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"Runde" Virus, a Coronavirus-Like Agent Associated With Seabirds and Ticks

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With 5 Figures

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Summary

From 206 *I. uriae* collected in the seabird colonies at Runde, Norway, two identical virus strains demonstrating no antigenic relationships to major arbovirus groups were isolated. The new strains demonstrated a corona-virus like morphology, haemagglutinated chicken red cells and were sensitive to sodium desoxycholate. Multiplication with CPE was demonstrated in BHK 21/c13 and BSC-1 cells, and without CPE in Vero and GMK cell cultures. The mouse pathogenicity was relatively low. In gel precipitation three to five specific lines were seen. Precipitating antibodies have been found in seabird species commonly infested by *I. uriae*.

The ecological circumstances of the isolates indicate an earlier unrecognized arbovirus circulating between seabirds and *I. uriae*. This corona-like virus has been tentatively termed Runde virus.

Introduction

In 1973 investigations were undertaken to evaluate the extent, distribution and ecological circumstances of arbovirus foci in Norway. Until then, no arbovirus isolates had been reported from this country, although ecological and clinical/epidemiological considerations (3, 24, 26) and a limited serological survey on bovine sera (28) indicated the existence of Central-European tick-borne encephalitis virus foci.

The early phase of the project concerns the study of the importance of ticks as vectors; later, mosquito-borne agents will be investigated.

In Norway five *Ixodes* species are abundant: *I. ricinus* (23), *I. trianguliceps* (24), *I. hexagonus* (17), *I. lividus* (16) and *I. uriae* (15, 23). The latter species is present at great numbers in the vast Norwegian seabird colonies. Its ability to

attack man and mammals has been questionable, but this has been recently documented (18).

This paper describes studies with *Ixodes uriae* as a possible vector. These studies were undertaken since:

a) The tick is part of rather confined ecosystem which might be suitable for studies on virus-vector-host interrelationships.

b) Virus-transmission by this tick might have mainly zoonotical but also some public health importance.

c) Reports of arbovirus isolates from seabird colonies have been presented earlier (9, 10, 12, 32, 33).

The seabird colonies at Runde were chosen from a great number of possibilities due to relative ease of access, and because an unusually high and unexplainable chick mortality had recently been reported.

From 206 *I. uriae* ticks collected at Runde in late September 1973, three virus strains have been isolated. One of these isolates belongs to the Uukuniemi group (30, 31).

The additional two strains are serologically closely related or identical. Some characteristics and ecological circumstances of the latter viruses are reported in the present article.

Materials and Methods

Description of the Biotope

The island Runde is situated at 62°25'N' 5°38'E, approx. 30 km from the city Ålesund (Fig. 1). The total area is about 6.4 km² and the circumference 20 km. There are two small settlements on the island, Runde in southeast and Goksøyr in northeast:

Most of the island consists of an uneven mountain plateau, and has altitudes varying between 100 and 330 metres above sea level. In the southwest part, the plateau is terminated by cliffs bordering the sea. Most seabird colonies are situated in this area. Approaching sea-level there is naked stone with niches and clefts inhabited mainly by *Rissa tridactyla*, *Sula bassana*, *Alca torda* and *Uria aalge*. Further up towards the plateau there are rockfalls covered by thin layers of soil supporting various gramnivor plants. These parts are inhabited by the Common puffin *(Fratercula arctica)*.

Collection of Ticks

We visited the island between September 25—27, 1973, and collected *Ixodes uriae* in the puffin rockfalls. According to local ornithologists, the puffins had migrated South about 3 weeks earlier. We found the ticks under and between the rocks. Signs of engorgement were never seen, and they seemed to have entered into diapause. Most of the ticks were to varying degrees surrounded by desiccated skinflaps indicating that they had newly emerged to their present stage. Some had not completed their development from nymph to adult, and were totally surrounded by nymph cutis. The ticks were kept on dram-vials with moist plaster of Paris, and transported alive to the laboratory. We collected a total of 257 *I. uriae*, 206 of which were processed for virus-isolation (Table 1).

Isolation Procedures

The ticks were divided according to stage and sex, pools of imagos consisting of 5 and nymph pools of 20 individuals. After rinsing in saline, the ticks were ground in a mortar in 0.4 per cent bovine albumin in PBS pH 7.4 (APBS) with mycostatin, streptomycin and penicillin. Suspensions were clarified by centrifugation at $1500 \times g$ and inoculated i.c. into baby mouse litters, Bom: NMRI (SPE), aged one to three days. Suspensions not inoculated the same day as processed were kept at $-70°$ C (Revco Ultralow). Mice were observed during 3 weeks for signs of illness. Diseased

mice were killed, brains removed aseptically and homogenated in APBS with antibiotics, centrifuged and passed into new baby mice. Litters which remained healthy were killed after 3 weeks, brains processed and passed as described. Two blind passages were performed routinely. Calculations of 50 per cent end point titers, LD_{50} baby mice ($BMLD_{50}$) were performed according to REED and MUENCH (20), based on ten-fold titrations in mice.

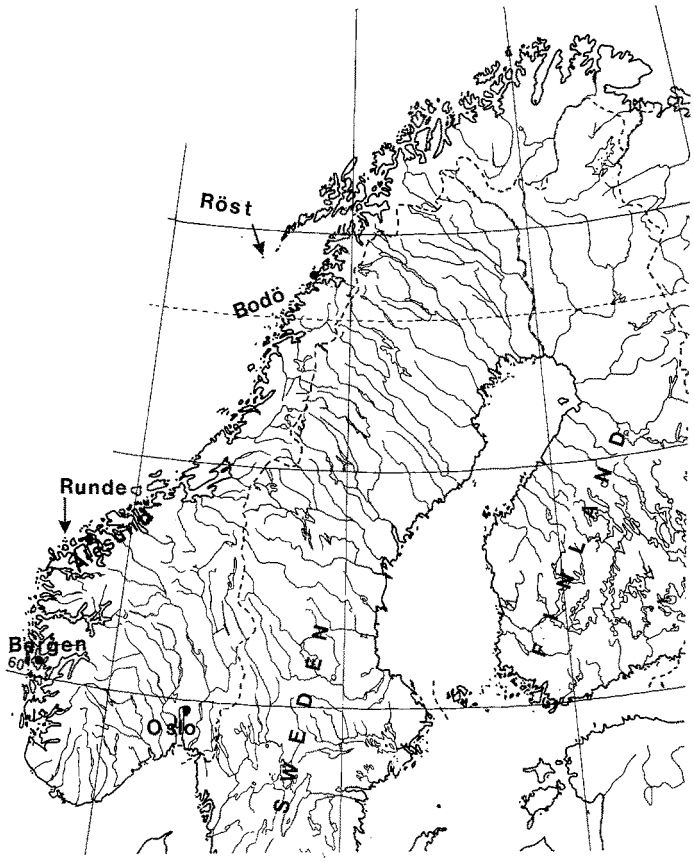


Fig. 1. The localizations of Runde and Røst

Table 1. *Unengorged* I. uriae *ticks collected in the Common Puffin* (Fratercula arctica) *colonies at Runde September 25—27, 1973, and processed for virus isolation*

Stage	No. of ticks	No. of pools	Isolates
Females	33	7	3[a]
Males	32	7	0
Intermediates[b]	24	4	0
Nymphs	117	6	0
Total	206	24	3

[a] Two Runde virus strains and one related to the Uukuniemi group
[b] Intermediated stage between nymph and adult

Cell Cultures

Virus-multiplication has been tested in BHK 21/c 13, HeLa Bristol, BSC-1, RK-13 and Vero cell lines and also in primary GMK cells.

BHK 21/c 13 cells were grown in the medium described by MACPHEARSON and STOKER (11). All other cultures were grown in Eagle's medium with 2 per cent inactivated calf serum. Tubes were seeded with 10^5 cells in 1 ml medium. Cultures were used when confluent monolayers were present. Cells were washed with saline, virus was diluted tenfold from 10^{-1} to 10^{-6} in the medium. A volume of 0.2 ml of each dilution was inoculated into three tubes and allowed to adsorb for 1 hour at room temperature before washing with saline and addition of new medium. Culture tubes were incubated for 8 days at 37° C and inspected daily for a Cytopathic effect (CPE). After 8 days culture fluids were inoculated i.c. in baby mice litters, 0.01 ml per mouse. Cell controls were treated in parallel.

BHK 21/c 13 cultures in Roux bottles were established by seeding 6×10^6 cells/ml.

Efforts to plaque the isolates were performed in BHK 21/c 13 cells under carboxymethylcellulose overlay by a method recently described (22).

Concentration of Cell Culture Fluids

One to three hundred times concentration of cell culture fluids were performed by precipitation with polyethylene glycol (PEG) 6000 and NaCl as described by MCSHARRY and BENZINGER (14). In some instances the precipitates were sonicated at 100 W for 3×15 seconds in a Branson B 12 sonifier.

Sensitivity Test

Sensitivity to sodium-desoxycholate (SDC) was determined in baby mouse litters by the method of THEILER (25).

Antigen Production

For haemagglutination (HA), Immunoelectroosmophoresis (IEOP) and closed hexagon immundiffusion (CHI) experiments, the following antigen preparations have been employed:

a) Crude suckling mouse brain (SMB) preparations: a 20 per cent infected suspension in APBS which has been centrifuged for 15 minutes at 10,000 rpm.

b) Sucrose-aceton (SA) extracted infected mouse brains: Prepared according to CLARKE and CASALS (4), but omitting the final lyophilization step.

c) Cell culture antigens: Culture fluids from infected BHK 21/c 13 Roux bottle cultures were precipitated with 6 per cent PEG 6000 (Macrogolum, Norsk Medisinaldepot) and 2.2 per cent NaCl (14). The 100—300 times concentrated virus-precipitates were sonicated.

Antibody Preparations

Antibodies were produced in adult white mice inoculated with infectious suckling mouse brain preparations.

Initially each mouse received a virus dose of approximately $2.5 \times 10^{4.5}$ $BMLD_{50}$ in 0.25 ml brain suspension mixed thoroughly with 0.25 ml Freund's complete adjuvant (Difco). Subsequently 3 weekly injections with approximately $5 \times 10^{4.5}$ $BMLD_{50}$ in 0.5 ml suspension were given. The last injection, identical to the initial one, was performed one week thereafter. Another 7—8 days later paracentesis and bleeding from the retro-orbital sinus were performed. Antibody preparations for Tahyna virus were produced in the same way.

A fowl antiserum to avian infectious bronchitis virus (AIB) with a NI of 6.1, was kindly provided by the Institute of Veterinary Medicine, Oslo. Reference mouse antibody preparations to TBE, Tribec, EEE, WEE and Uukuniemi (S 23) were supplied by the Yale Arbovirus Research Unit.

Seabird Sera

An ornithological expedition to Hernyken, Røst (approx. 67° 28° N, 12° E (Fig. 1), collected 19 seabird sera in early May 1975. The composition of this material is cited in Table 6.

Serological Methods

Haemagglutination (HA) and haemagglutination inhibition (HAI) tests were performed according to CLARKE and CASALS (4), modified for microtitration equipment (Cooke Eng. Co.). HA activity was tested within the pH range 5.6—7.2 at 4°, 22° and 37° C. Erythrocytes from a variety of species have been investigated. These results will be reported separately, and all HA and HAI titers in the present paper refer to the employment of 0.5 per cent chicken erythrocytes.

Immunoelectroosmophoresis (IEOP)

Twelve ml gel was poured onto precoated 8 × 8 cm lantern slides. For antigen detection 1 per cent agarose (L'Industr. Biol. Franc.) and for antibody detection a mixture of 0.6 per cent agarose and 0.4 per cent Difco Bacto agar was used (29). Three rows of paired wells with 3 mm diameter were punched out. The well interdistance was 3 mm. In the Hepascreen electrophoresis apparatus (Spectra Biologicals, Oxnard, Calif., 9303) 2 slides were run simultaneously for 1—2 hours. In screenings, this allows testing of 58 unknown samples in one run.

Gel Precipitation

This was performed by a sensitive modification of the micro Ouchterlony technique termed closed hexagon immunodiffusion (CHI) (27), and also with varying patterns on lantern slides.

To obtain the maximum number and intensity of the precipitation lines, the antigens were applicated several times (2—5) during the 16—24 hours prior to antiserum application. Due to the lower diffusion rate of antigens, an interval of 2—4 hours between the last antigen and the antiserum application was adopted. The first lines then could be seen 4—5 hours after antiserum application. The gels were incubated at room temperature overnight, and then several days at +4° C before staining and final reading.

IEOP and CHI gels were stained by 4 per cent tannic acid as previously described (8).

Electron Microscopy

Negative Contrast EM

Infected culture fluids from BHK 21/c 13 cells grown in Roux bottles were concentrated 300 times by PEG 6000/NaCl precipitation (14), resuspended in borate saline pH 9 or PBS pH 7.4 and sonicated. Then 0.3 ml suspension was diluted $^1/_{10}$ in PBS and centrifuged for 1 hour in a Sorvall RC 2-B with rotor 55—34 at 20,000 rpm. The pellet was suspended in a few drops of distilled water and negatively stained with 2 per cent phosphotungstic acid pH 7.0 or 0.5 per cent uranyloxalate pH 6.0. One drop virus-stain mixture was placed on a formvar-carbon coated grid and excess fluid was withdrawn with filterpaper. The grid was examined in a JEM 100 B Electron Miscroscope at a magnification of 50,000 ×.

Thin Section EM

Infected and control BHK 21/c 13 cultures were harvested 4 days p.i. by means of a rubber policeman. The cells were fixed in 3 per cent glutaraldehyde for 1 hour at +4° C, washed three times in cacodylate buffer, postfixed in 1 per cent osmiumtetroxyde for 1 hour at room temperature and centrifuged for 3 minutes at 2500 rpm. The cell pellet was resuspended in a few drops of cacodylate buffer and centrifuged in micro capillary tubes in a hematocrit centrifuge for 3 minutes at 12,500 rpm. The pellets were removed from the capillary tubes, dehydrated in acetone and embedded in Spurr by a rapid procedure (6). Ultrathin sections (silver) were cut on a U 2-Reichert Ultramicrotome and doubly stained by saturated uranylacetate in 50 per cent ethanol and Reynolds lead citrate (21). Electron microscopy was carried out in a JEM 100 B at a magnification of 50,000 ×.

Results

Isolation of Runde Viruses

Two pools, both processed from 5 unengorged female *I. uriae*, termed E81 and E85 were inoculated into mouse litters 2 and 3 days old respectively. The mice seemed unaffected during the 3 weeks observation period. However, in the first passage (M1) the mice were moribund after 14 days for E81 and 13 days for E85. In the second passage (M2), incubation periods were reduced to 10 and 9 days respectively and in the subsequent passages to 5—7 days for both strains. The viruses were reisolated twice from the original tick pools during the following year. These results are summarized in Table 2.

Table 2. *Isolation histories of the Runde virus strains*

	Ru E81	Ru E85
Pool consist. of	5 female *I. uriae*	5 female *I. uriae*
Primary inocul.	November 9, 1973	December 4, 1973
Effects by i.c. inocul. on 1—3 days old mice		
Primary inoc.	No effect	No effect
Passage 1	Moribund after 14 days	Moribund after 13 days
Passage 2	Moribund after 10 days	Moribund after 9 days
Passage 3	Moribund after 5 days	Moribund after 6 days
Reisolation from original pools	June 27, 1974 August 13, 1974	April 17, 1974 October 23, 1974

The original tick pools were diluted 1/10 in BHK-medium and inoculated on five BHK 21/c 13 tube cultures. The tubes were harvested after five days. No CPE was recorded. The culture fluids were incubated into three baby mouse litters each. The mice were moribund after 8—10 days. The viruses were shown to be serologically identical to the strains isolated in mice by HAI, CHI and IEOP.

The Ru E81 tick pool was diluted 1/100 in BHK-medium, and three BHK cultures in Roux bottles were infected. The cultures were harvested after four days. Infected culture fluid was inoculated i.c. into 5 baby mouse litters, which were paralysed at day 10 p.i. The rest of the culture fluids were concentrated 100 times by PEG 6000/NaCl, and used as antigen. The isolates were shown to be serologically identical, and also identical to the mouse-passaged strains by HAI, CHI and IEOP.

Sensitivity of Mice to Runde E81 Virus

A 20 per cent brain suspension from the third suckling mouse brain passage (M3) titered $10^{5.8}$ $BMLD_{50}$/ml in 1 day old mice. The virus suspension was titrated in parallel in 7 and 14 days old mice. As shown in Table 3, 1 week old mice were nearly as sensitive as the newborn, while there was a drop in infectivity of 1.2 logs and a prolongation of average survival time from 6.3 to 10.5 days for 2 week old mice. During production of antibody preparations it was demon-

strated that adult mice were refractary to intraperitoneally injections with both E81 and E85.

Table 3. *Sensitivity of suckling mice to a Runde E81 infected mouse brain suspension*

Mice aged	Titer in $\log_{10}$ LD_{50}	Average survival time
1 day	5.8	6.3 days
7 days	5.5	7.3 days
14 days	4.6	10.3 days

Growth of Runde E81 in Cell Cultures

BHK 21/c13, BSC-1 and Vero cell cultures demonstrated CPE to a varying extent after inoculation with Runde E81M2 brain suspension. The culture fluids killed suckling mice when intracerebrally inoculated. In GMK no CPE was registered, but some mouse pathogenicity was demonstrated in the culture fluids after 300 times concentration.

In HeLa Bristol and RK-13 cultures no signs of virus multiplication were detected during primary inoculation and 3 consecutive blind passages. No CPE was present, and culture media concentrated 1—300 times by PEG 6000/NaCl precipitation did not affect 1—3 days old suckling mice. As mentioned before, virus multiplication but no CPE was recorded after infection of BHK 21/c13 cultures with the original tick pools.

Plaquing in BHK 21/c13 cells was not successful by the method used.

Sensitivity to Desoxycholate

Both Runde E81 and E85 virus demonstrated a very marked sensitivity to treatment with sodiumdesoxycholate. The titers were reduced from 5.5 to 3.4 in the case of Ru E81 and 5.8 to 3.6 in the case of Ru E36.

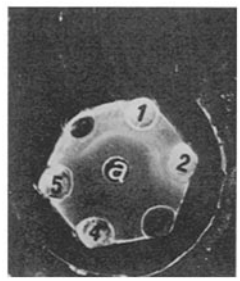
Antigenicity of Runde Virus

Runde virus produces antigens in suckling mouse brains as well as in BHK 21/c13. The antigens demonstrate specific reactions with mouse antisera and immune ascitic fluids in HAI, CHI and IEOP. The antigenic preparations have been kept at $-20°$ C for 11 months without loss in reactivity.

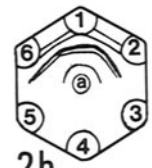
Haemagglutinating (HA) antigens can be produced from infected SMB by the SA extraction method of Clarke and Casals (4), but also suspensions of crude infected SMB and PEG 6000/NaCl precipitated cell culture fluids contain haemagglutinating activity. The ability to agglutinate chicken erythrocytes is relatively independent of pH and temperature. The highest titers and most clear-cut endpoints have however been attained by pH 6.4—6.8 at 4° C.

In HAI no serologic differences between RuE81 and E85 could be demonstrated. The mouse antisera and immune ascitic fluids were inhibitory to 4HA units of antigen to dilutions of 1/80—640. Normal mouse sera were not inhibitory, neither was a fowl antiserum to AIB virus or antisera to TBE, Tribec, EEE, WEE and Tahyna.

Precipitating antigens, as revealed by CHI and IEOP, also were present in the same preparations as cited above. In CHI 3—5 specific lines have been noted (Fig. 2), while in IEOP double lines were seen. No antigenic difference between the two strains was detected. Virus from mouse brains and cell culture demonstrated total identity by these methods. No reaction was observed with an antiserum against avian infectious bronchitis virus.



2a



2b

Fig. 2 a and b. Closed hexagon immunodiffusion in 1 per cent agarose

Well 1: Suckling mouse brains infected with Ru E 81 virus

Well 2: Suckling mouse brains infected with Ru E 85 virus

Well 3: BHK 21/c 13 control antigen

Well 4: Suckling mouse brain control antigen

Well 5: Suckling mouse brains infected with Uukuniemi virus (strain Ru E 82)

Well 6: Ru E 81 virus from the 6th to 9th passage in BHK cells and concentrated 100 times by PEG 6000/NaCl

The antigen wells were filled 5 times to capacity. The antisera well (a) was filled 4 hours after the last antigen application

Antibodies in Seabirds

The 19 seabird sera were screened by IEOP, and the specificity secured by CHI. Four out of 19 birds had precipitating antibodies to RuE81. The composition of the material and the results are shown in Table 4.

Table 4. *Precipitating antibodies to Ru E 81 in seabird sera*

Bird species	No. positive/No. tested
Common Puffin *(Fratercula arctica)*	2/7
Razorbill *(Alca torda)*	1/6
Common Murre *(Uria aalge)*	1/2
Black Guillemot *(Cepphus grylle)*	0/2
Shag *(Phalocrocorax arrslatolis)*	0/2
Total	4/19

Electron Microscopy

Negative Contrast EM

A series of electron micrographs of negatively stained preparations are presented in Figs. 3, 4. Fig. 3 shows corona-virus-like structures in a preparation from Ru E81 infected cell culture supernate. Similar structures were never seen in uninfected controls. Most of the particles have an approximate total diameter of 170 nm, but in other preparations particles with diameters varying from 110 to 220 nm have been seen. Fig. 4A demonstrates an intact particle at a greater magnification. It seems to contain an inner structure 80 nm in diameter. Fig. 4B and 4C demonstrate partly and completely disrupted particles with loss of knobs and release of inner helical material. Staining with 0.5 per cent uranyloxalate (Fig. 4D) produces a sort of 3-dimensional picture of the virus-like particle.

Thin Section EM

Thin sections of Ru E81 infected BHK 21/c13 cultures 4 days p.i., demonstrate virus-like particles in the cytoplasm and extracellularly between broken and intact cells (Fig. 5). The particles have a diameter of 100—110 nm. Some of them look empty, while others are penetrated by stain and expose inner structures. The particles seem to have a double outer membrane, but no knobs are visible by thin sections and positive staining.

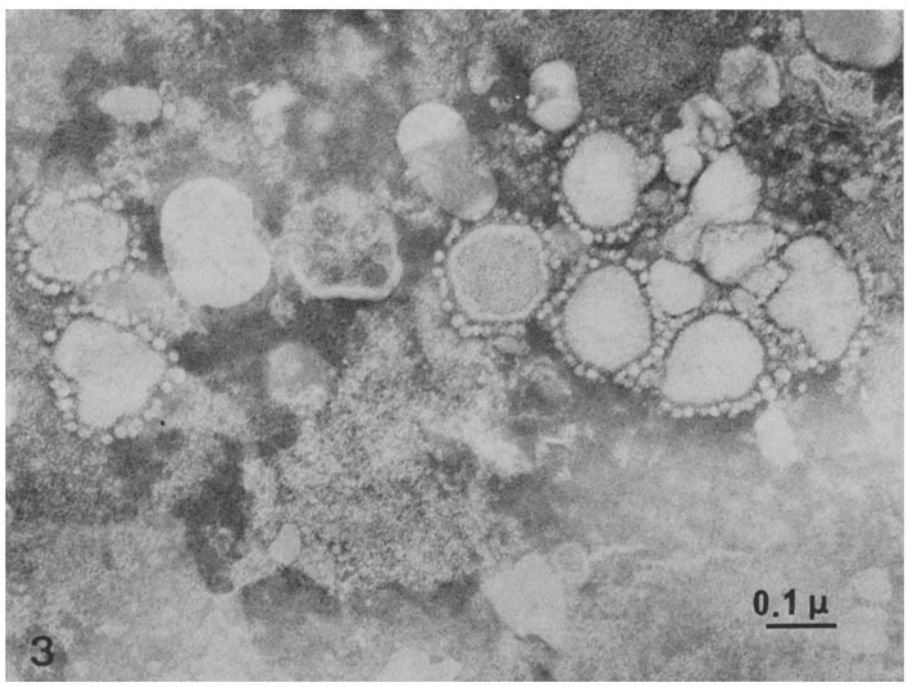


Fig. 3. Virus-like particles from BHK 21/c 13 cell cultures infected with Ru E 81 virus. Stained with 2 per cent PTA pH 7.0. Magnification 90,000×

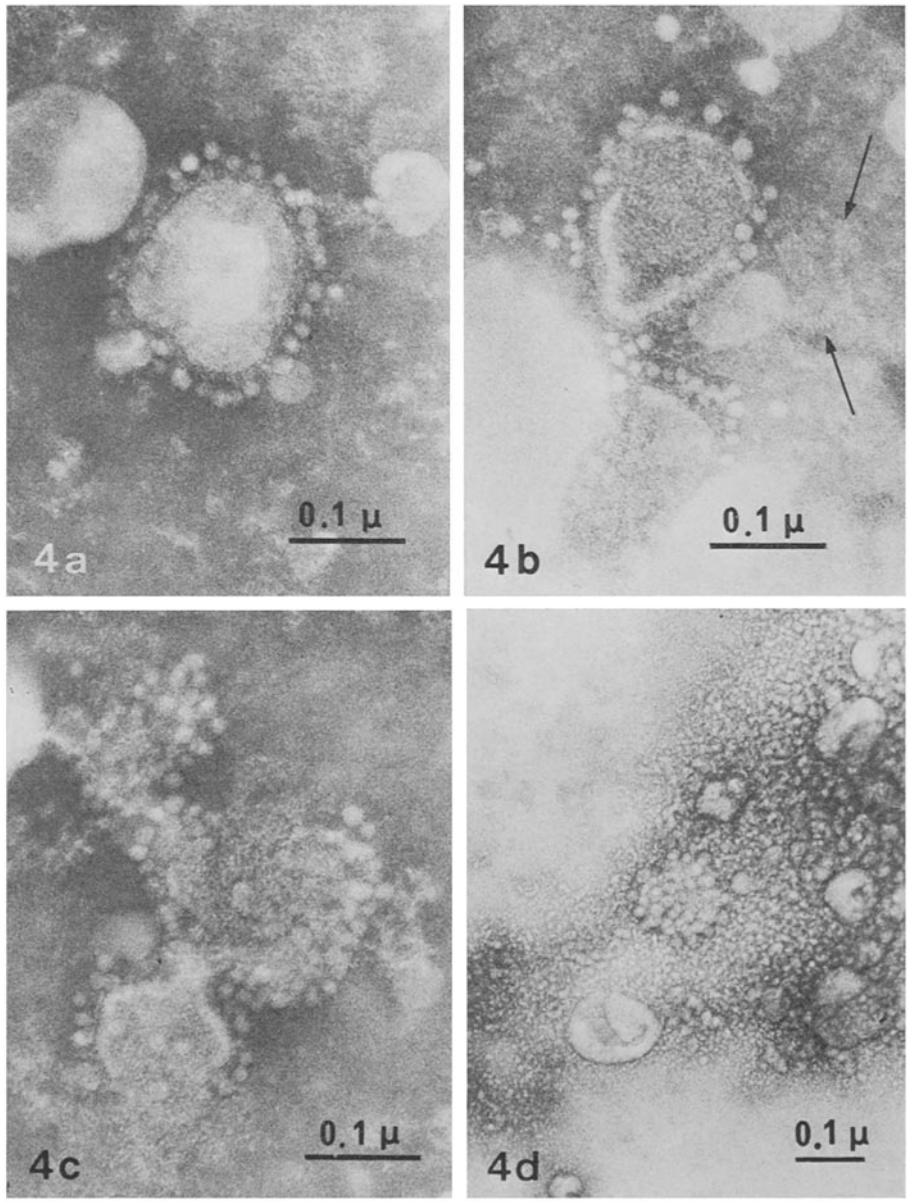


Fig. 4. Particles negatively stained as in Fig. 4

a. Intact particle. Magnification 150,000×

b. Partly disrupted particle releasing inner helical material (arrows). Magnification 180,000×

c. Completely disrupted particle. Magnification 150,000×

d. Particle negatively stained with 0.5 per cent uranyloxalate. Magnification 90,000×

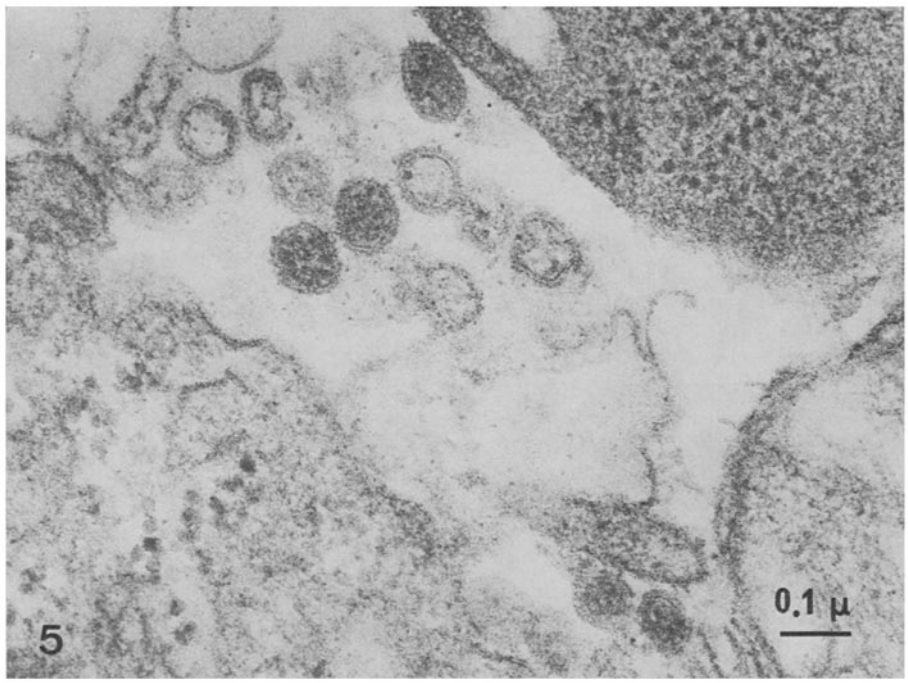


Fig. 5. Thin section of Ru E 81 infected BHK 21/c 13 cells, demonstrating virus-like particles. Magnification 90,000×

Discussion

When the Runde virus strains were first isolated, it had to be kept in mind that they might originate from the staff handling the tick pools, or from the baby mice used for isolation. The reisolations from the original tick suspensions (Table 2), and the properties differing from known human, rodent and also avian coronaviruses (2, 19), could not entirely rule out these possibilities. But the fact that antibodies to the virus were demonstrated in seabirds and the isolation of virus also in cell cultures seem to settle this question. Circumstances of tick-collection strongly support that Runde virus is an arbovirus in the ecological sense, and not a mechanical pick-up:

1. It is highly unlike that a relatively labile virus should survive for 3 weeks in *I. uriae* without active multiplication.

2. The lack of engorgement or pregnancy of the female *I. uriae*, and the fact that they were in diapause and surrounded by desiccated skinlaps, strongly indicate that Runde virus may have passed interstadially in the virus-carrying ticks.

Still, the final inclusion of Runde virus among the arboviruses must await the demonstration of true biological transmission by *I. uriae*.

Although the morphology of Runde virus strikingly resembles that of the coronavirus group, some characteristics of the virus differ considerably from

those of known coronaviruses. This holds true for the pathogenicity to mice (2, 13), the ability to grow in cell cultures (1, 2, 5) and the haemagglutinating properties (2, 7). Besides, the size of the virion seems larger than for other members of the coronavirus group (19).

The discrepancy in size between negative contrast and thin sections may be partly due to shrinkage during fixation and dehydration prior to embedding and thin sectioning, and partly to collapse of the particles by negative staining adding to their actual size. The most reliable staining procedure for size calculations probably is negative staining by uranyloxalate which also stabilizes the structure of the particles.

Detailed taxonomic considerations for Runde virus must await further characterization of biological, serological and molecular properties. But Runde virus is probably a "new" coronavirus on the grounds of: its morphology, the lack of cross reaction with avian infectious bronchitis virus and the lack of serological reactions with control mouse sera probably eliminating the possibility of MHV isolates. Also, our Department of experimental animals have no indications of mouse hepatitis in the mouse colony which have been used in these studies.

The demonstration of this hitherto unrecognized virus associated with seabirds and ticks also necessitates further efforts to clarify the ecological and possible epizootological implications. As already mentioned, an unusually high chick mortality in the seabird colonies at Runde has been reported during the last few years. Bacteriological and toxocological research has not been able to reveal any definite etiological factor, although pesticide residues have been demonstrated to some extent. This must of course bring viruses into consideration. A synergistic action between pesticides and virus seems one possible working hypothesis in this connection. Since Norwegian seabird colonies in general are rather frequently visited by scientists, students, holiday travellers and the local public, the recent documentation of the ability of *I. uriae* to attack man (18) raises the question of public health implications. Isolations from *I. uriae* of Uukuniemi viruses (30, 31, 33) orbiviruses (12, 32) and flaviviruses (9, 33) in addition to Runde virus further stresses this point.

At present nothing is known concerning the distribution and general infection rates of Runde virus. Some indications that it might be widespread in Norwegian seabird colonies can be given, however. Firstly, the isolation of two virus strains from only 206 *I. uriae* collected at Runde indicates a rather high infection rate of the vectors in this location. Secondly, the demonstration of a high rate of seropositive birds at Hernyken, Røst, proves that virus-circulation is not restricted to Runde.

Further field work and experiments which may throw more light upon the ecological interrelationships of Runde virus as well as upon the characteristics of the virion are in progress.

Acknowledgments

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References

1. Bradburne, A. F., Tyrrell, D. A. J.: The propagation of "coronaviruses" in tissue-culture. Arch. ges. Virusforsch. **28**, 133—150 (1969).
2. Bradburne, A. F., Tyrrell, D. A. J.: Coronaviruses of man. Progr. med. Virol. **13**, 373—403 (1971).
3. Brennaas, O., Raeder, S.: Meningoencephalomyeloradiculitis in Sunnhordland. Tidsskr. for Den norske lægeforening **11**, 739—744 (1962).
4. Clarke, D. H., Casals, J.: Techniques for haemagglutination inhibition with arthropod-borne viruses. Amer. J. trop. Med. Hyg. **7**, 561—573 (1958).
5. Cunningham, C. H., Spring, M. P., Nazerian, K.: Replication of avian infectious bronchitis virus in African green monkey kidney cell line Vero. J. gen. Virol. **16**, 423—427 (1972).
6. Doane, F. W., Anderson, N., Chao, J., Norman, A.: Two-hour embedding procedure for intracellular detection of viruses by electron microscopy. Appl. Microbiol. **27**, 407—410 (1974).
7. Kaye, H. S., Dowdle, W. R.: Some characteristics of haemagglutination of certain strains of "IBV-like" virus. J. inf. Dis. **120**, 576—581 (1969).
8. Kjeldsberg, E., Traavik, T., Siebke, J. C.: A serological tool for the diagnostics of virus B hepatitis. Acta path. microbiol. scand. (Section B) **82**, 396—402 (1974).
9. Lvov, D. K., Timopheeva, A. A., Chervonsky, V. I., Gromashevsky, V. L., Klisenko, G. A., Gostinschikova, G. V., Kostyrko, I. N.: Tuleniy virus—a new group B arbovirus isolated from *Ixodes* (Ceratixodes) *putus* Pick.—Camb. 1878 collected on Tuleniy Island, Sea of Okhotsk. Amer. J. trop. Med. Hyg. **20**, 456—460 (1971).
10. Lvov, D. K., Timopheeva, A. A., Gromashevsky, V. L., Chervonsky, V. I., Gromov, A. I., Tsyrkin, Yu. M., Progrebenko, A. G., Kostyrko, I. N.: Sakhalin virus—a new arbovirus isolated from *Ixodes* (Ceratixodes) *putus* Pick.—Camb. 1878 collected on Tuleniy Island, Sea of Okhotsk. Arch. ges. Virusforsch. **38**, 133 to 138 (1972).
11. MacPherson, I., Stoker, M.: Polyoma transformation of hamster cell clones—an investigation of genetic factors affecting cell competence. Virology **16**, 47—51 (1962).
12. Main, A. J., Downs, W. G., Shope, R. E., Wallis, R. C.: Great Island and Bauline: Two new Kemorovo group orbiviruses from *Ixodes uriae* in Eastern Canada. J. med. Ent. **10**, 229—235 (1973).
13. McIntosh, K., Becker, W. B., Channock, R. M.: Growth in suckling mouse brain of "IBV-like" viruses from patients with upper respiratory tract disease. Proc. nat. Acad. Sci. (Wash.) **58**, 2268—2273 (1976.)
14. McSharry, J., Benzinger, R.: Concentration and purification of vesicular stomatitis virus by polyethylen glycol "precipitation". Virology **40**, 745—746 (1970).
15. Mehl, R.: Lopper og lundelus på sjøfugl på Røst 1968. Fauna (Oslo) **21**, 197—198 (1968). (In Norwegian.)
16. Mehl, R.: Records of ectoparasitic insects and mites on birds and mammals in Norway. Norsk. ent. Tidsskr. **17**, 109—113 (1970).
17. Mehl, R.: Lopper, fått og midd på piggsvin i Norge. Fauna (Oslo) **25**, 186—196 (1972). (In Norwegian.)
18. Mehl, R., Traavik, T.: Manuscript in preparation.
19. Oshiro, L. S.: Coronaviruses. In: Dalton, A. J., Haguenau, F. (eds.), Ultrastructure of animal viruses and bacteriophages: An atlas, 331—343. New York-London: Academic Press 1973.
20. Reed, L. J., Muench, H.: A simple method of estimating fifty percent endpoints. Amer. J. Hyg. **27**, 493—497 (1938).
21. Reynolds, E. S.: The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. **17**, 208—212 (1963).

22. SAIKKU, P., BRUMMER-KORVENKONTIO, M.: Arboviruses in Finland. II. Isolation and characterization of Uukuniemi virus, a virus associated with ticks and birds. Amer. J. trop. Med. Hyg. **22**, 390—399 (1973).
23. TAMBS-LYCHE, H.: Notes on Norwegian ticks. Bergens Mus. Årbok **3**, 1—8 (1943).
24. TAMBS-LYCHE, H.: *Ixodes ricinus* som mulig vektor for sykdommer hos mennesker i Norge. Nord. Medicin **62**, 1217—1222 (1959). (In Norwegian.)
25. THEILER, M.: Action of sodium desoxycholate on arthropod-borne viruses. Proc. Soc. exp. Biol. Med. **96**, 380—382 (1957).
26. TRAAVIK, T.: Arbovirusencephalitt overført av *Ixodes ricinus*. Norsk Veterinærtidskrift **82**, 705—712 (1970). (In Norwegian.)
27. TRAAVIK, T., SIEBKE, J. C., KJELDSBERG, E.: A sensitive modification of the Ouchterlony technique. Detection of hepatitis associated antigen by immunodiffusion in a closed hexagonal system. Acta path. microbiol. scand. (Section B) **80**, 773—774 (1972).
28. TRAAVIK, T.: Serological investigations indicating the existence of tick-borne encephalitis virus foci along the Norwegian coast. Acta path. microbiol. scand. (Section B) **81**, 138—142 (1973).
29. TRAAVIK, T.: Development of a modified immunoelectroosmophoresis method for Uukuniemi and Runde virus serology. Arch. Virol. (1977, in press).
30. TRAAVIK, T., MEHL, R.: Tickborne viruses in Norway. Med. Biol. **53**, 321—324 (1975).
31. TRAAVIK, T., MEHL, R.: Uukuniemi group viruses isolated in Norway. Arch. Virol. (1977, in press).
32. YUNKER, C. E., CLIFFORD, C. M., KEIRANS, J. E., THOMAS, L. A., CORY, J.: Tickborne viruses in Western North America. II. Yaquina Head, a new arbovirus of the Kemorovo group isolated from *Ixoedes uriae*. J. med. Ent. **10**, 264—269 (1973).
33. YUNKER, C. E., CLIFFORD, C. M., KEIRANS, J. E., PHILIPS, R. N., THOMAS, L. A.: Tick-borne viruses from seabird nesting sites in Oregon and Alaska. Abstr. Comm., 9th Int. Congr. Trop. Med. and Malar., p. 37, Athens. 1973.

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