groups dosed on day 51 and their controls. At least six days elapsed between dosing and slaughter of individual pigs.

After slaughter the stomach contents and washings were made up to 3 litres with water and representative 3 per cent samples abstracted. The stomach mucosa was then scraped off and digested for eight hours with pepsin/hydrochloric acid at 38°C and washed through a 150 µm sieve to salvage any *Hyostrongylus* species remaining. The small intestine contents were sieved to retrieve *Ascaris* species and the whole of the contents of the large intestine, except the last foot of rectum, were made up to 3 to 6 litres with water and 2 per cent or 3 per cent samples extracted for *Oesophagostomum* species worm counts depending upon the amount of material involved.

Results

The results of the trial are displayed in Tables 1 and 2. In addition 56 *A suum* were recovered at slaughter from one pig in the first group of controls, one and 13 from two pigs dosed at 3 mg per kg on day 20 and two more from one pig dosed on day 20 with 4·5 mg oxfendazole per kg.

A dose rate of 3 mg oxfendazole per kg almost completely removed L4, L5 and adult *Oesophagostomum dentatum* and *O quadrispinulatum* which were present in a 4:1 ratio in the controls. L5 and adult *H rubidus* were equally susceptible. There was a fairly flat dose response over the 3 to 6 mg per kg range against the less susceptible L3 *Oesophagostomum* species and L3 and L4 *Hyostrongylus* species, ranging from about 78 to 93 per cent in the case of the former and from about 60 to 83 per cent in the case of the latter. The infestation levels with *Ascaris suum* were too poor to give useful results. The only indications were that 20-day-old stages were not completely susceptible to dose rates as high as 4·5 mg oxfendazole per kg.

The medicated meal was eaten without hesitation over a period ranging from 20 minutes to two hours.

Discussion and conclusions

The results of this trial confirmed the 100 per cent efficacy of oxfendazole against oesophagostomum adults already published by Corwin (1977) and Corwin and others (1979) in the USA. These authors also reported 100 per cent efficacy against adult ascaris and 86 to 100 per cent against adult metastrongylus with a 3 mg per kg dose rate of oxfendazole. The efficacy shown in Table 2 against immature hystrongylus was inferior to that found by Connan (1978) over a similar dose range on days 5, 10 and 16 after infection but was better against immature oesophagostomum, probably because no evidence of asyuchronous development was experienced.

It was concluded that a dose rate of 4·5 mg oxfendazole per kg provides adequate anthelmintic for treatment of adult and immature hyostrongylus and oesophagostomum infestations. This dose rate would be only slightly less efficacious against immature parasites if food intake by a few pigs in a group were reduced by up to one third. More experience is needed to establish the efficacy of oxfendazole against immature ascaris in pigs.

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Failure to isolate rotavirus from bovine meconium

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In order to ascertain whether rotavirus could pass through the placenta and infect the bovine fetus in utero, meconium from 66 newborn calves was examined for the presence of rotavirus using counter-immunoelectroosmophoresis, immunofluorescence and virus isolation on cell culture. No rotavirus could be isolated or detected.

DIARRHOEA is an important condition when it affects neonatal calves and its aetiology appears to be more and more complex. Rotavirus is often detected in diarrhoeic faeces but its pathogenic action is not fully understood since healthy calves excrete the virus (McNulty and others 1976, Scherrer and others 1977, Bégin and others 1978). McNulty and coworkers (1976) reported excretion of rotaviruses in two calves as soon as 48 hours after birth. They wondered if the infection was due to contamination by the mother's faeces or to a transplacental passage of the virus. There remain several obscure points in the source of infection and, in order to verify the hypothesis of transplacental passage of the virus, 66 meconiums of newborn calves were examined for the detection of rotavirus.

Materials and methods

Source of meconiums

Forty-three meconiums were collected from calves born in the faculty of veterinary medicine of the University of Liège (divisions of genetics, obstetrics, bacteriology and the clinic of large animals), mainly after caesarean section.

Twenty-three other meconiums were taken from farms where numerous calves were regularly suffering from diarrhoea associated with rotavirus infection (Pastoret and others 1977, 1978). The samples were taken from calves born by caesarean operation, immediately after the surgical intervention, and were provided by Dr Vanesse.

Rotavirus antigen and antibody

Two cultivated strains of rotavirus were used as controls; the American strain from Dr C. A. Mebus of the University of Nebraska and the Quebec strain from Dr M. E. Bégin of the University of Montreal, St-Hyacinthe, Québec. Also, four diarrhoeic faeces (Dr H. Antoine, Laboratoire de Virologie, Marloie, Belgium), found positive by immunodiffusion and counter-immunoelectroosmophoresis (CIEOP), were tested.

The sera used for the CIEOP and immunofluorescence techniques were rabbit antibovine rotavirus antisera kindly supplied by Professor Reginster (University of Liège) and Dr H. Antoine (Marloie) respectively.

Cell culture

Rhesus monkey kidney cells (MA 104), provided by Dr

Bohl (Ohio, USA) were grown in Eagle's minimal essential medium (MEM) supplemented with 0·086 per cent sodium bicarbonate, penicillin 100 units per ml, streptomycin 100μg per ml and 10 per cent fetal calf serum.

Treatment of meconium samples

All the meconiums were diluted in 5 to 10 volumes of distilled water, thoroughly homogenised and centrifuged at 10,000*g* for 30 minutes at 4°C. Then the supernatants were submitted to two different treatments; concentration of the antigens with polyethylene glycol 6000 (PEG 6000) or trypsin treatment for inoculation on cell culture. These two types of treatment will be described later.

Counter-immunoelectroosmophoresis

The CIEOP test was carried out according to Middleton and others (1976). Reference serum was diluted 1 in 5. Usual controls were included; the American and the Quebec strain of rotavirus and the supernatants of four stools of positive faeces. Negative controls consisted of buffer alone.

To increase the sensitivity of the CIEOP test the samples were concentrated with PEG 6000 as described by VanOpdenbosch and others (1978) and Dagenais and others (1980). One millilitre of a solution of 70 per cent PEG 6000 was added to 9 ml of the clarified faeces and thoroughly shaken. The mixture was then incubated at 4°C overnight and centrifuged at 40,000*g* for 30 minutes at 4°C. The pellet, resuspended in 0·05 ml phosphate buffered saline (PBS), was tested by CIEOP. The gels were read after fixation in 1 per cent tannic acid.

Virus isolation in cell culture

Virus isolation was performed on confluent MA 104 cells in microtitre plates. For this method, as for the immunofluorescent test, the clarified supernatants of the meconiums were treated with 500μg trypsin per ml as described by Babiuk and others (1977). A volume of 0·1 ml of the treated stools was inoculated in duplicate on MA 104 cells and allowed to adsorb for one hour at 37°C. After that, MEM containing 2μg trypsin per ml was added. Plates were read up to four days after inoculation under light microscopy. Three passages were done. The supernatants of the third passage were tested by CIEOP for the presence of rotavirus. Cell controls and positive controls with reference strains and the four Belgian isolates were included.

Direct immunofluorescence

MA 104 cells were grown in Lab-teks chamber slide cultures and inoculated by the technique described above. Thirty-six hours after infection, the slides were fixed with isopropanol at 4°C for 10 minutes, dried and incubated with the antirotavirus conjugate for 30 minutes at 37°C. This step was followed by a wash of 30 minutes in PBS pH 7·2 and a rapid rinse in distilled water. After drying and mounting, the slides were examined with an ultra-violet microscope. Positive controls were cells infected with reference rotaviruses and negative controls were uninfected cells.

Results

Counter-immunoelectroosmophoresis

Some samples of clarified meconiums, tested by the CIEOP method using a rabbit antibovine rotavirus serum, gave doubtful results. But, when concentrated with PEG 6000, none of them gave a precipitating band.

With the two reference rotaviruses, the American and the Quebec strains, a clear precipitating line was observed. The four faeces from diarrhoeic calves gave the same results. Controls with buffer alone and fetal calf serum were negative.

Virus isolation in cell culture

No cythopathic effect was observed during the three blind passages with meconiums. The reference rotaviruses and the four Belgian isolates showed a cytopathic effect characteristic of rotavirus from the second passage.

Positive bands were obtained by CIEOP only with the supernatants of cell cultures inoculated with the third passage of reference rotaviruses strains and the four Belgian isolates, but none with the meconium samples.

Direct immunofluorescence

No fluorescent cells were observed when inoculated with the 66 meconiums. Positive reactions were obtained with the two reference rotaviruses and the four Belgian isolates. Uninfected cells were negative.

Discussion

From these results rotavirus particles do not appear to cross the placental barrier during the gestation period in the bovine species since no rotavirus was detected in the 66 meconiums of newborn calves examined. Our observations agree with those of Wellemans and others (personal communication). This seems to be logical since up to now no viraemia had been described with rotavirus. Viruses are restricted to the digestive tract and circulating antibodies do not influence the development of the illness (Pastoret and Schoenaers 1977).

Another way to ascertain if transplacental passage occurs is to search for specific antibodies in newborn calf serum collected before the colostral intake. We were not able to find reports of the presence of significant quantities of antirotavirus antibodies in newborn calf serum although the bovine fetus is perfectly able to produce an immune response to rotavirus antigens from the 127th day of intrauterine development (Schlafer and others 1979).

It would be more likely that calves are contaminated after birth probably from the mother's faeces or from other sick calves (VanOpdenbosch and others 1979). Excretion of rotaviruses by adult cattle has been reported (Moreno-Lopez 1979, Woode and Bridger 1975) and we observed it using the CIEOP test (unpublished data). In humans, child-mother transmission had been suggested by Zissis and others (1976).

Rotavirus epidemics are usually a result of poor hygiene. VanOpdenbosch and others (1979) noticed that the incidence of rotavirus diarrhoea could be reduced if certain hygienic precautions were taken just after birth and if the calf was isolated afterwards. On the other hand, Lecce and coworkers (1978) concluded that the frequency of diarrhoea due to rotavirus in piglets was directly related to the pressure of infection which depended on hygienic procedures.

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Seasonal and age-related changes in semen quality and testicular morphology of bulls in a tropical environment

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The spermiogram of 10 mature bulls, ranging in age between three and 10 years and comprising five indigenous (*Bos indicus*) and five exotic (*Bos taurus*) bulls, was studied for 12 months. There were no significant seasonal variations in sperm cell concentration, percentage live sperm cells and sperm cell abnormalities in the indigenous bulls but the exotic breed showed significant seasonal fluctuations with higher sperm cell abnormalities, lower percentage live-sperm cells and lower sperm cell concentration during the hot periods. In both groups the sperm cell concentration was significantly higher in the relatively younger, mature bulls (three to seven years) than in the older bulls (seven-and-a-half to 10 years). Histological study of the testes of 240 indigenous bulls over the same period showed that percentage spermatogenesis was lower in the older than in the younger bulls. This lower sperm output was associated with degenerative changes in the seminiferous tubules. It is suggested that thermal stress in the tropics has significant adverse effects on spermatogenesis only in bulls of non-indigenous breeds. Senile testicular degeneration could be a significant factor in the low reproductive performance of the local bulls.

AGE-related changes in the testes of *Bos taurus* bulls have been documented (McEntee 1958, Collins and others 1962, Bishop 1970). These investigators observed that permanent and progressive senile atrophy or degeneration of the testes occurs fairly frequently in all species without indication of its pathogenesis. It was further observed that aged bulls, seven to 13 years old, had a lower sperm output per ejaculate than younger bulls, two to six years old (Hahn and others 1969).

A seasonal pattern in spermatogenesis has been observed. The so called "summer sterility" in bulls in temperate climates is related to season (Roberts 1971). A seasonal reproductive pattern has been reported from tropical and subtropical regions in East African and South American cattle (Wilson 1946, Carneiro 1950).

There is scanty information on the seasonal and age-related changes in spermatogenesis of both indigenous (*Bos indicus*) and exotic (*Bos taurus*) bulls under Northern Nigerian (tropical) conditions. The lack of such basic information has been a major obstacle to any programme aimed at improving the low reproductive performance of the indigenous cattle. This study was conducted to document seasonal and age-related changes in the testes and to assess the importance of such changes to the problem of infertility.

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Materials and methods

SECTION A

Five indigenous (*Bos indicus*) and five exotic (*Bos taurus*) sexually mature bulls, ranging in age from three to 10 years, were studied for a period of 12 months from January to December. The indigenous breed comprised five Sokoto Gudali bulls aged four to nine years. The exotic group consisted of three South Devon bulls aged three to seven years and two Friesian bulls aged five to 10 years. Age was determined from birth records. The management was such that, in addition to daily grazing (0900 hours to 1500 hours approximately) on improved pasture, bulls were given mineral licks, hay and concentrates (guinea corn and groundnut cake). The animals were housed in individual pens at night.

Physical examination which included palpation of the scrotum and rectal palpation of the pelvic genitalia was carried out twice a month. Semen was collected twice a month by electroejaculation (South Devon) and/or artificial vagina (five Sokoto Gudali and two Friesian). Semen evaluation was done by the method of Zemjanis (1970).

SECTION B

Clinical and anatomical studies of 240 Sokoto Gudali bulls were carried out for the same period of 12 months in local abattoirs. The ages of the bulls were determined by tooth eruption patterns and the animals were assigned to the following groups: A—one to three years; B—three-and-a-half to seven years; C—seven-and-a-half to 12 years. Twenty bulls were studied every month for 12 months.

Before slaughter, clinical examination, including palpation of external genitalia and rectal palpation of pelvic genitalia, was carried out. Only bulls with apparently normal genitalia were selected. Soon after slaughter the entire genitalia were removed and closely examined for gross abnormalities.

The testes and epididymides were removed by cutting the spermatic cord level with the most proximal part of the caput epididymides. The combined testis and epididymis was weighed on a spring balance. Transverse sections (2 mm thick by 2 mm long) of both testes and epididymides were then fixed in Bouin's solution for 36 hours. The tissues were processed in a routine manner for light microscopy and stained with haematoxylin and eosin, periodic acid Schiff and van Gieson stains. The sections were examined at a magnification of × 450 and the presence of histopathological lesions—interstitial and tubular degeneration, atrophy, fibrosis, calcification—was recorded. Spermatogenic activity was assessed by the presence of the germinal cells at various stages of spermatogenesis. To determine percentage spermatogenesis 40 tubules per slide were selected. Percentage spermatogenesis was determined by the number of tubules per 40 tubules containing spermatids under low power mag-