

From the Department of Bacteriology and Epizootiology and the  
Department of Animal Nutrition, Genetics and Hygiene, Royal  
Veterinary College, Stockholm, Sweden.

## THE INTESTINAL FLORA IN PIGS WITH PARAKERATOSIS

### V. CLOSTRIDIUM PERFRINGENS ALPHA ANTITOXIN LEVELS IN BLOOD SERUM

By

*Ingmar Månsson*

In earlier papers (*Månsson* 1964, I, II, III) some investigations have been described concerning the intestinal flora in pigs with experimental parakeratosis. It was shown that the composition of the intestinal flora was changed in pigs which developed the disease. By the method used this was indicated by a significant increase in the clostridial component of the flora, particularly of atypical *Clostridium perfringens*. Furthermore, the erythrocyte sedimentation rate was raised, the amounts of serum protein fractions changed (the  $\gamma$ -globulin fraction was particularly increased) and in blood serum antibodies against the atypical *Clostridium perfringens* could be demonstrated. Antibodies have been found by using agglutination and precipitation technique. Though it was possible to determine the serum titres the technique used involved difficulties. The precipitation reactions were doubtful and in the agglutination tests complete clearing of the bacterial suspension was seldom obtained. The occurrence and demonstration of antibodies (in connection with the appearance of the skin lesions) against some of the intestinal microbes is of great importance in determining whether or not they have antigenically influenced the host. The *Clostridium perfringens* microbes are known to produce different antigens (alpha, theta,

kappa, etc), *Oakley* (1943), *Oakley & Warrack* (1953), *Brooks et al.* (1957). Considering that alpha antitoxin can be demonstrated in vitro and that the technique might give more clear cut results it seemed worthwhile completing earlier serological studies by investigating the alpha antitoxin values in serum from the pigs.

### MATERIAL

Serum samples were examined from pigs used in studies on experimental parakeratosis (*Månsson* 1964, I, II, III). By using these serum samples the results obtained could be correlated to other findings which have already been reported. Sera from group I and group III were examined even if a few samples were not available. The pigs in group I developed parakeratosis but the pigs in group III did not. For further details concerning diet, bacteriological and clinical findings and so on, see *Månsson* 1964 I.

### METHODS

The method has in principle been described by *Oakley & Warrack* (1941) and therefore it will only be summarized here. The sera were heated at 50°C for 30 min. Test toxin B 607C *Cl. welchii* dried filtrate\*) without detectable amounts of *Cl. welchii* theta toxin was used. Calciumgelatine saline (Cagsal) was used as a diluent and sheep red cells as indicator.

To obtain an estimate of the amount of alpha antitoxin in the serum the serum samples were first tested in small tubes (Dreyer's tubes). Equal amounts of sample were assayed against various dilutions of the test toxin. If the alpha antitoxin values were greater than 0.1 units per ml serum the sera were retested at 0.01 unit alpha toxin in the following way: 0.5 ml of test toxin (containing 0.01 unit alpha toxin) was placed in test tubes (Lambeth tubes), decreasing amounts of the serum, suitable diluted, were added and the total volume was made up to 1.5 ml with Cagsal. After mixing, the tubes were left at room temperature for 30 min. 0.5 ml of 6 % sheep red cells was added. After shaking (to get a mixture), the tubes were heated at 37°C for 60 min. in water bath and after that were stored at 4°C over night. 20 % hemolysis was taken as end point when the tubes were read.

---

\*) The test toxin has been kindly placed at our disposal by the Wellcome Research Laboratories, England.

## RESULTS

The alpha antitoxin levels (units per ml serum) are given in Table 1 and Fig. 1. As can be seen in Table 1 alpha antitoxin levels between 0.10 and 0.27 units per ml serum were found at the start of the experiment. The pigs in group III were also tested three weeks before the beginning of the experiment. The antitoxin levels at this time varied between 0.13 and 0.51 units per ml serum. During the experiment different results were obtained in the two groups.

*Group I.* On day 9 the antitoxin levels in two pigs, nos. 293 and 302, were 0.77 and 0.65 units per ml serum. Pigs nos. 292 and 299 showed on the 20th experimental day titres of 6.96 and 2.35 units per ml serum. An elevation from 0.23 to 0.71 units per ml serum can also be seen in pig no. 300. On day 26 the antitoxin level in serum from pig no. 292 was 6.27 (this figure not given in Table 1). Pig no. 292 had been given zinc intravenously between day 20 and day 26. This treatment did not seem to have decreased the alpha antitoxin level. From day 26 onwards the antitoxin values in pigs nos. 292 and 299 are reduced. A decrease can also

Table 1. *Clostridium perfringens* alpha antitoxin levels of serum, units per ml.

Pig no.	No of days after the beginning of the experiment				
	1	8	20	63	91
Group I					
292	0.22		6.96	1.74	0.96
293	0.26	0.77			
299	0.26		2.35	0.87	0.44
300	0.23		0.71	1.48	0.96
302	0.29	0.65			
303	0.27		0.68	1.74	1.67
	No of days before (—) and after the beginning of the experiment				
Group III	—21	1	14	28	42
42	0.22	0.27	0.13	0.15	0.10
50	0.41	0.18	0.05	0.05	0.02
51	0.22	0.22	0.15	0.15	0.10
52	0.32	0.05	0.05	0.05	0.02
53	0.26	0.10	0.12	0.13	0.05
55	0.51	0.22	0.05	0.05	0.05

Values below 0.1 unit per ml serum have not been retested at 0.01 toxin unit level.

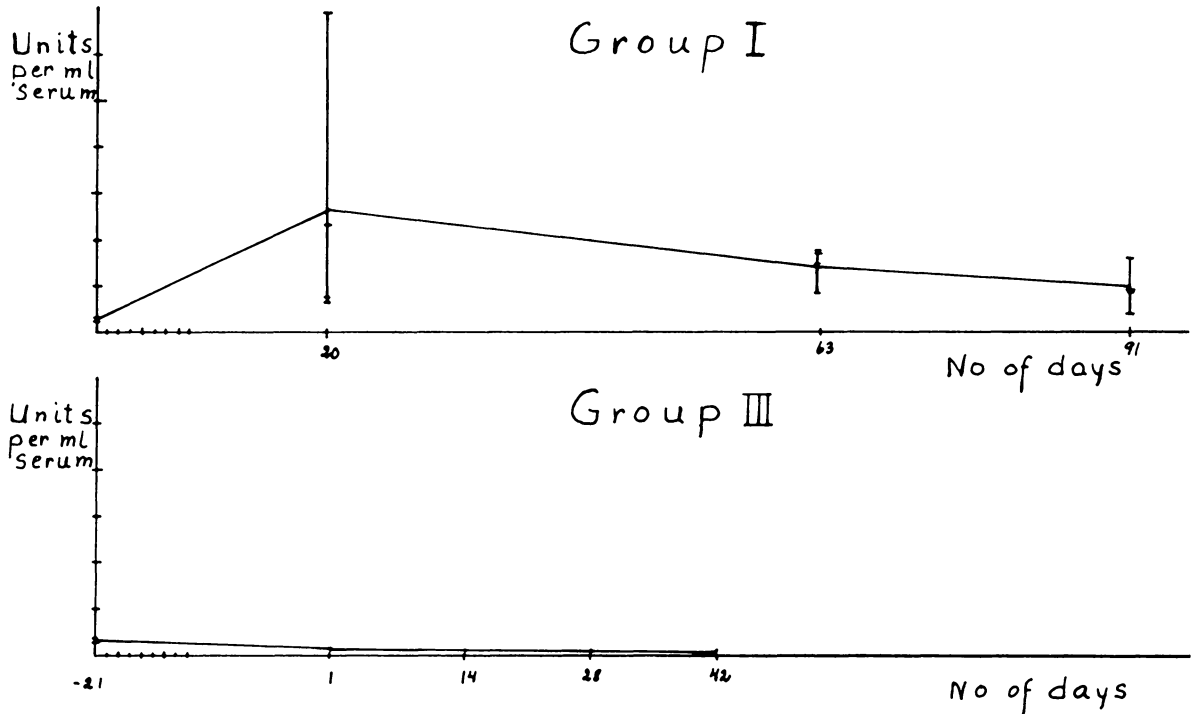


Fig. 1. *Clostridium perfringens* alpha antitoxin levels in serum, units per ml. Group I includes pigs nos. 292, 299, 300 and 303. Group III includes pigs nos. 42, 50, 51, 52, 53 and 55. The curves demonstrate the mean values.

be seen in the values of pigs nos. 300 and 303, if the results for day 63 and 91 are compared. As no sera between day 20 and 63 are available, it has not been possible to determine when — during the experiment — the antitoxin values had reached their peak.

*Group III.* The alpha antitoxin levels have as a rule fallen during the experiment. Details concerning the figures obtained, are given in Table 1.

#### DISCUSSION

The purpose of the investigation was to study the occurrence of alpha antitoxin in serum from pigs with experimental parakeratosis. With the method used it has been possible to demonstrate alpha antitoxin levels in both group I and group III, two groups of pigs which have been studied from different points of view to investigate the composition of the intestinal flora and its

significance in pigs contracting experimental parakeratosis. In *Group I* there is an increase of the alpha antitoxin levels on day 8 in pigs nos. 293 and 302 which at this time were developing acute parakeratosis and were at that day slaughtered for other purposes. The alpha antitoxin levels in pigs nos. 292, 299, 300 and 303 were increased (more or less) on day 20. The increase of the alpha antitoxin values is concomitant with the increase in number of the intestinal *Clostridium perfringens*, the onset of the clinical signs of parakeratosis (less pronounced in pigs nos. 300 and especially 303), the elevation in ESR-values, the changes in the serum protein fractions, and the occurrence of agglutinins etc. in serum (Månsson 1964, I, II, III). The correlation between the alpha antitoxin values and the parakeratotic syndrome is obvious. Intravenous injection of zinc to pig no. 292 between days 20 and 27 (Månsson 1964, I) did not seem to decrease the alpha antitoxin level (6.96 and 6.27 alpha antitoxin units per ml serum).

From day 27 onwards the pigs in group I were treated with zinc orally. The symptoms disappeared and the ESR-values etc. normalized. As has been pointed out (Månsson 1964, I) the zinc therapy does not reduce the number of intestinal *Clostridium perfringens*. The alpha antitoxin values, however, decreased during this period of the experiment. The mere existence of the *Clostridium perfringens* microbes in the bowel does not seem to be enough to keep the alpha antitoxin titres in serum from the pigs. The zinc therapy probably affects the antigenic influence (including formation and resorption of alpha toxin) and/or the antibody response of the host. In *Group III* the values obtained were lowered during the experiment. No alteration of the intestinal flora could be demonstrated, nor did the parakeratotic syndrome develop.

In all pigs in the experiment alpha antitoxin levels were found at the beginning of the experiment. In *Group III* this was observed in samples taken three weeks before the experiment was started. From birth until weaning the pigs have shown no clinical symptoms indicating the occurrence of infections known to give this antitoxin response. It is known, however, that during this period of life pigs develop an intestinal flora rich in *Clostridium perfringens*, but that these microbes decrease in number after weaning (Van der Heyde & Henderickx 1964; and own investigations). Remembering this fact it seems worthwhile studying in more

detail the antigenic influence and the immunological response acquired during this period of life.

From a methodological point of view the determination of alpha antitoxin values have given more clear cut results than the agglutination and precipitation tests. Furthermore, a confirmation of earlier results concerning antibodies against intestinal *Clostridium perfringens* was obtained.

The results argue in favour of a connection between the occurrence and antigenic influence of the intestinal *Clostridium perfringens* and the host. Further studies are, however, necessary to elucidate the immunology of the disease parakeratosis.

#### REFERENCES

- Brooks, M. E., M. Sterne & G. H. Warrack: A re-assessment of the criteria used for type differentiation of *Clostridium perfringens*. J. Path. Bact. 1957, 74, 185—195.
- Van der Heyde, H. & H. Henderickx: Quantitative Untersuchungen der Flora des Verdauungskanals von Saugferkeln. Zbl. Bakt., I. Abt. Orig. 1964, 195, 215—226.
- Månsson, I.: The intestinal flora in pigs with parakeratosis. I. The intestinal flora with special reference to atypical *Clostridium perfringens* and clinical observations. Acta vet. scand. 1964, 5, 279—286.
- Månsson, I.: The intestinal flora in pigs with parakeratosis. II. Electrophoretic studies of blood serum, the erythrocyte sedimentation rate and hemoglobin determinations. Acta vet. scand. 1964, 5, 287—294.
- Månsson, I.: The intestinal flora in pigs with parakeratosis. III. Serological studies of blood serum and some skin tests. Acta vet. scand. 1964, 5, 295—303.
- Oakley, C. L.: The toxins of *Cl. welchii*. A critical review. Bull. Hyg. 1943, 18, 781—806.
- Oakley, C. L. & G. H. Warrack: Factors affecting the activity of the  $\alpha$ -toxin of *Clostridium welchii*. J. Path. Bact. 1941, 53, 335—370.
- Oakley, C. L. & G. H. Warrack: Routine typing of *Clostridium welchii*. J. Hyg. 1953, 51, 102—107.

#### SUMMARY

Pigs which develop parakeratosis acquire increased alpha antitoxin values in blood serum. The determination of the occurrence of alpha antitoxin in serum has confirmed earlier results concerning the presence of antibodies against intestinal *Clostridium perfringens* and has, furthermore, — from a methodological point of view — given more clear cut results than the agglutination and precipitation tests. The observation that even at the beginning of the experiment all the pigs showed alpha antitoxin in serum is discussed.

## ZUSAMMENFASSUNG

*Die Darmflora bei Schweinen mit Parakeratose.**V. Bestimmung der Alpha-Antitoxin-Mengen in Blutserum.*

Bei Schweinen die an parakeratose erkranken sind die Alpha-Antitoxinmengen in Blutserum erhöht. Frühere Untersuchungen über das Vorkommen von Antikörpern gegen *Clostridium perfringens* sind dadurch bestätigt. Das Verfahren, die Alpha-Antitoxinmenge in Blutserum festzustellen, scheint distinktere Resultate als die Agglutinations- und Präzipitationsmethoden zu geben. Schon im Anfang des Versuches vorkommende Alpha-Antitoxin ist diskutiert.

## SAMMANFATTNING

*Tarmfloran hos grisar med parakeratos.**V. Förekomst av alfa antitoxin i serum.*

Hos grisar, som insjuknat i parakeratos, har påvisats förhöjda alfa antitoxin värden i serum. Tidigare gjorda iakttagelser beträffande förekomsten av cirkulerande antikroppar mot *Clostridium perfringens* vid denna sjukdom har därigenom bekräftats. Undersökningen av alfa antitoxin i serum har skett med en metod, som givit klarare resultat än agglutinations- och precipitationsundersökningarna. Det har kunnat iakttagas, att grisarna redan vid försökets början visade positiva alfa antitoxin värden. Detta förhållande diskuteras.

*(Received March 26, 1965).*