoea when bled at 3 weeks of age; their mean serum gamma globulin concentrations were not significantly different from the levels found in the entire population.

## DISCUSSION

Protein concentrations were determined in 29 normal pigs from 3 litters from 2 days to 6 months of age. The same pigs were bled throughout the experiment. When compared with the serum protein concentrations of the dams the results suggest that adult serum protein levels are reached by 21—24 weeks of age. The serum protein concentrations reported here are similar to those reported by others (*Rook et al.* 1951; *Miller et al.* 1961). Significant litter to litter differences in protein concentrations were found in the various age groups.

In an attempt to evaluate the significance of serum gamma globulin levels in relation to *E. coli* associated diarrhoea in young pigs, 327 pigs from 5 herds with and without enteric colibacillosis were bled at 2 days and 3 weeks of age. The serum gamma globulin concentrations followed a normal distribution. Almost 16 % of the 2-day-old pigs had serum gamma globulin concentrations below 1 standard deviation from the mean of the entire population (hypogammaglobulinaemia), 12.5 % had a concentration above 1 standard deviation from the mean (hypergamma-globulinaemia); 1.7 % and 3.4 % respectively, of the pigs were below or above 2 standard deviations.

All pigs with hypogammaglobulinaemia had a lower total serum protein concentration with a lower percentage of gamma globulins than had the entire population, while hypergamma-globulinaemic pigs had increased total serum protein concentration with a higher percentage of gammaglobulins. The presence or absence of *E. coli* associated diarrhoea did not appear to influence these differences and they are probably due to individual variations in the ability to absorb gamma globulins from the intestinal tract or may reflect differences in quantities of gamma globulin ingested by the newborn pig (*Svendson et al.* 1971).

There was no apparent correlation between the serum gamma globulin content and enteric colibacillosis. Forty-three of the pigs bled at 2 days of age suffered from *E. coli* associated diarrhoea at the time of bleeding, but their serum protein values were the same as in the normal 2-day-old pigs from the same herds. Pigs from herds with *E. coli* diarrhoea (herds 1 and 2) had as high serum gamma globulin concentrations as were found in pigs from herds free of the disease. Of the 51 pigs with hypogammaglobulinaemia, only 3 suffered from *E. coli* diarrhoea whereas



of the 40 pigs with hypergammaglobulinaemia, 4 had *E. coli* associated diarrhoea. These findings contrast those of *Breitling* (1965) and *Meese* (1967) who found a higher incidence of disease, including enteritides, in pigs with hypogammaglobulinaemia. — The pigs bled at 3 weeks of age had only 1/7 of the gamma globulin concentration found in 2-day-old pigs (see Tables 2 and 4), but only 7 of the pigs bled had enteric colibacillosis at that time.

The majority of the immunoglobulins, and hence antibodies, migrate in the beta and gamma region of the electrophoretic field, and most immunoglobulins in sera of young pigs are found in the gamma region. It can be concluded from the results presented here that no quantitative relationship exist between serum gamma globulin concentrations and the presence or absence of *E. coli* associated diarrhoea in pigs up to 3 weeks of age. A qualitative relationship might exist, but is unlikely since the 3-week-old pigs have an extremely low serum gamma globulin concentration.

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## SAMMENDRAG

*Undersøgelser over serum proteinkoncentrationer hos grise i alderen fra 2 dage til 2 år og hos 2 dages- og 3 ugers grise med og uden „Enteric Colibacillosis“.*

Ændringer i serum proteinkoncentrationer, der optræder med alderen, blev studeret hos 29 normale grise fra 3 hold. Ved en alder

af 21—24 uger havde afkommet samme serum proteinkoncentrationer, som fandtes hos deres mødre. Der var ofte signifikant forskel i serum proteinkoncentrationerne fra hold til hold indenfor samme aldersgruppe.

Serum proteinkoncentrationerne blev undersøgt hos 327 grise 2 dage og 3 uger gamle med og uden „Enteric Colibacillosis“. Gamma-globulinkoncentrationerne i de 2 dage gamle grise fulgte en normal fordeling, og der var øjensynlig ingen korrelation mellem serum gammaglobulinkoncentrationer og hyppighed af „Enteric Colibacillosis“.

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