Experimental Infection of Newborn Calves with Coccidia and Reinfection after Weaning

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SUMMARY

Four newborn Hereford calves were orally inoculated five times with 100 sporulated oocysts of coccidia, predominantly Eimeria zurnii. Each calf was kept isolated with its dam until weaned at the age of 13 weeks. Three other newborn calves were similarly isolated but not experimentally infected. The calves were then challenged with 300,000 sporulated oocysts at the age of five, seven and nine months. The previously unexposed calves developed marked clinical coccidiosis after the first challenge, but resisted the second and third challenge. The neonatally exposed calves were susceptible to infection at the first challenge as well as to the next two challenges at seven and nine months of age, but the clinical signs following the last two challenges were milder than those of the first challenge. These findings suggest that under conditions where calves become infected with coccidia when very young, such calves may, by shedding oocysts in large numbers for long periods, be a continuing source of coccidial infections to other animals.

INTRODUCTION

It is well known that survivors of coccidial infections become resistant to reinfection with the same species. This resistance appears to be immunologically mediated, but its mechanism is rather poorly understood. In experimental infections of calves with *Eimeria bovis* the development of resistance to clinical coccidiosis has been well demonstrated (1, 6). Fitzgerald (1) has shown that different levels of the initial coccidial infections confer demonstrable but varying degrees of resistance to reinfection with *E. bovis* in calves. In most of his experiments the calves when first infected were several weeks old and the resistance was assessed on the response to one or two large challenge inoculations.

Since calves of the beef breeds remain with their dams for months after birth it is possible for these newborn calves to become infected if the dams had coccidial infections at the time of parturition. The work reported herein was conducted to ascertain the effects of low level neonatal exposure of calves to coccidia on the animals' ability to resist reinfection after weaning.

MATERIALS AND METHODS

Experimental Animals and Housing

Seven pregnant Hereford cows were isolated individually in solid-walled and concrete-floored boxstalls and allowed to calve. The calving period lasted nine days. Each calf was then held with its dam in these separate boxstalls. The boxstalls were cleaned and the straw bedding changed twice weekly. In cleaning, inoculation and sampling procedures, the control animals were handled first and the stable was kept in a sanitary state to minimize possible natural infection with coccidia.

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The calves were weaned when they were approximately 19 weeks old and then stanchioned in an isolation building where they were challenged with coccidia.

Infection of Calves

Coccidial oocysts were obtained from fecal material passed by calves with experimental or natural infections with coccidia. The material was washed through a series of screens and after overnight sedimentation the oocysts were harvested by the method of Jackson (2) and allowed to sporulate at room temperature in 2.5% $K_2Cr_2O_7$ solution in shallow trays. Several lots of oocysts were pooled and mixed thoroughly to form a single batch of washed oocyst suspension. The species distribution of this homogeneous suspension was as follows: *Eimeria zurnii*, 87%; *E. bovis*, 8%; *E. auburnensis*, 2%; and *E. ellipsoidalis*, 3%. Aliquots of this suspension were dispensed in vials and stored at 4°C until needed for initial inoculations and for challenge use.

Four calves were inoculated orally with 100 oocysts by means of a pipette three to 40 hours after birth. The inoculation was repeated four times at intervals ranging from three to 11 days. These calves were termed "exposed". Three other newborn calves were kept isolated as control animals and were designated as "unexposed" individuals.

At five months of age (one week after weaning) each calf was given a challenge dose of 300,000 sporulated oocysts. The oocysts were inoculated directly into the rumen via a stomach tube. This challenge was repeated at seven and nine months of age.

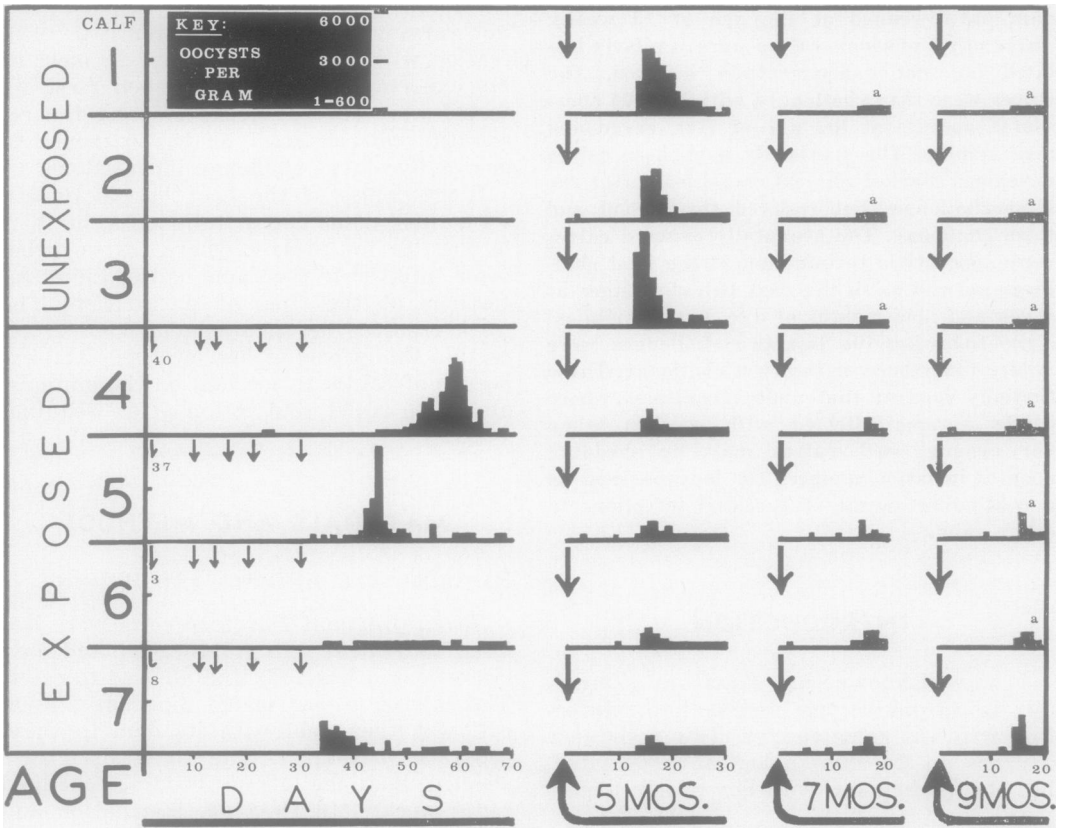

Fig. 1. Inoculation schedules and the results of coccidial oocyst counts of the neonatally exposed calves and of the unexposed calves. The downward arrows indicate time of inoculations. The numbers below the arrows at the extreme left are the ages in hours of the calves when first inoculated.
[a] In these counts at least 50% of the coccidial fauna was composed of species other than E. zurnii. The oocyst counts of calves 1, 2 and 3 at 7 and 9 mos. were less than 60 oocysts/gm although shown as a minimum range of 1—600.

Fecal Examinations

Fecal samples were examined twice in 12 days after the inoculations were started; then daily for 15 days and twice weekly or weekly until the end of the experiments. The feces of the cows were examined weekly. The coccidial oocysts were counted with a McMaster counting chamber.

Intensity of diarrhea in each calf was judged daily by the following scale: slight diarrhea, 1; moderate diarrhea, 2; profuse diarrhea with tenesmus, 3; and diarrhea with blood, 4.

RESULTS

Neonatal Infections

The inoculation schedule and the oocyst discharge in the feces of the calves are shown graphically in Fig. 1. Oocysts were first detected in the feces of calf 4 when it was 23 days of age, 21 days after the first inoculation. The oocyst count was 20 per gram. Oocysts were not detected on days 25 to 44 but were found again at 45 days of age. A peak oocyst discharge occurred at 58 days. In the other three inoculated calves, the oocysts were first detected at 32 to 35 days. The numbers of the oocysts discharged in the feces varied widely. The lowest peak recovery was 290 oocysts per gram of feces at 47 days from calf 6 and the highest peak of 5900 oocysts per gram was counted at 45 days from calf 5. From the 63rd day until weaning, oocyst discharge in the feces of the four inoculated calves became intermittent with counts ranging from one to 38 oocysts per gram. However, no inoculated calf was negative on fecal examinations for a period of more than two weeks. The predominating coccidial species found in the feces was *E. zurnii*, but a small percentage of other species which were originally present in the inoculum were also observed. All the animals remained free of diarrhea and other clinical signs during the period of isolation.

The feces of the three control calves remained negative for oocysts until the 11th week. Then, a few oocysts were detected in calf 2 at 11, 12, 17 and 20 weeks and in calf 1 at 15 and 16 weeks. The highest count was 15 oocysts per gram. The oocysts found were those of *E. bovis* and *E. ellipsoidalis*.

The cows occasionally discharged a few oocysts of *E. bovis*, *E. ellipsoidalis* and *E. canadensis*.

Challenge

The degree and duration of diarrhea of the exposed as well as the unexposed calves following each of the three challenges are shown in Fig. 2. After the first challenge, the previously unexposed animals suffered from diarrhea for a longer period than did the exposed calves. In the next two challenges at seven and nine months of age, the former group did not respond with any clinical sign whereas the neonatally exposed animals experienced mild diarrhea for up to four days.

Oocysts counted in the feces of the calves during the challenge periods are shown in Fig. 1. The highest counts were obtained after the first challenge of unexposed calves 1, 2 and 3 and the lowest counts were observed after the second and third challenge

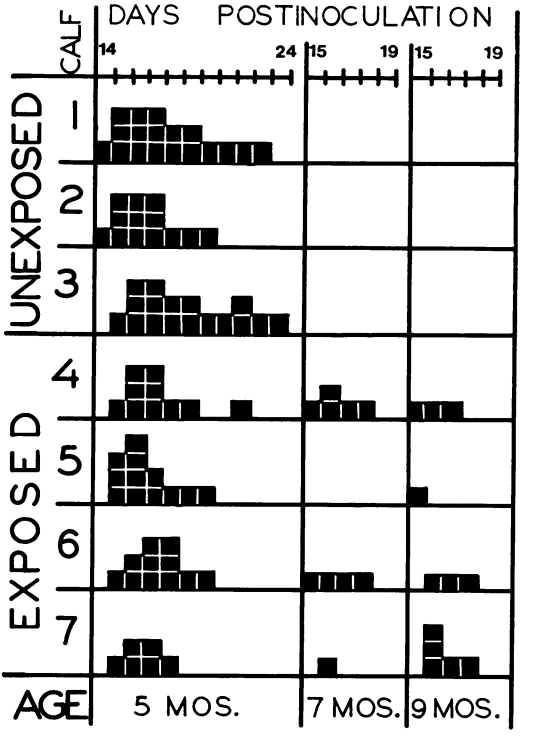

Fig. 2. Representation of degree of diarrhea in unexposed (control) calves and calves exposed neonatally to low level coccidial infection after three challenge inoculations at the age of five, seven and nine months with 300,000 sporulated oocysts. Each solid black square represents a daily assessment of diarrhea as described in the text under "Fecal Examinations."

of these calves. The exposed calves gave a comparable response to all these challenges in terms of oocyst counts.

During the peak oocyst discharge after the first challenge *E. zurnii* formed 75% to 98% of the oocysts present in the fecal samples of all seven calves. Following the next two challenges, the coccidial fauna in the unexposed calves was predominantly other than *E. zurnii*. In the exposed calves after the third challenge, *E. bovis* and *E. ellipsoidalis* combined accounted for more than 50% of the coccidial oocysts present in samples from calves 4, 5 and 6.

DISCUSSION

Fitzgerald (1) reported that calves inoculated continuously with ten, 100 or more oocysts of *E. bovis* per day for up to 62 days developed fair to good resistance to reinfection when challenged with 500,000 oocysts. Calves receiving ten oocysts per day experienced no clinical signs, but those given 100 or more oocysts showed clinical signs of coccidiosis and discharged a large number of oocysts. The degree of resistance to challenge inoculation was related to the number of oocysts given in the immunizing infection. His experiments were conducted on Holstein-Friesian calves and the inoculations started when the calves were between three and four weeks of age. In the present study the previously unexposed calves responded with marked clinical coccidiosis following the first challenge, despite the fact that two of them had minor inadvertent infections during their isolation period. This challenge, which could be viewed as a large immunizing dose for the control calves, had the effect of inducing an excellent resistance against subsequent clinical coccidiosis when challenged again. However, the pattern of challenge response in the neonatally exposed calves suggests that the degree of immunity was insufficient to protect against the development of clinical coccidiosis on repeated challenges.

The immunological behaviour of the neonatally exposed calves may be difficult to classify. The persistence of clinical signs of coccidiosis on repeated challenges, although the oocyst production was only moderate, may be interpreted as a degree of specific immunological tolerance or unresponsiveness. From a practical viewpoint, the immune response was poor.

Kerr and Robertson (4) reported that young calves did not respond with antibody production to injected *Trichomonas foetus* antigen until they were at least one month old. However, Pierce (5) was able to demonstrate antibody production with this antigen within the first week of life in calves deprived of colostral antibody. Smith and Ingram (7), working with three antigens, concluded that acquisition of immunological competence in young calves occurred at different ages depending on the characteristics of the antigens. The interval varied from one day of age for a soluble protein antigen to about one month for a polysaccharide antigen. In neonatal coccidial infections one or two theoretical complications arise: (i) due to the relatively long life cycle of bovine coccidia the parasite antigen may not be immediately available or it may not reach an immunologically-competent host tissue until a week or more after the inoculation. If the host lacks immunological competence, a degree of immune tolerance may be expected; (ii) colostral antibody, if present, may influence the subsequent immune response of the neonatally infected calves.

The challenge of animals with infective parasites as a criterion to measure resistance to reinfection was reported by Soulsby (8). He infected newborn calves with *Cysticercus bovis* and challenged them nine months later with animals first infected at four to six months of age. Those exposed neonatally were unable to resist reinfection. A similar approach has been used to demonstrate immunological unresponsiveness of rats to intestinal parasitism with *Nippostrongylus brasiliensis* (3). Although this procedure may not be sufficiently sensitive to detect minor differences in the immune response, it should serve to recognize a degree of resistance that would be of practical significance in disease control.

On the basis of the results of the study reported herein, one of the practical implications would be that those calves which become infected with coccidia when very young may, by shedding oocysts in large numbers, serve as a source of coccidial infections to other susceptible animals for extended periods.

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REFERENCES

1. **FITZGERALD, P. R.** Results of continuous low-level inoculations with **Eimeria bovis** in calves. Am. J. vet. Res. **28**: 659-665. 1967.
2. **JACKSON, A. R. B.** The isolation of viable coccidial sporozoites. Parasitology **54**: 87-93. 1964.
3. **KASSAI, T. and I. D. AITKEN.** Induction of immunological tolerance in rats to **Nippostrongylus brasiliensis** infection. Parasitology **57**: 403-418. 1967.
4. **KERR, W. R. and M. ROBERTSON.** Passively and actively acquired antibodies for **Trichomonas foetus** in very young calves. J. Hyg., Camb. **52**: 253-263. 1954.
5. **PIERCE, A. E.** Electrophoretic and immunological studies on sera from calves from birth to weaning. II. Electrophoretic and serological studies with special reference to the normal and induced agglutinins to **Trichomonas foetus.** J. Hyg., Camb. **53**: 261-275. 1955.
6. **SENGER, C. M., D. M. HAMMOND, J. L. THORNE, A. E. JOHNSON and G. M. WELLS.** Resistance of calves to reinfection with **Eimeria bovis.** J. Protozool. **6**: 51-58. 1959.
7. **SMITH, A. N. and D. G. INGRAM.** Immunological responses of young animals. II. Antibody production in calves. Can. vet. J. **6**: 226-232. 1965.
8. **SOULSBY, E. J. L.** Immunological unresponsiveness to helminth infections in animals. Proc. 17th World Vet. Congr. (Hannover) **1**: 761-767. 1963.

Book Reviews

ABNORMAL BEHAVIOUR IN ANIMALS. *Edited by M. W. Fox. Published by W. B. Saunders Company, Toronto. 1968. 563 pages; illustrated. Price $21.10.*

Few Canadian veterinarians have had the opportunity to study ethology during their undergraduate years in university. Ethology (the science of animal behaviour) has become increasingly relevant for veterinarians in all facets of veterinary medicine and what was once considered to be armchair philosophy should now be considered to be outmoded when supplanted by the information contained in this book.

Abnormal animal behaviour is frequently observed in all species of animals but how should we interpret this behaviour? Twenty authorities on this subject have contributed a total of 27 chapters on subjects concerning hypnosis, nutrition, neurosis, abnormal sexual behaviour, human-pet relationships, restraint and applications to small and large animal practice. Clinicians will find many clarifications of behaviour syndromes and others will find technical and theoretical material applicable to their specialties.

This book will be of considerable aid in the recognition, treatment and control of problems associated with behaviour in domestic animals. *C. A. V. Barker.*

KLEINTIER-VADEMEKUM (SMALL ANIMAL VADE-MECUM). *K. Schochow. Published by VEB Gustav Fischer Verlag Jena. 1968. 229 pages with 6 illustrations. Price M13,20. (In German).*

This pocket size handbook is written by a small animal practitioner for quick orientation on small animal diseases. The text is written in terse, precise wording and includes many useful hints based on the author's experience.

Part I lists clinical values: determination of age, rectal temperatures, respiratory types and rates and data on reproduction. Part II outlines handling procedures for dogs and cats: positioning of animals for examination, restraining, oral administration and injection sites for drugs. Part III describes examination methods for small animals under field conditions. Examinations requiring elaborate facilities such as x-rays or laboratory tests are not given and the reader is referred to standard textbooks. Part IV comprises the main part of the book describing 787 symptoms and syndromes in alphabetical order with many cross references. Therapy is given for most minor diseases. In the more complex diseases the reader is advised to consult appropriate textbooks. A table of symptoms in part V further facilitates rapid orientation.

An addendum describes handling and examination of pet birds and lists 87 symptoms and syndromes of these, most of them with suggestions of therapy.

The book will be exceedingly useful to students and to practitioners who are only occasionally called upon to treat small animals. It is not intended for those specializing in small animal medicine. — *F. Tittiger.*