



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

TRANSMISSIBILITY OF THE CONTAGIOUS EQUINE METRITIS ORGANISM FOR THE CAT

P. J. TIMONEY*, S. J. SHIN, D. H. LEIN and R. H. JACOBSON

Diagnostic Laboratory, New York State College of Veterinary Medicine, Cornell University,
Ithaca, NY 14850, U.S.A.

Abstract—A group of SPF cats were moderately susceptible to the causal organism of contagious equine metritis (CEM) following intra-uterine or intrapreputial challenge with an Irish streptomycin resistant strain isolated from a clinically infected mare. Subclinical infections were established in only 50% of the cats, none of which became long-term carriers of the organism. Cytological examination of vaginal smears was of no diagnostic value in confirming infection in inapparently infected cats. Bacteriological responses after primary or secondary challenge with the CEM organism were essentially similar, with one exception, a female cat in which there was possible evidence of local immunity persisting after the primary infection. Efforts to reactivate shedding subsequent to the immediate post-challenge period were unsuccessful. Throughout the experimental period, the cats remained sero-negative to the complement-fixation test, and they failed to develop any significant increase in the levels of antibody activity as measured by the kinetics-based ELISA or KELA system. On day 89 after primary challenge, the cats were euthanized and various sites in the genitourinary tract and the internal iliac lymphatic glands subjected to bacteriological and pathological examination for evidence of CEM infection with negative results.

The findings of this study, although establishing the transmissibility of the CEM organism for the cat, demonstrate the limited value of this species as an experimental model system for the disease in the horse.

Key words: Contagious equine metritis, experimental transmission study, cat

TRANSMISSIBILITE DU GERME DE LA METRITE CONTAGIEUSE EQUINE CHEZ LE CHAT

Résumé—Un groupe de chats SPF s'est montré modérément sensible au germe de la Métrite Contagieuse Equine (EMC), après avoir été contaminé par voie intra-utérine ou par inoculation dans le fourreau avec une souche irlandaise résistante à la streptomycine et isolée d'une jument présentant des signes cliniques de métrite. Une infection inapparente n'a été établie que chez 50% des chats, aucun de ceux-ci n'étant devenu porteur chronique. L'examen cytologique de frottis vaginaux n'est d'aucune valeur pour confirmer l'infection inapparente des chattes. Les réponses bactériologiques suivant la première ou la deuxième inoculation du germe EMC étaient comparables, à l'exception d'une chatte pour laquelle une immunité locale s'est installée après la première inoculation. Les tentatives de réactivation immédiatement après l'inoculation n'ont pas réussi. Durant toute l'expérience, les chats ont présenté une sérologie en fixation du complément négative et il n'y a eu aucune élévation significative des titres en anticorps détectés par les réactions KELA. 89 jours après la première inoculation, les chats ont été sacrifiés et différents prélèvements du tractus génito-urinaire et des ganglions lymphatiques iliaques internes ont été soumis aux examens bactériologiques et histologiques pour rechercher l'infection par l'EMC sans résultat.

Les résultats de cette étude, bien qu'établissant la transmissibilité de l'organisme responsable de l'EMC au chat, démontrent l'intérêt limité de cette espèce comme modèle expérimental de cette maladie équine.

Mots-clefs: Métrite Contagieuse Equine, Etude de transmission expérimentale, chats

* Present address: Department of Veterinary Science, University of Kentucky, Lexington, KY 40546-0076, U.S.A.

INTRODUCTION

Various studies have been undertaken to establish the natural and experimental host range of the causal organism of contagious equine metritis (CEM). The latter is a transmissible venereal disease of the horse, first reported in Britain and Ireland in 1977 [1, 2] and shown to be caused by a gram-negative coccobacillus [3, 4] which still awaits taxonomic classification [5]. The horse remains the only species in which spontaneous disease with this organism has been reported. Whereas CEM has been experimentally reproduced in donkey mares [6], cattle, sheep and pigs have been refractive to experimental challenge [7]. Limited subclinical infections have been established in mice, rabbits and guinea-pigs, with mice the most susceptible of the three species examined [8, 9].

As part of a long-term study of the biology of the casual agent of CEM, and in an effort to obtain a suitable model system of the disease in the horse, a group of young adult domestic cats were investigated for their susceptibility to this organism. Clinical, bacteriological and serological responses were monitored after primary and secondary challenge with CEMO. Persistence of CEMO in the genital tract and the possibility of associated pathological changes were investigated.

MATERIALS AND METHODS

Cats

A group of 9 to 11 month-old male and female SPF cats were kept in individual isolation cages under controlled environmental conditions. All were culture negative for CEMO on three successive intrauterine/cervical/clitoral area or preputial swabbings. Before challenge, the female cats were synchronized in oestrus with oestradiol cypionate (Estradiol Cyp 5; Interstate Drug Exchange, Plainview, NY) after the method of Lein and Concannon [10].

Bacterial strain

The test strain of CEMO was an Irish streptomycin resistant isolate (77/T/M12) cultured from the vaginal discharge from a mare in the acute phase of CEM and passed once on Eugon chocolate agar (Baltimore Biological Laboratories, Cockeysville, MD) and once in Robertson's cooked meat medium (Oxoid, London, England). Cultures were incubated at 35.5°C in 5% carbon dioxide in air in a humidified CO₂ incubator.

Challenge inoculum

The experimental inoculum was prepared from a 48-h growth of CEMO on Eugon chocolate agar. Growth was suspended in phosphate buffered saline, pH 7.2 and viability counts were carried out according to the method of Miles and Misra [11]. The inocula for the primary and secondary challenge experiments contained 2.2×10^9 and 6.5×10^{11} colony-forming units per ml, respectively, each representing the mean of pre- and post-challenge counts.

Experimental procedures

Prior to challenge, female and male cats were tranquilized with benzethonium chloride (Vetalar; Park Davis), swabbed twice within a 3-day interval, and pre-bled by jugular venepuncture. Female cats were exposed to CEMO by depositing 0.5 ml (primary

challenge) or 0.25 ml (rechallenge) of infective suspension either directly into the uterus or in the cervical canal, using a syringe fitted with a disposable udder infusion canula (Bayvet Div., Cutter Labs., Inc., Shawnee, KS). They were held head downwards for several minutes after challenge to minimize back-flow and leakage of a variable amount of the inoculum to the exterior. Male cats were challenged by introducing the infective suspension into the preputial cavity.

Primary challenge experiment

A total of six cats, five female and one male, were exposed to CEMO. They were examined and swabbed daily for 14 days and three times weekly thereafter until rechallenged. Induction of oestrus with oestradiol cypionate was attempted between weeks 3 and 4 and subsequently, weeks 7 and 8 after challenge, in an effort to determine if shedding of the organism could be reactivated in any latently infected cat. Occurrence of oestrus in the cats was based on clinical assessment and on serum progesterone levels. Progesterone (P_4) determinations were carried out on sera collected throughout the experimental period according to the method of Concannon *et al.* [12]. At 4 weeks, one of the female cats was paired with a male previously cultured negative for CEMO.

Rechallenge experiment

Four of the primary challenge group, comprising 3 females and one male cat, were rechallenged at 72 days. The cats were examined and swabbed daily for 8 days after reexposure and then 3 times weekly until the termination of the experiment.

In both challenge experiments, the cats were bled twice a week for the first 2 weeks and once weekly thereafter until the respective studies were concluded. Appropriate controls were included with each experimental group.

Bacteriological responses in the cats were monitored by taking individual intrauterine/cervical and clitoral area swabs from the female cats and a preputial swab from the males. Calcium alginate swabs premoistened with Amies transport medium without charcoal (Calgiswab Type 1; Inolex Corp, Glenwood, IL) were used. Intrauterine/cervical swabs were obtained with the aid of a disposable otoscope head (Welch Allyn, Skan. Falls, NY) and a pencil flashlight. An additional vaginal swab was taken for cytological examination during the first 3 weeks of the primary challenge experiment. Smears were prepared according to a rapid differential staining technique (Diff-Quik; Harleco) previously described by Timoney *et al.* [13]. The caudal reproductive tract was examined at time of swabbing for evidence of inflammation or discharge.

Swabs were plated out within 30 min on Eugon chocolate agar without antibiotics and on Eugon chocolate agar containing 200 μ g per ml of streptomycin sulphate (Sigma Chemical Co., St. Louis, MO). Procedures used to isolate and identify CEMO were as described by Timoney *et al.* [14] with the modification that cultures were held for 10 days before being discarded.

Serological responses in the cats were investigated by means of the complement-fixation and computer-assisted kinetics-based ELISA (KELA) tests. The complement-fixation test was based on the micro-technique of Pitre *et al.* [15] modified by the use of three corrected 50% haemolytic units of complement.

The KELA procedure was essentially similar to that described by Jacobson *et al.* [16] and Barlough *et al.* [17]. Assays were conducted on Gilford EIA-PR50's (Gilford Instruments, Inc., Oberlin, OH) interfaced with a PDP11/34A computer (Digital Instru-

ment Corp, Maynard, MA). A sequencing program was developed in which the EIA-PR50 mode selection and operation were placed under direct computer control. The rate of reaction between bound peroxidase and substrate was determined from three absorbance readings taken and transmitted to the computer at 4 min intervals beginning 5 min after addition of substrate solution. These intervals provided a linear relationship between absorbance values and time so that the resultant sample regression coefficient or slope, representing the rate of enzymatic conversion of the substrate, was directly proportional to the quantity of antibody present in the sample [16].

The antigen used in the KELA was a sodium desoxycholate extract of an English streptomycin resistant strain (NADC, E-CMO) of CEMO. The organism was grown in Eugon broth (Baltimore Biological Laboratories, Cockeysville, MD) with 1% Isovitalax (Baltimore Biological Laboratories, Cockeysville, MD) and 10% horse serum for 48 h at 35.5°C in 5% carbon dioxide in air in a rotating shaker incubator. Extraction of antigen was based on the method of Barber *et al.* [18]. The antigen was diluted to a final concentration of 5 µg/ml in 0.1 M carbonate-bicarbonate buffer (pH 9.6), and then dispensed using an automatic pipette in 0.1 ml quantities into cuvettes and dried at 37°C overnight. Coated paks were either used the following day or were stored at room temperature in sealed plastic bags until use. Just prior to use, cuvettes were rinsed in 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 0.05% Tween 20. This solution was used in all washing steps throughout the assay.

The enzyme-antibody conjugate consisted of the immunoglobulin G (IgG) fraction of goat antiserum against cat IgG (both heavy and light chains) conjugated to horse radish peroxidase (Lot No. 19558, Cappel Laboratories, Cochranville, PA). Following reconstitution, it was diluted 1:10 in PBS and stored at -20°C until use.

Since no serologically positive cat sera to CEMO were available, optimal concentrations of reagents were predetermined by box titration of four antigen concentrations against five concentrations of enzyme-antibody conjugate (equine IgG specificity) and five working dilutions of known CEM positive and negative equine sera. After optimal conditions of the assay were determined for the equine system, a feline conjugate of a concentration known to be optimal for toxoplasmosis and feline infectious peritonitis serodiagnosis by KELA, was substituted for the equine-specific conjugate. Optimal conditions of the assay were found to be 0.1 ml of a 5 µg/ml concentration of antigen, 0.1 ml of a 1:100 dilution of test serum, 0.1 ml of conjugate diluted 1:3000 in PBS-Tween 20, and 0.4 ml of substrate solution per cuvette.

All samples were tested for specific antibody content in one test run, thus precluding the necessity of normalization of the data. Five replicates of each sample were tested and the mean slope value, standard deviation, and coefficient of variation were determined. A running mean of the slope values was developed to establish trends in antibody production as a function of time for individual animals. Accordingly, the mean values for samples 1-5, followed by those of 2-6, 3-7 etc., were used in calculation of the running mean.

At the conclusion of the rechallenge experiment, all of the cats were euthanized, necropsied and the entire genito-urinary tract and internal iliac lymphatic glands removed aseptically. The following sites were swabbed and cultured for CEMO as previously described: female (ovaries, oviducts, uterus, cervix, vagina, urethra, clitoral area, bladder, kidney and internal iliac lymphatic gland); male (testes, epididymi, urethra, sheath, bladder, kidney and internal iliac lymphatic gland). Portions of each tissue were transferred to Bouin's fixative and processed for histopathological examination. Sections were cut at 7 µm and stained with haematoxylin and eosin (HE).

Table 1. Bacteriological responses of cats after primary challenge with the contagious equine metritis organism

Cat I.D.	Sex	Days on which isolations made†
291*	Male	1, 5, 6
294	Female	1-16
383	Female	1-2, 6, 8-9
393	Female	1
394	Female	None
396	Female	1
G81‡	Male	None
Control	Female	None

* Cats challenged by the intrauterine/cervical or preputial route with 1.1×10^9 CFU/0.5 ml.

† Isolations of CEMO from cultures of uterus/cervix and clitoris or penis and prepuce.

‡ CEM-negative male paired with female No. 383 on day 28 after challenge.

RESULTS

Clinical findings

No clinical evidence of infection was observed in any of the male or female cats challenged with CEMO. Three of the female cats had a moderate degree of vulvar oedema on day 1 after challenge, which could be attributable to the fact that the cats were in oestrus. It could also have been the result of mechanical trauma sustained during the challenge procedure. One cat developed slight evidence of a vulvar discharge on days 12 and 13, which was associated with a heavy growth of *E. coli* cultured from the intrauterine/cervical swabs taken during this period. All the cats remained clinically normal following rechallenge with CEMO.

Cytological findings

Examination of smears of vaginal swabs collected from the cats during the first 3 weeks after primary challenge, disclosed nothing of significance. Neither polymorphonuclear leucocytes nor CEMO were observed. Smears contained a variable number of desquamated epithelial cells, some amorphous material and on occasion, bacterial contaminants e.g. coliforms, streptococci and staphylococci. The latter were especially evident in smears from one of the cats between days 11 and 13 after challenge.

Bacteriological responses

Primary challenge experiment. Although CEMO was recovered from four of the five female cats in the immediate post-challenge period, in only two animals was there evidence of active multiplication of the organism in the genital tract (Table 1). The duration of detectable infection in both cases and in the male cat challenged in this experiment, was relatively short-lived and did not exceed 16 days. Shedding of CEMO was not reactivated in any of the cats following the induction of artificial oestrus.

Rechallenge experiment. Of the one male and three female cats reexposed to CEMO, only the male and one female cat shed the organism when rechallenged 10 weeks after primary exposure (Table 2). There was little difference in the duration of the respective infections in these cats compared with the corresponding responses detected in the primary challenge experiment. One cat that had shed CEMO for 9 days following primary challenge, remained completely culture negative after reexposure to the organism.

Table 2. Bacteriological responses of cats after rechallenge with the contagious equine metritis organism

Cat I.D.	Sex	Days on which isolations made†	Necropsy‡ culture result
291*	Male	1, 4	Neg
294	Female	1-6, 8, 14	Neg
383	Female	None	Neg
393	Female	None	Neg
G81§	Male	None	Neg
Control	Female	None	Neg

* Cats rechallenged by the intrauterine/cervical or preputial routes with 1.6×10^{11} CFU/0.25 ml.

† Isolations of CEMO from cultures of uterus/cervix and clitoris or penis and prepuce.

‡ Mice necropsied day 17 after rechallenge and genito-urinary and int. iliac lymphatic glands cultured for CEMO.

§ Unchallenged male paired with female No. 383.

In both experimental studies, CEMO was recovered with almost equal frequency from intrauterine/cervical and clitoral area swabs from infected cats. In only one instance was the organism isolated from a clitoral area swab several days after it had last been cultured from the anterior genital tract of that animal. The cultivation time for CEMO was usually 2 to 4 days and rarely 5 days, on either of the two isolation media.

The profuseness of growth of CEMO obtained from swabs varied between cats. In the three infected cats, the number of colonies observed on daily cultures decreased in the first three days from an initial high of over 100 to less than 1 to 20 per plate, only to increase again to over 100 colonies a week to 10 days later, before finally falling off sharply at the end of the shedding period.

A heavy mixed growth of contaminating bacteria, mainly *E. coli* and to a lesser extent, *Staphylococcus* and infrequently, *Streptococcus*, *Bacillus* and *Pseudomonas spp.* was obtained from clitoral area swabs from most of the cats during the experimental period. In a few animals, these organisms were also cultured on several occasions from intrauterine/cervical swabs.

Bacteriological culture of the range of genito-urinary tract tissues and the internal iliac lymphatic glands harvested from each cat at necropsy, failed to indicate persistence of CEMO in any site. A profuse growth of a non-haemolytic species of *Streptococcus* was obtained from both anterior and caudal portions of the reproductive tract of two of the female cats, one of which had been infected with CEMO in the primary challenge experiment.

Serological responses

Complement-fixing antibody responses were not detected in any of the cats in either experimental study. Pronounced anti-complementary activity was not encountered in the test sera.

A plot of the absolute and running mean KELA slope values for each cat over the experimental period did not reveal any detectably significant antibody responses after primary or secondary challenge with CEMO. Slope values for one of the female cats that was shown to harbour CEMO for up to nine days after primary challenge (Table 1) rose transiently between days 42 and 72, with a peak around day 63 after challenge. There was no evidence of an anamnestic response in this cat following rechallenge with the organism.

Pathological findings

Other than moderate turgidity of the uterine horns in four cats including the control, nothing of significance was observed on gross examination of the individual genito-urinary tracts or internal iliac lymphatic glands. Histopathologically, minor infiltration of the endometrial stroma with monocytes was detected in one cat. Another cat, which had remained culture negative for CEMO in both primary and rechallenge experiments, had cystic hyperplasia of the uterus and a mild pyometra. Moderate numbers of polymorphonuclear leucocytes and macrophages were observed in the endometrium and uterine lumen.

DISCUSSION

Attempts at experimental transmission of CEM to male and female cats met with limited success. There was evidence of active multiplication of CEMO in the genital tract of only 50% of the cats in the primary challenge experiment and in each instance, infection was relatively short-lived.

Of the various factors that could have influenced susceptibility of the cat to CEMO, *viz.* bacterial strain, *in vitro* passage history, challenge dose, route of exposure, sexual maturity and in the female, stage of the oestrous cycle at times of challenge, none were considered to have played a critical role in the establishment of infection under the conditions of this experimental study. The same strain of CEMO at a higher *in vitro* passage level had previously been used to transmit CEM to a group of experimental pony mares [14]. Whilst the challenge dose may possibly have been sub-liminal for the cat, this was thought unlikely as it closely approximated those used in other transmission experiments in which CEM was successfully reproduced [6, 19, 20]. In an earlier study in which evidence of dose-dependency had been noted, the inoculum contained approximately 10^4 fewer organisms [19]. The variability in response of individual cats observed in the present study was considered primarily attributable to intrinsic host factors, since the route of challenge, stage of sexual development and in the female, stage of the oestrous cycle at time of exposure, were regarded as optimal for the successful transmission of CEMO.

Responses of the cats to rechallenge with CEMO can be compared with those reported in mares following experimental reexposure to the organism. Sahu *et al.* [19] and Timoney *et al.* [21] successfully reinfected pony mares using large inocula. Clinical, bacteriological and serological responses were less than those originally observed after primary challenge and evidence was detected of local immunity in the genital tract. Failure to reestablish infection in one of the female cats after rechallenge is not regarded as reflective of dose-dependency [19] since the inoculum contained in excess of 10^{11} organisms. The presence of residual local immunity in this cat following primary challenge 72 days earlier, though unconfirmed, would appear a more likely explanation.

None of the cats developed clinical signs or cytological evidence of infection in this experimental study. Subclinical CEM infection has previously been reported in barren and foaling mares [22, 23] and in experimentally infected albino Swiss [8] and CBA/N and LAF₁/J mice (P. J. Timoney, S. J. Shin and R. H. Jacobson, submitted for publication). The limitations of cytology for the detection of animals inapparently infected with CEM, has been reported earlier by Timoney *et al.* [13] in respect of the carrier mare.

The patterns of the bacteriological responses of the cats following primary or secondary challenge with CEMO were essentially similar, with one exception which has been

previously discussed. Whilst individual variation in persistence of the organism was observed, none of the cats became long-term carriers. The duration of CEM infection was appreciably shorter than that reported in strains of albino Swiss [8], ICR [9] or CBA/N and LAF₁/J mice (P. J. Timoney, S. J. Shin and R. H. Jacobson, submitted for publication). In contrast to the response of pony mares following reexposure to CEMO [21], there was no significant reduction in the shedding period compared with the corresponding interval after primary challenge. The method and frequency of sampling [24] were not considered to have had any major influence on the pattern of isolations of CEMO. Two of the cats that were refractive to CEM had intercurrent infections of the genital tract primarily with *E. coli* and to a lesser extent *Staphylococcus* and *Streptococcus* spp. Although strains of staphylococci and streptococci have been shown to have a selective inhibitory effect on the growth of CEMO *in vitro* [25, 26], it is uncertain whether such organisms could have a similar effect *in vivo*, precluding the establishment of infection in these cats. Failure to establish long-term CEM infection in any of the cats prevented a comparison of the relative persistence of the organism in the anterior and caudal genital tract; ruled out an assessment of the possible effect of oestrus on reactivation of shedding in the carrier animal; and negated attempts at venereal transmission of CEMO.

Inability to culture CEMO from any of the selected sites in the genitourinary tract and the internal iliac lymphatic glands at the conclusion of the study, contrasts with the long-term persistence of the organism in the reproductive tract of the mare [20] and female CBA/N mice (P. J. Timoney, S. J. Shin and R. H. Jacobson, submitted for publication). Recovery of a heavy growth of *Streptococcus* spp. from the genital tract of two of the female cats though surprising, is of questionable significance in relation to the negative findings for CEM. It is conjectural whether CEMO might have been detected in some of the cat tissues using the immunofluorescence technique. Acland *et al.* [20] found immunofluorescence much more sensitive than culture for demonstrating organisms in a greater range of sites in CEM infected mares. They observed stained organisms in tissues harvested 116 days after challenge, 62 days after the last positive culture had been obtained from an experimental mare.

The absence of a detectable complement-fixing antibody response and failure to demonstrate a significant increase in KELA slope values, i.e. antibody activity in any of the cats after primary or secondary challenge with CEMO, may be reflective of the limited susceptibility of the species to this organism. Various groups of workers have found the complement-fixation test to be insensitive for the demonstration of serological responses in CEM-infected pony mares [21, 27, 28] and donkey mares (P. J. Timoney, P. J. O'Reilly, J. F. McArdle and J. Ward, submitted for publication). In the experimental study by Rommel and Sahu [28], pony mares did not develop detectable complement-fixing antibodies after primary or secondary challenge with CEMO, and only two of five mares responded with low to moderate titres after a third intrauterine inoculation. Sahu *et al.* [29] found the ELISA markedly superior to the complement-fixation and certain other serological tests for the detection of CEM-infected mares. However, even using the kinetics-based ELISA or KELA system, which is a highly sensitive technique for quantitation of serum antibody levels [17], very little evidence was found to indicate that the cats in this study, even those that became infected, responded serologically on single or repeated exposure to CEMO.

Failure to demonstrate any CEM-related gross or microscopic lesions in the genitourinary tract of the cats is perhaps not surprising in view of the subclinical nature and

relative transience of the infection in this species. The mild pyometra detected in one cat was associated with an intercurrent streptococcal infection. There was no evidence of the mild or multifocal lymphocytic endometritis observed by Acland and Kenney [30] in mares killed up to 116 days after challenge, nor the additional endometrial changes described by Ricketts *et al.* [31] in uterine biopsy specimens from field cases of CEM 2 to 205 days after infection.

The results of this study indicate that the cat is not very susceptible to CEMO. It is unlikely to act as a source of infection and would be of questionable epidemiological significance in the natural history of this disease. Furthermore, it has considerable limitations as an experimental model system for CEM in the horse.

Acknowledgements—The excellent technical assistance of Ms. B. Hodgson, S. Kanaly, L. Terraciano and Mr. J. Tashjian is gratefully acknowledged. Special thanks are due to Mr. C. Geissinger, Cornell Feline Research Laboratory, for providing the SPF cats and to Mr. J. Ward, Veterinary Research Laboratory, Abbotstown, Castleknock, Co. Dublin, Ireland, who kindly supplied the strain of CEMO.

REFERENCES

1. Crowhurst R. C. Genital infection in mares. *Vet. Rec.* **100**, 476 (1977).
2. O'Driscoll J. Venereal infection in Thoroughbreds with *Bacillus proteus mirabilis*. *Vet. Rec.* **101**, 534 (1977).
3. Platt H., Atherton J. G., Simpson D. J., Taylor C. E. D., Rosenthal R. O., Brown D. F. J. and Wreghitt T. G. Genital infection in mares. *Vet. Rec.* **101**, 20 (1977).
4. Timoney P. J., Ward J. and Kelly P. A contagious genital infection of mares. *Vet. Rec.* **101**, 103 (1977).
5. Mackintosh M. Bacteriological techniques in the diagnosis of equine genital infections. *Vet. Rec.* **108**, 52–55 (1981).
6. Timoney P. J., McArdle J. F., O'Reilly P. J., Ward J. and Harrington A. M. Successful transmission of CEM to the donkey. *Vet. Rec.* **104**, 84 (1979).
7. Timoney P. J., O'Reilly P. J., McArdle J. and Ward J. Attempted transmission of contagious equine metritis 1977 to other domestic animal species. *Vet. Rec.* **102**, 152 (1978).
8. Timoney P. J., Geraghty V. P., Dillon P. B. and McArdle J. F. Susceptibility of laboratory animals to infection with *Haemophilus equigenitalis*. *Vet. Rec.* **103**, 563–564 (1978).
9. Arko R. J. and Wong K. H. Murine infection model for contagious equine metritis: A new venereal disease of horses. *Am. J. vet. Res.* **41**, 989–993 (1980).
10. Lein D. H. and Concannon P. W. Infertility and fertility treatments and management of the queen and tom cat. In *Current Veterinary Therapy, XIII, Small Animal Practice* (Ed. by Kirk R. W.) W. B. Saunders, pp. 936–942 (1983).
11. Miles A. A. and Misra S. S. The estimation of the bactericidal power of the blood. *J. Hyg. (Lond.)* **38**, 732–749 (1983).
12. Concannon P. W., Butler W. R., Hansel W., Knight P. J. and Hamilton J. A. Parturition and lactation in the bitch, serum progesterone, cortisol and prolactin. In *Biology of Reproduction*, Vol. 19 (Ed. by Nalbandov A. Z.). Society for the Study of Reproduction, pp. 1113–1118 (1978).
13. Timoney P. J., O'Reilly P. J., McArdle J. F., Ward J., Harrington A. M. and McCormack R. Responses of pony mares to the agent of contagious equine metritis 1977. *J. Reprod. Fert. Suppl.* **27**, 367–375 (1979).
14. Timoney P. J., McArdle J. F., O'Reilly P. J. and Ward J. Infection patterns in pony mares challenged with the agent of contagious equine metritis 1977. *Equine Vet. J.* **10**, 148–152 (1978).
15. Pitre J., Legendre M. R. and Voisin G. Informations et réflexions sur le diagnostic microbiologie et sérologique de la métrite coccobacillaire des équides. *Prat. Vét. Equine* **11**, 11–26 (1979).
16. Jacobson R. H., Downing D. R. and Lynch T. J. Computer-assisted enzyme immunoassays and simplified immunofluorescence assays: applications for the diagnostic laboratory and the veterinarian's office. *J.A.V.M.A.* **181**, 1166–1168 (1982).
17. Barlough J. E., Jacobson R. H., Downing D. R., Marcella K. L., Lynch T. J. and Scott F. W. Evaluation of a computer-assisted, kinetics-based enzyme-linked immunosorbent assay for detection of coronavirus antibodies in cats. *J. Clin. Microbiol.* **17**, 202–217 (1983).
18. Barber C., Dimitriu O., Vasilescu Th. and Cerbu A. L. Contribution à l'étude de la structure antigénique des *Brucella*. 1. Séparation d'antigène M et de quelques complexes qui le contiennent. *Arch. Roum. de Pathol. Expér.* **20**, 201–213 (1961).
19. Sahu S. P., Pierson R. E. and Dardiri A. H. Contagious equine metritis: effect of intrauterine inoculation of contagious equine metritis agent in pony mares. *Am. J. vet. Res.* **41**, 5–9 (1980).

20. Acland H. M., Allen P. Z. and Kenney R. M. Contagious equine metritis: distribution of organisms in experimental infection of mares. *Am. J. vet. Res.* **44**, 1197–1201 (1983).
21. Timoney P. J., O'Reilly P. J., McArdle J. F., Ward J. and Harrington A. M. Responses of mares to rechallenge with the organism of contagious equine metritis 1977. *Vet. Rec.* **104**, 264 (1979).
22. Crowhurst R. C., Simpson D. J., Greenwood R. E. S. and Ellis D. R. Contagious equine metritis. *Vet. Rec.* **102**, 91–92 (1978).
23. Timoney P. J. Contagious equine metritis in Ireland. *Vet. Rec.* **105**, 172–173 (1979).
24. Timoney P. J., Ward J. and McArdle J. F. Daily variations in the shedding of the agent of contagious equine metritis 1977 by a carrier mare. *Vet. Rec.* **103**, 210–211 (1978).
25. Atherton J. G. Inhibition of CEM organism in mixed cultures. *Vet. Rec.* **103**, 432 (1978).
26. Swerczek T. W. Inhibition of the CEM organism by the normal flora of the reproductive tract. *Vet. Rec.* **103**, 125 (1978).
27. Swerczek T. W. Contagious equine metritis—outbreak of the disease in Kentucky and laboratory methods for diagnosing the disease. *J. Reprod. Fert. Suppl.* **27**, 361–365 (1979).
28. Rommel F. A. and Sahu S. P. Contagious equine metritis: antibody response of experimentally infected pony mares. *Vet. Immunol. Immunopathol.* **2**, 201–213 (1981).
29. Sahu S. P., Rommel F. A., Fales W. H., Hamdy F. M., Swerczek T. W., Youngquist R. S. and Bryans J. T. Evaluation of various serotests to detect antibodies in ponies and horses infected with contagious equine metritis bacteria. *Am. J. vet. Res.* **44**, 1405–1409 (1983).
30. Acland H. M. and Kenney R. M. Lesions of contagious equine metritis in mares. *Vet. Pathol.* **20**, 330–341 (1983).
31. Ricketts S. W., Rossdale P. D. and Samuel C. A. Endometrial biopsy studies of mares with contagious equine metritis 1977. *Equine Vet. J.* **10**, 160–166 (1978).