

Literature review: SARS-CoV-2 and COVID-19—The role of domestic animals in transmission and the associated risks in the context of reproductive technology

Sarah Van Dyk, Animal Biosecurity Branch, Biosecurity Animal Division, Department of Agriculture, Water and the Environment, 18 Marcus Clarke Street, Canberra, ACT, 2601, AUSTRALIA.

Executive summary

In December of 2019, numerous cases of undiagnosed pneumonia in people became apparent in Wuhan city, Hubei region, China. It was determined that this emerging disease was caused by a novel coronavirus, SARS-CoV-2, causing the disease COVID-19. On January 30, 2020, due to the large scale of the spread of the disease, the World Health Organisation (WHO) declared it to be a Public Health Emergency of International Concern, and on March 11, 2020, as global spread became apparent, SARS-CoV-2 was labelled a pandemic (WHO, 2020).

Members of the International Embryo Technology Society (IETS) are consistently on the front line, exposed to animals and their derivatives, and given the suspected animal origin of SARS-CoV-2, it is relevant to review all literature currently available relating to the role of animals in infection and transmission of SARS-CoV-2, particularly in the context of reproduction, and assess the potential role of reproductive material (germplasm, embryos) in transmission.

The current evidence shows that the only animals that have been demonstrated to develop infection with SARS-CoV-2 either naturally or experimentally are members of the families *Felidae*, *Canidae* and *Mustelidae*, and golden Syrian hamsters in two experimental papers. There have been a few isolated cases of natural infection in companion animals (cats, dogs), zoo animals (big cats), and mink, which all have evidence of contact with a human with COVID-19. It is not clear whether horizontal transmission was possible or occurred in some of these cases involving companion animals; however, there is evidence that horizontal transmission in mink led to the widespread outbreaks observed in these farms.* There are a number of experimental studies under laboratory conditions demonstrating infection, production of infectious virus and transmission in cats, ferrets and golden Syrian hamsters.

The limited available evidence suggests that it is unlikely that the other species typically dealt with (dogs, pigs, horses) can be infected and transmit the virus; however, at this point there are no studies looking at infection and transmission in ruminants, zoo animals or wild animals, and the absence of this evidence does not preclude the possibility for other animals to be susceptible to infection with SARS-CoV-2. Some human studies suggest that it is highly unlikely that the virus could be transmitted through germplasm; however, these are limited in number and the majority have not been subjected to peer review. Currently, there are no studies examining the transmission of SARS-CoV-2 in animal germplasm.

Extrapolating from the limited available literature in animals, there is potential for SARS-CoV-2 to be present in faeces and urine. The evidence of viral-RNA-positive environmental samples and indirect horizontal transmission in mink farms suggests that particular care should be taken if any assisted reproductive technologies are undertaken in species of the *Mustelid* family.* Despite the lack of evidence of domestic animal to human transmission, and the low likelihood that an animal presented

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for breeding could be infected with SARS-CoV-2, it is recommended that vigilance be maintained, including exercising routine hygiene, wearing of standard personal protective equipment (mask, gloves, eye protection, disposable gown), and adhering to thorough biosecurity practices, such as hand washing.

Introduction

In December of 2019, numerous cases of undiagnosed pneumonia in people became apparent in Wuhan city, Hubei region, China. It was determined that this emerging disease was caused by a novel coronavirus, SARS-CoV-2, causing the disease COVID-19. Since then, the virus has spread to more than 213 countries around the world, with almost 3 million confirmed cases, and over 202,000 deaths attributed to COVID-19 worldwide, as of April 28, 2020 (WHO, 2020).

Since this virus emerged, global research has been hastily conducted to acquire, collate and disseminate rapidly as much information about this virus and disease as possible. This provides a valuable means to inform governments, scientists, public health professionals, medical professionals and the general public. However, it should be noted that most of the literature currently available on this subject has not been subjected to peer review, and some reports have not yet been verified.

As the international collaborative organisation for advanced reproductive techniques in animals, members of the International Embryo Technology Society (IETS) are consistently on the front line, exposed to animals and their derivatives. Given the suspected animal origin of the novel coronavirus, it is pertinent to review all literature currently available related to the role of animals in infection and transmission of SARS-CoV-2, particularly in the context of reproduction, which include domestic (livestock and companion animals), laboratory, zoological and endangered species, and assess the potential role of reproductive material (germplasm, embryos) in transmission.

Background

- On December 31, 2020, the World Health Organisation (WHO) was notified by Chinese authorities of cases of pneumonia in people linked with a seafood and live animal “wet” market in Wuhan city, Hubei region of China (WHO, 2020).
- On January 9, 2020, WHO identified that the causal pathogen for the cases of pneumonia was a new emerging coronavirus. This coronavirus was temporarily labelled 2019-nCoV (novel coronavirus) (WHO, 2020).
- On February 11, 2020, the novel coronavirus was officially labelled SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) by WHO. The subsequent disease was labelled COVID-19 disease (coronavirus disease) (WHO, 2020).
- On January 30, 2020, due to the large scale of the spread of the disease, WHO declared it to be a Public Health Emergency of International Concern.
- On March 11, 2020, as global spread became apparent, SARS-CoV-2 was labelled a pandemic (WHO, 2020).
- Coronaviruses are enveloped, single-stranded, positive-sense RNA viruses that infect a wide variety of species, including humans, livestock and companion animals.
- Coronaviruses are classified in four genera—*Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus* and *Deltacoronavirus*.

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- *Alpha-* and *Betacoronaviruses* usually infect mammals, and *Gamma-* and *Deltacoronaviruses* usually infect birds and fish.
- SARS-CoV-2 is classified as a *Betacoronavirus*.
- Zhou and others (2020) reported that 2019-nCoV (now named SARS-CoV-2) shares 79.6% sequence identity with SARS CoV and shares 96.2% sequence identity at a whole-genome level to Bat CoV RaTG13, which was previously detected in *Rhinolophus affinis* (horseshoe bats) from Yunnan province, China. To date, this provides the most substantial evidence that SARS-CoV-2 originated from an animal reservoir, a bat.
- Additionally, it was found that SARS-CoV-2 likely uses the same cell entry receptor, angiotensin converting enzyme 2 (ACE2), as SARS-CoV (Zhou et al., 2020).

Evidence of infection in animals

At the time of writing (May 1, 2020), there have been a few isolated reports of natural infection with SARS-CoV-2 in animals, which have all been associated with close contact with a human diagnosed with COVID-19. The majority of reports of SARS-CoV-2 infection in animals have been experimental “proof of concept” studies under laboratory conditions.

Natural conditions

The following are reports from the World Organisation for Animal Health (OIE):

1. February 29, 2020 – Hong Kong, China – A Pomeranian dog owned by a confirmed COVID-19 patient showing no clinical signs of COVID-19 tested positive to SARS-CoV-2 with qRT-PCR on nasal and oral swabs. Virus isolation negative (OIE, 2020a).
2. March 18, 2020 – Belgium, Netherlands – One cat owned by a confirmed COVID-19 patient presented with clinical respiratory and gastrointestinal signs and tested positive to SARS-CoV-2 by PCR (OIE, 2020b).
3. March 20, 2020 – Hong Kong – A German Shepherd dog, one out of two dogs owned by a confirmed COVID-19 patient, neither showing any clinical signs, tested positive to SARS-CoV-2 with qRT-PCR and virus isolation. The other dog in the household tested negative (OIE, 2020a).
4. March 31, 2020 – Hong Kong, China – One cat owned by a confirmed COVID-19 patient, showing no specific clinical signs, tested positive to SARS-CoV-2 with qRT-PCR (OIE, 2020a).
Note: As of April 3, 2020, Hong Kong has tested more than 29 dogs and 17 cats from households with human COVID-19 cases or close contacts of cases, and apart from the two dogs and one cat, no further animal cases have been found (AFCD, 2020).
5. April 6, 2020 – Bronx, New York, USA – Tiger – Three lions and five tigers presented with a dry cough and wheezing, and had presumed exposure to an asymptomatic zoo employee with COVID-19. One tiger and one lion were tested; both were positive for SARS-CoV-2 with qRT-PCR (OIE, 2020a). Subsequently, there has been an unconfirmed report that the remaining four tigers and two lions also tested positive to SARS-CoV-2 using an unspecified faecal test (WCS, 2020). It is not known whether all animals contracted the virus from the same source or whether transmission between animals occurred.
6. April 15, 2020 – Richmond, New York, USA – Dogs – One out of two German Shepherd dogs from a household with known COVID-19-affected inhabitants showed respiratory illness and tested positive to SARS-CoV-2 with PCR. Virus neutralizing antibodies were detected in both the clinically affected and clinically unaffected dog (OIE, 2020a).*

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7. April 19 and 20, 2020 – Netherlands – Two mink farms had mink showing respiratory disease and increased mortality rates. Epidemiological investigations and viral sequencing revealed that the mink were infected with SARS-CoV-2 through two separate introductions into the two farms, most likely from contact with infected farm workers (Oreshkova et al., 2020). Workers on the farms were reported to have previously shown symptoms of COVID-19 or have been hospitalized with confirmed COVID-19 preceding clinical signs in the mink. However, concerningly, preliminary sequencing results suggest that one person may have contracted the virus from the mink; the investigation is ongoing (Oreshkova et al., 2020). Following introduction, it is thought that widespread infection occurred through horizontal transmission between mink, although in this instance, direct contact between mink was precluded by partitions between cages, implicating fomites, respiratory droplets or faecally contaminated dust particles in transmission (Oreshkova et al., 2020). Viral RNA was detected by PCR in the conchae, lungs, liver, intestines, and throat and rectal swabs of diseased mink. Viral RNA was also detected in dust samples from inside the mink farm buildings. A survey of 24 stray cats in the proximity of the mink farms found seven with antibodies against SARS-CoV-2 and one cat with positive viral RNA on an oropharyngeal swab sample (Oreshkova et al., 2020). This suggests that environmental transmission has likely occurred in this instance and may also present a hazard to humans. Following this initial outbreak in two mink farms, a further nine mink farms in the Netherlands have become infected (Oreshkova et al., 2020).*
8. April 22, 2020 – New York, USA – Two cats from separate households, owned by confirmed COVID-19 patients, presented with respiratory signs, tested positive to SARS-CoV-2 with qRT-PCR and had virus neutralizing antibodies (reported on April 29, 2020) (OIE, 2020a).
9. April 30, 2020 – Paris, France – One cat owned by a suspected COVID-19 patient with mild respiratory and digestive signs tested positive to SARS-CoV-2 on rectal swab but negative on nasopharyngeal swab (Zientara et al., 2020). Currently (May 3, 2020), the number of cats that were tested has not been published.
10. May 18, 2020 – Russia – One cat tested positive to SARS-CoV-2 with RT-PCR of throat and nasal swabs (OIE, 2020a). *
11. May 19 and 20, 2020 – Illinois and Minnesota, USA – In two separate incidences, one cat from a household with known COVID-19-affected inhabitants showing respiratory signs tested positive to SARS-Co-2 with PCR (OIE, 2020a).*

Serological surveys under natural conditions

Cats

A serological survey of cats in Wuhan included 102 cats sampled after the COVID-19 outbreak from animal shelters and pet hospitals in Wuhan and 39 cat samples from the laboratory serum bank that were collected in Wuhan in 2019, prior to outbreak, which were tested for SARS-CoV-2 (Zhang et al., 2020).

No positive samples for viral RNA using a SARS-CoV-2-specific qRT-PCR commercial kit were detected from nasopharyngeal and anal swabs (Zhang et al., 2020).

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Using an indirect ELISA based on the SARS-CoV-2 receptor binding domain, 15 from 102 surveyed (14% of cats) were positive for antibodies against SARS-CoV-2. Of the 15, 11 had SARS-CoV-2 neutralising antibody (73% of cats) with titre ranging from 1/20 to 1/1080 (Zhang et al., 2020).

The positive ELISA and VN tests were from cats in a veterinary hospital (6), strays (6), or cats owned by a person diagnosed with COVID-19 (3). The cats with the three highest VN titres were owned by COVID-19 patients, contrasting with relatively low titres in cats in animal shelters or pet hospitals, demonstrating that close contact with an infected human led to higher VN titres (Zhang et al., 2020).

This study demonstrates that cats produced neutralizing antibodies under natural conditions (Zhang et al., 2020).

Cats, dogs, horses

During the validation process of Idexx SARS-CoV-2 RealPCR Test, more than 3500 canine, feline and equine specimens from the United States of America and South Korea were tested, with no positive results (Idexx, 2020). By mid-April more than 5000 samples from Europe, Canada, USA and South Korea had been tested, with no positive results (Idexx, 2020).

Other animals

In a serological survey of 1914 samples from 35 different species of animals,¹ using a double antigen sandwich ELISA, all tested negative for antibodies against SARS-CoV-2, leading the authors to conclude that all of the animal species included in the study could not act as intermediate hosts (Deng et al., 2020). The ELISA test was validated for specificity using samples from pig, mouse and rabbit, and sensitivity using samples from ferrets, but was not validated in all 35 of the animal species. It is not clear where some of the animals were sourced from or whether they could have had contact with a COVID-19 patient, except for one dog, who was owned by a COVID-19 patient and tested negative (Deng et al., 2020). This study does not necessary preclude the involvement of all of these animal species in the epidemiology of SARS-CoV-2.

Laboratory conditions

Ferrets

Experimental studies conducted in ferrets demonstrate that ferrets are highly susceptible to SARS-CoV-2 infection and effectively transmit the virus by direct or indirect contact. Ferrets were intranasally inoculated with $10^{5.5}$ TCID₅₀ of NMC-nCoV2 strain isolated from a patient in South Korea (Kim et al., 2020). Ferrets subsequently developed mild clinical signs, viral RNA was detected in blood, nasal washes, saliva, urine and faeces for up to eight days and infectious virus was detected in urine and faecal specimens of the inoculated ferrets (Kim et al., 2020). Direct contact ferrets developed viral RNA in their blood two days after contact, demonstrating effective transmission to naïve ferrets (Kim et al., 2020). Indirect-contact naïve ferrets also tested positive to viral RNA in nasal washes and faecal samples, suggesting airborne transmission (Kim et al., 2020).

¹Species tested (number of samples): Livestock – pig (187), cow (107), sheep (133), horse (18); Poultry – duck (153), chicken (153), goose (25); Lab animals – mouse (81), rat (67), guinea pig (30), rabbit (34), monkey (39); Companion animal – dog (487), cat (87); Wild animals – camel (31), fox (89), mink (91), alpaca (10), ferret (2), bamboo rat (8), peacock (4), eagle (1), tiger (8), rhinoceros (4), pangolin (17), leopard cat (3), jackal (1), giant panda (14), masked civet (10), porcupine (2), bear (9), yellow-throated marten (4), weasel (1), red pandas (3), and wild boar (1).

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Shi and others (2020) also concluded that SARS-CoV-2 replicates efficiently in ferrets, finding that for ferrets inoculated intranasally and intratracheally in separate studies, viral RNA and infectious virus were detected in intranasal washes, and viral RNA was detected in only one rectal swab. All ferrets developed antibodies detected by ELISA and neutralisation assay (Shi et al., 2020).

Beer and others (2020) also demonstrated that ferrets were efficiently infected, showed no clinical signs and were capable of transmitting the virus to other animals.

Cats

Shi and others (2020) concluded that SARS-CoV-2 replicates efficiently in cats. Two repeat experiments using five inoculated cats and one naïve exposed cat repeatedly demonstrated that the exposed cat became infected, apparently through respiratory droplets (Shi et al., 2020).

Other animals

Experimental infection under laboratory conditions demonstrated that SARS-CoV-2 replicates poorly in dogs, pigs, chickens and ducks. Animals were inoculated with 10^5 PFU of samples of SARS-CoV-2 intranasally (Shi et al., 2020). Five inoculated beagles housed with two uninoculated beagles resulted in detection of viral RNA in rectal swabs of the inoculated dogs but no infectious virus detected (Shi et al., 2020). Two inoculated dogs seroconverted, but the rest were antibody negative by ELISA, demonstrating that dogs were not efficiently infected and did not transmit the virus (Shi et al., 2020). The experiment was replicated for pigs, chickens and ducks, all of which tested negative for viral RNA and anti-SARS-CoV-2 antibodies on day 14, leading to the same conclusion (Shi et al., 2020).

Research at the Friedrich Loeffler Institut found that under experimental conditions where animals were inoculated intranasally, Egyptian fruit bats became infected, with no clinical signs, but did not infect other animals efficiently and pigs and chickens were not susceptible to infection (Beer et al., 2020).

Under experimental conditions, golden Syrian hamsters were shown to be infected and transmit the virus effectively, and develop antibodies against SARS-CoV-2 (Sia et al., 2020) (Chan et al., 2020). Rhesus macaques did not develop overt clinical signs, but viral RNA was detected in upper respiratory tract and anal swab samples (Shan et al., 2020).

Currently, all of the experimental studies describing infection in animals used inoculations with exceptionally large amounts of virus. Exposure to this viral load is unlikely under natural conditions, and therefore, such studies must be interpreted with caution.

Receptors

It appears that SARS-CoV-2 has similar structure to SARS-CoV and also uses the host receptor angiotensin converting enzyme 2 (ACE2) (Wan et al., 2020). Analyses based on modelling of the receptor binding protein and ACE2 interaction using atomic level structures of SARS-CoV isolated from different animal species over 10 years suggest that the structure of the ACE2 receptor in a number of animal species, including pigs, ferrets, cats and non-human primates, may indicate that these animals could act as intermediate hosts (Wan et al., 2020). However, it is noted that other factors affect the infectivity and pathogenesis of SARS-CoV-2.

Additionally, Sun and others (2020) describe high ACE2 expression levels in cats and dogs in various different tissues including the skin, testes, retina and ear tips, suggesting the potential for these species

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and tissues to become infected. However, it may be unwise to extrapolate that these animals could be a source of transmission.

Evidence of SARS-CoV-2 in semen or embryos

Currently, there is no literature published related to the presence of SARS-CoV-2 in animal germplasm; however, there are some studies in human COVID-19 patients assessing the potential effects of SARS-CoV-2 on sexual transmission and reproductive function.

Single cell RNA-seq profiling of human testes indicates that ACE2 is expressed in Leydig cells, Sertoli cells and spermatogonia, which indicates that, theoretically, the testes are a potential target for SARS-CoV-2 (Wang and Xu, 2020). In contrast, using single cell transcriptome analysis on single testicular cells from testes of three healthy young adults, it was determined that there was sparse expression of ACE2 and serine protease TMPRSS2 in testicular cells; therefore, the authors concluded that ACE2-mediated viral entry into target host cells was unlikely to occur in the testes (Pan et al., 2020).

It was theorized that high ACE2 receptor expression in the testes, specifically the Leydig cells, Sertoli cells and spermatogonia, may cause the testes to be vulnerable to damage and/or dysregulation from SARS-CoV-2 infection (Ma et al., 2020). A retrospective study of the sex hormone profiles of 81 male COVID-19 patients and 100 healthy male patients (age 20–54 years) detected some significant aberrant results in COVID-19 patients. However, this study did not address whether SARS-CoV-2 could be detected in semen and is, therefore, of less relevance to this review (Ma et al., 2020).

In another study, single ejaculate semen samples were collected from 34 male patients at a median of 31 days after diagnosis of COVID-19 and tested by qRT-PCR for identification of SARS-CoV-2; however, no virus was detected on any of the semen samples. Six of the patients had experienced scrotal discomfort at the time of COVID-19 diagnosis (Pan et al., 2020).

Similarly, semen samples were obtained during the recovery phase from 12 patients diagnosed with COVID-19 (age 22–38 years) by ejaculation and from the testes of a patient who died from COVID-19 (age 67 years). The deceased man had test results consistent with acute phase of infection. No SARS-CoV-2 RNA was identified in any of the semen samples or testes of the deceased patient, demonstrating that transmission of the virus via semen in the recovery phase is unlikely (Song et al., 2020). However, whether the virus could be shed in human semen during the acute phase of infection is still unknown in the absence of significant evidence.

In females, it also appears that sexual transmission of SARS-CoV-2 is unlikely. In 35 female patients diagnosed with COVID-19, vaginal discharge, cervical or vaginal residual exfoliated cells and anal swab samples all tested negative for SARS-CoV-2 RNA except for one anal swab sample (Cui et al., 2020).

A study of the potential for intrauterine vertical transmission of COVID-19 in nine pregnant women found that there was no evidence of transuterine infection caused by vertical transmission in women with COVID-19 in late pregnancy (Chen et al., 2020).

However, an analysis by Colaco and others (2020) of single cell RNA seq data sets of human embryos for a number of key cell entry mechanisms for SARS-CoV-2 concluded that human embryos express receptors for SARS-CoV-2 and also have the necessary machinery for virus internalization and replication. The analyses included single cell RNA-seq data sets of human embryos for SARS-CoV-2 receptors ACE2 (receptor for viral entry) and BSG (receptor for viral entry), serine protease TMPRSS2 for

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viral spike protein priming (entry to cell) and endosomal protease CTSL for spike protein activation via the endosomal route. The results were that ACE2 and TMPRSS2 are co-expressed in the proportion of epiblast cells and BSG and CTSL are expressed during embryonic development.

Infection and transmission during reproductive techniques

There is no literature that specifically relates to the transmission of SARS-CoV-2 in animal germplasm or the risks of exposure associated with veterinary reproductive techniques.

Available evidence suggests that SARS-CoV-2 virus may be present in nasal and ocular discharge, respiratory secretions, faeces and urine (Kim et al., 2020) (OIE, 2020). Currently, there is no conclusive evidence of transmission of SARS-CoV-2 by venereal contact, semen or embryos or intrauterine transmission.

However, although there is no literature published that specifically investigates animals as fomites for transmission of the virus, as was also found by O'Connor and others (2020), following the recent outbreaks of SARS-CoV-2 infection in mink farms in the Netherlands and Denmark, there is evidence that transmission occurred amongst mink through fomites, infectious respiratory droplets and dust particles potentially containing faecal matter. This presents a potential exposure risk for farm and animal health personnel. This could also include those involved with assisted reproductive technology in species of the *Mustelid* family, such as mink or ferrets.*

Conclusion

The current evidence shows that the only animals that have been demonstrated to develop infection with SARS-CoV-2 either naturally or experimentally are members of the families *Felidae*, *Canidae* and *Mustelidae* and golden Syrian hamsters in two experimental papers (Sia et al., 2020) (Chan et al., 2020).

There are very few isolated cases of natural infection in companion animals (cats, dogs), zoo animals (big cats), and mink, which all have evidence of contact with a human with COVID-19; however, it is not clear whether horizontal transmission was possible or occurred in any of these cases. There are a number of experimental studies under laboratory conditions demonstrating infection, production of infectious virus and transmission in cats, ferrets and golden Syrian hamsters. It is important to note that a positive viral RNA with PCR does not imply that the animals have produced infectious virus or that transmission could occur.

The limited available evidence suggests that it is unlikely that the other species typically dealt with (dogs, pigs, horses) can be infected and transmit the virus. However, at this point there are no studies looking at infection and transmission in ruminants, zoo animals (other than big cats) or wild animals, and the absence of this evidence does not preclude the possibility for other animals to be susceptible to infection with SARS-CoV-2. Currently, there is no evidence of transmission from domestic animals to humans.

The very few studies that have been done in humans suggest that it is highly unlikely that the virus could be transmitted through germplasm, and of these, many have not been subjected to peer review. Currently, there are no studies examining the transmission of SARS-CoV-2 in animal germplasm.

Extrapolating from the small number of studies in animals, there is potential for SARS-CoV-2 to be present in respiratory secretions, faeces and urine. The presence of viral RNA in environmental samples

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associated with mink farms demonstrates that fomites and dust must also be considered a potential route for transmission, although infectious virus was not isolated in these instances.* Despite the low likelihood that an animal presented for breeding could be infected with SARS-CoV-2, the nature of our work in animal reproduction involves exposure to secretions that may carry infectious agents. Therefore, it is recommended that vigilance be maintained, including exercising routine hygiene, wearing standard personal protective equipment (mask, gloves, eye protection, disposable gown) and adhering to thorough biosecurity practices, such as hand washing.

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