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Electron microscopy as an aid to diagnosis

How many viruses do we know, and how many are there in nature? The first part of this question is easy to answer—you only need to count them in the scientific literature (there are about 500 in animals and man); the second part must be considered a 'forbidden' question since there are no statistical procedures available which would permit such an estimate. In addition, viruses are genetically very flexible, they can mutate and recombine which may enable them to infect new hosts, target organs and cell species; new viruses have made their appearance, eg, the canine parvovirus (1978) or the virus causing human AIDS (1981), and others will certainly follow.

Which viruses do we know so far? In essence only those which (1) lead to distinct disease signs, (2) can be grown in experimental animals, embryonated eggs or cell culture, and/or (3) occur at high concentrations in body fluids or excreta so that they can be detected by electron microscopy. A disease where uncharacteristic symptoms would develop slowly and progressively and which would occur only sporadically (eg, schizophrenia, diabetes) is usually not thought of being caused by a virus. The standard methods of isolation in culture do not work with viruses which require highly differentiated cells for their replication (eg, entero-absorptive cells at a defined degree of maturity). For enteric virus infections the electron microscope is the diagnosis instrument of choice since very high particle concentrations can be expected to occur in intestinal contents and faeces. After all, the gut can be considered as an 'organ culture' of considerable length with a homogeneous cell composition, and a virus released from one infected cell has ample opportunity to encounter other susceptible cells on its way out.

For several years now, Dr Herbst and Prof Dr Krauss have used electron microscopy for the study of enteric infections in animals; their results show that hitherto unrecognised enteric viruses occur in cats; their pathogenic potential must, of course, be established through experimental or epidemiological study. With its
present contribution of an original research paper, FELINFO wants to draw the readers' attention to this diagnostic possibility.

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Electron microscopy in the diagnosis of enteritis in cats

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Viral gastroenteritis is a frequent cause of morbidity and mortality in young animals and human babies worldwide (Banatvala, 1979; Eugster and Sneed, 1980). Cats, especially kittens, are no exception. Post-mortem examinations performed between 1969 and 1982 on 4,561 cats have resulted in the diagnosis of panleucopenia (16 per cent) and gastroenteric conditions (5 per cent) as causes of death, both associated with enteritis (Landes and others, 1984). The best known and most frequent cause of diarrhoea is feline panleucopenia virus; the disease has been formerly called feline infectious enteritis (Gillespie and Scott, 1973). In addition, some viruses have been discussed as causative agents of diarrhoea and enteritis; as in other mammals, faeces and intestinal contents from cats with enteritis have been found to contain rotavirus (Snodgrass and others, 1979; Yasutaka and others, 1981), feline enteric coronavirus (Pedersen and others, 1981), astrovirus (Hoshino and others, 1981), reovirus (Scott and others, 1979), herpesvirus (Waber and Bestetti, 1981) and coronavirus-like particles (Hoshino and Scott, 1980), with the aid of electron microscopy and cell culture.

Experimental studies have shown that rotavirus is pathogenic for kittens and its widespread distribution in feline populations has been evidenced by sero-epidemiology (Snodgrass and others, 1979). The feline enteric coronavirus is closely related with feline infectious peritonitis (FIP) virus and can cause severe enteritis in antibody-free kittens; this virus, however, does not cause FIP (Pedersen and others, 1981). Reovirus induces minor signs in cats, eg, conjunctivitis and increased lachrymation (Scott and others, 1979). The significance of coronavirus-like particles, of astro- and herpesviruses as causative agents of enteritis in cats has not been documented in the literature.

For several years now we have routinely examined faeces and intestinal contents of cats by electron microscopy in our institute. Little is known about the occurrence of viruses in the gut of cats with enteritis—parvovirus excepted; we thought it timely and useful to give an account of our results.

MATERIALS AND METHODS

Our studies are based on sample material submitted to our laboratory for diagnosis between 1981 and 1985. In total we have examined 346 faecal and intestinal samples of cats with enteritis.
TABLE 1. Results of electron microscopic examinations of faecal and intestinal samples from cats with enteritis

| Year (number of samples) | 1981 (28) | 1982 (31) | 1983 (55) | 1984 (110) | 1985 (122) | Total (346) |
|-------------------------|-----------|-----------|-----------|------------|------------|-------------|
| Particles present in number of samples | 12 | 13 | 22 | 50 | 47 | 144 |
| Identified as | | | | | | |
| parvovirus | 11 | 12 | 18 | 40 | 36 | 117 |
| rotavirus | | 2 | | | 6 | 2 |
| coronavirus | 1 | 1 | 1 | 4 | 6 | 13 |
| coronavirus-like particles | 2 | 3 | 5 | 6 | 10 |
| picornavirus-like particles | 1 | 1 | | | | 2 |

Electron microscopy was performed as described by Arens and Krauss (1980). Faecal material and enteric mucosa triturated with quartz sand, respectively, was suspended in Eagle's Minimal Essential Medium to result in a 10 per cent (w/v) suspension. After clarification at low speed the supernatant was centrifuged for 1 hour at 200,000 x g. The pellet was resuspended in a small volume of phosphate-buffered saline and sonicated (Branson Sonifier); 50 microlitres of this material was mixed with an equal volume of Feliserin (Behringwerke Marburg, West Germany) and kept at 37°C for 30 minutes. The mixture was applied to a Formvar-coated, carbon stabilised copper grid resting on a prewarmed D.S.T. agar plate (Oxoid, code CM 261). Shortly before the fluid had completely diffused into the agar the preparation was stained with phosphotungstic acid and subsequently examined in a Siemens Elmiskop II.

RESULTS

In 144 of the 346 samples (41.6 per cent) we were able to detect virus particles. As shown in Table 1, parvovirus was most prevalent in our material. In the 144 virus-containing samples, parvovirions have been detected in 117 (81.3 per cent) instances. In the remaining 27 (18.7 per cent) samples we found rotaviruses, coronaviruses, coronavirus-like and picornavirus-like particles (Figs 1a to e). Table 1 also shows a listing of the results per year.

In Table 1 a faecal sample from a cat with diarrhoea and vomiting is not listed which we have obtained only recently (1987). The animal came from a group of five cats showing similar signs. Using electron microscopy, particles were visualised which we identified as astrovirions on the basis of their size and morphology (Fig. 1f).

DISCUSSION

The results presented show a widespread occurrence of parvovirus in our cat population. In 117 out of 346 faecal samples from cats with diarrhoea this virus was
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FIG. 1. Electron micrographs of virus particles in faecal and intestinal samples from cats (negative staining with 1 per cent phosphotungstate; bar represents 100 nm). a. Parvovirus; b. picornavirus-like particles; c, rotavirus; d, coronavirus; e, coronavirus-like particles; f, astrovirus.

demonstrated by immune electron microscopy. Comparatively rare were cases of rotavirus (2), coronavirus (13), coronavirus-like particle (10) and picornavirus-like particle (2) detection; in one sample examined recently, astrovirus particles were seen for the first time.

Feline parvovirus is the causative agent of panleucopenia, a disease which has been controlled by vaccination for many years now. Persistence of panleucopenia virus in the cat population may be due to the extreme stability of the virus to environmental conditions and to the fact that protection of kittens by vaccination is
possible only after maternal antibodies have disappeared. Demonstration of parvovirus particles in such a large number of samples from diarrhoeic cats was very unexpected indeed. It is still possible, however, that the parvovirus particles detected are not panleucopenia virus, since diagnosis by electron microscopy is based on morphology only (size, shape, substructure). This question should be answered using other virological methods.

We were able to detect only two cases of infection with rotavirus, which on the basis of seroepidemiological studies is widespread in feline populations (Snodgrass and others, 1979; Yasutaka and others, 1981). This is a rather low incidence, especially when comparing it with our finding of 20 per cent rotavirus-positive samples from calves with diarrhoea (Herbst and Krauss, unpublished observations). Rotavirus infections in cats do not often lead to laboratory examination, in contrast to the situation in calves. Most field infections seem to occur without signs, as indicated also by experimental studies (Yasutaka and others, 1981).

Present knowledge about coronavirus infections in cats is too limited to permit conclusions to be drawn from our results. In the literature, FIP virus, feline enteric coronavirus and coronavirus-like particles have been reported. Feline enteric coronavirus may cause inapparent infections and mild enteritis in kittens (Pedersen and others, 1981). Cases of enteritis have also been observed in connection with FIP virus infections (Hayashi and others, 1982). The agents cannot be distinguished by electron microscopy and both may have been present in the 13 instances of coronavirus identification in our material.

Coronavirus-like particles are similar in morphology to coronaviruses, but differ in size, shape and appearance of the surface projections (Schnagl and Holmes, 1978). The particles have been described in connection with enteritis in the pig (Turgeon and others, 1980), horse (Huang and others, 1983), rabbit (Eaton, 1984), and man (Rousset and others, 1984). Also feline coronavirus-like particles have been reported (Dea and others, 1982) which, however, were present in faecal samples of healthy cats, too (Hoshino and others, 1981). Our coronavirus-like particles were similar in size and shape to genuine coronaviruses (Figs 1d and 1e) but showed shorter and more numerous surface projections. The particles are morphologically different from those described by Hoshina and Scott (1980); it remains to be shown whether they play a role in the aetiology of diarrhoea and, in fact, whether they are viruses at all.

Members of the Picornaviridae family (Matthews, 1982) have not been identified in cats. The two cases in which we found picornavirus-like particles may be compared to the situation in dogs, where human strains have been isolated (Binn, 1970).

The particles shown in Fig. 1f have been encountered in a faecal sample from a cat showing vomition and diarrhoea (spring 1987). Based on the size (25-30 nm) and a star-shaped surface pattern, they were identified as astrovirions. Morphologically indistinguishable particles have been described by Hoshino and others (1981) in a cat with diarrhoea. A connection between astroviruses and diarrhoea has been
found in the dog (Hammond and Timoney, 1983), sheep (Snodgrass and Gray, 1977), calf (Wood and Bridger, 1978), red deer (Tzipori and others, 1981), mouse (Kjeldsberg and Hem, 1985), duck (Gough and others, 1984) and in man (Kurtz and others, 1979). In our material, this virus was detected for the first time.

The present study confirms that direct examination of faecal material using electron microscopy is an important diagnostic tool. In about 40 per cent of the faecal samples from cats with diarrhoea we were able to detect virus particles. Results are rapidly obtained, and the agents can be directly identified with the aid of specific immune sera. The methodology can be easily adapted for use in other domestic animals.

GLOSSARY

The term 'infection' appears familiar and self-evident to all of us; we know what it means and use the word frequently, sometimes indiscriminately. At a closer look, however, the expression 'infection' does not only apply to cough, febrile conditions and suppurative wounds—these are the obvious examples. Leukaemias, sarcomas, warts, immune complex and autoimmune diseases as well as acquired immunodeficiencies may all be the consequences of infections. This contribution will focus on one aspect of the infection—on its duration.

We are most accustomed to cyclic or generalised infections. In these cases the infectious agents invade the organism using a characteristic portal of entry, such as the mucosal surfaces of the mouth, nose or genital tract, but also through a break in the skin caused by some form of trauma. Next, the virus multiplies locally, spreads throughout the organism via the blood and lymph, upon which it occupies the target tissues and organs. Fever develops and the signs characteristic for the infection follow. The animal will either die or survive—after having mobilised its defences. This type of infection is of a transient nature.

Another type of infection is not limited in time. All infectious agents, be it bacteria, mycoplasms or viruses are of a parasitic nature which have progressively adapted to their hosts during evolution. Some of them have developed strategies to elude the host's defence mechanisms and establish persistent infections. From the infectious agent's viewpoint this strategy offers the advantage of being able to spread in a population by causing many infectious—as long as the host is alive. In animal species with a predominantly solitary behaviour—such as the cat—persistence is a necessity for the survival of the infectious agent. Virtually all feline viruses cause persistent infections; this guarantees continuation of the chain of infection irrespective of the fact that cats are territorial predators which—under natural conditions—meet each other only infrequently.

Different mechanisms of viral preservation have been identified, one of them being the chronic infection. In this case the virus can be demonstrated in the host organism for a long period (often for lifetime), during which it is excreted. One group of viruses causing feline upper respiratory disease (URD)—the caliciviruses—may
establish chronic infections after the clinical signs have subsided. In some cases, clinical signs may be absent and detection of the chronic infection is a chance event.

Another virus of the URD complex, the rhinotracheitis herpesvirus, causes a latent infection. In this case—and in contrast to the calicivirus—the virus disappears into specific cells of the central nervous system after primary infection and may be occasionally activated, usually in stress situations. Activation means production of virus accompanied by clinical signs, virus excretion and infection of other animals.

Another type of persistent infection is called 'slow infection'—the term is used also in other languages. Disease signs appear only months or even years after infection and the virus hides in the cells of its host for some time before producing progeny in small quantities. Originally coined for the maedi-visna infections of sheep, the label 'slow infection' has been attached also to, eg, feline leukaemia and the acquired immunodeficiency syndrome (AIDS) of man.

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QUESTIONS AND ANSWERS

Q: Practitioners have occasionally reported inflammatory lesions in the omentum, on liver and spleen of clinically healthy cats, which they discovered at the occasion of spaying; these lesions were very much like those found in cases of feline infectious peritonitis (FIP). The question is asked whether an infection with FIP virus (FIPV) could and should be diagnosed by laparotomy.

A: The development of clinical FIP signs can take several weeks, and the owner will frequently overlook the rather uncharacteristic early signs, eg, intermittent fever in the beginning, more or less pronounced anorexia, sometimes difficult mastication. It cannot be excluded that inflammatory changes have already occurred in some organs at this early stage of the infection. Taking this into consideration, the question for a diagnosis by laparoscopy may appear justified, even more so since a latent infection with FIPV cannot be diagnosed at present. However, the procedure does not appear rational for the following reasons:

1. Laparoscopy as such causes severe stress in a cat. It has been shown by German authors (Neu, H. and Pfeifer, E. G. (1985) Kleintierpraxis 30, 307–314, 1985) that a situation of stress usually precedes the clinical appearance of FIP. Consequently, stress should be avoided, especially in cats that are suspected latent carriers.

2. Nothing is known about the diagnostic efficiency of laparoscopy—ie, the numbers of correct diagnoses in relationship to the numbers of animals examined. Also, we know very little about the incubation period of FIP under field conditions; it can be assumed, however, that the characteristic changes are present for only a short time period which makes the efficiency of this diagnostic procedure doubtful.

3. There is an urgent need for a diagnostic procedure able to detect latent infections in cats which may serve as a source of infection for other animals. Most
infections are thought to originate from clinically healthy animals (see eg, Pedersen, N. C. and Floyd, K. [1985] Continuing Education 7, 1001–1011, 1985)—and in these laparotomy will probably fail to give a result.

4. We think that the cost of a laparotomy is not justified by the result it can be expected to provide.

Q: Clinicians are occasionally requested to tattoo single cats (eg, to prevent theft). The question was asked whether virus diseases can be transmitted by the instruments used in the process.

A: Different procedures of tattooing cats are in use; in most cases the ears are marked. Special pincers containing inserts with short needles are employed for punching the numbers and letters into the skin. The ear and the needles are wetted with ink and pressure is applied with the pincers. Also, tattooing devices are in use which permit free marking (Dermograph Sidac MR 3695). In this tool needles contained in a pen-like holder are set in rapid motion by a magnet or motor. For marking, ink is applied which is then imprinted into the skin by the moving needle. Although transmission of virus infections by tattooing needles has so far not been reported, it is a good practice to clean and disinfect them after each use. The cleaned needles should be rinsed in a 35 to 50 per cent solution of methanol in water (see below).

Q: Which disinfectants may be used for destroying viruses on floors, walls and cages?

A: Resistance to disinfectants depends primarily upon the absence or presence of a lipoprotein membrane in the virus particle. Enveloped viruses are far less resistant than naked viruses. Important enveloped viruses are feline leukaemia virus, corona- and herpesviruses; Parvo- and caliciviruses are devoid of a membrane. It should be remembered that efficient disinfection can be expected only after the respective surfaces had been thoroughly cleaned. Some disinfectants and their effects are given in the table overleaf which has been compiled by the Cornell Feline Health Centre, Cornell University, Ithaca, N.Y.

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Selected virucidal disinfectants

| Disinfectant       | Concentration | Parvo | Calici | Herpes | Corona | FeLV |
|--------------------|---------------|-------|--------|--------|--------|------|
| **Alcohols**²      |               |       |        |        |        |      |
| Methanol           | 35%           | 0     | 0      | +++    | +++    | +++  |
| Ethanol            | 50%           | 0     | +      | ?      | ?      | ?    |
| **Halogen compounds** |           |       |        |        |        |      |
| Betadine           | 0/+/0-5%      | +/++  | ++     | +++    | +++    | +++  |
| Sodium hypochloride³ | 0-1-5%        | ++    | +++    | +++    | +++    | +++  |
| **Phenolic compounds** |           |       |        |        |        |      |
| Cresol preparations⁴ | 1-5%          | 0     | +++    | +++    | +++    | +++  |
| **Aldehydes**⁵     |               |       |        |        |        |      |
| Formaldehyde       | 4%            | +++   | +++    | +++    | +++    | +++  |
| Glutardialdehyde   | 1%            | +++   | +++    | +++    | +++    | +++  |
| Hydrogen peroxide  | 1.5%          | +     | +      | ?      | ?      | ?    |

Results expressed in per cent inactivation of infectivity: 0—less than 10; 0/+—10 to 90; +—90 to 99.9; ++—99.9 to 999; +++—more than 999.9; ?—not tested.

² Suitable for disinfection of small surfaces, eg, examination tables. Not corrosive for steel and other metal or plastic.

³ Suitable especially for disinfection of larger surfaces, eg, floors, since the compound is inexpensive. Not corrosive for steel, concrete and certain plastics.

⁴ Suitable for large surfaces, non-corrosive for most metals, fabrics, plastics, brick walls.

⁵ Universal disinfectant; slow action, needs several hours.