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VIRAL DISEASES CHALLENGING THE CANINE AND FELINE FAUNA AND PROSPECTIVE METHODS TO TRACE THEIR ONSET

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Review Article

ABSTRACT

Over the last century there has been an alarming increase in the number and diversity of epizootic diseases. Pathogens associated with these diseases may be bacterial, viral or parasitic and can spread by direct contact, food, water and/or environment. Zoonosis can lead to disturbances in the production and trade of animal products. Zoonosis comprise of many newly identified infectious diseases as well as many existing ones ex. Canine distemper virus (CDV). These newly identified and less studied viruses are constantly mutating and is a cause of concern for many virologists and veterinarians. Although, with advancement in science and technology, identification of new or previously unknown viruses has become precise and efficient, however, the need of the hour is to focus on identification of potential pathogenicity of the existing and new viruses for development of cure well in advance, to prevent an onset of endemic or a pandemic like ongoing COVID-19. Furthermore, exercising precautionary measures in addition to development of remedial measures will be like developing a double layered defense system as this will promote healthy practices in handling wild animals, maintaining hygiene in their shelter and routine health checkup of technicians working closely with these animals.

Keywords: Animal health; environment; feline species; India; Lion; viral disease; molecular biology; zoonosis.

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1. INTRODUCTION

The effects of the newly emerging wild and domestic animals diseases are huge in terms of public health and economic investment. Animals including felines and canines can fall victim to pathogenic microorganisms and can get infected. Pathogens like bacteria, fungi, parasites, viruses, and prions lead to zoonotic diseases which can spread between vertebrate mammals and humans [1]. Spread of these zoonotic diseases can have a profound consequences on public health and can lead to illness and/or death of millions of people every year [2]. Disease outbreaks in exotic wild animals like lions can result in panic and tremendous economic damage for identification of pathogen and development of its remedial measure, if the pathogen is new [3]. The recent COVID-19 caused by coronavirus, believed to be originated from a wild animal host [4], is estimated to cost between 5–9 trillion USD globally [5].

Out of the bacterial and viral infections affecting the wildlife, the fight against emerging viral diseases is exhausting because vaccines approved for human use are limited, and those available for animal use, may prevent disease symptoms but do not prevent the spread of the infection [6, 7]. Preventive measures are based on early identification of infection onset through surveillance and application of available medication [6].

In recent past, we have witnessed several viral diseases that challenged virologists and veterinarians and have been a threat to lion health. Due to their pathogenic potential and absence of absolute treatment for native species, these disease causing viruses were included in list of pathogens deserving the attention of the animal health personnel, virologists along with biotechnologists for development of treatment and monitoring techniques to predict these diseases in advance.

Disease can be considered as endemic or epidemic or pandemic based on its presence in the population. Mankind has witnessed epidemics like SARS (severe acute respiratory syndrome) and MERS (Middle east respiratory syndrome) in 21st century. SARS-Cov-2 has caused damage to mankind to an extent unmatched with SARS or MERS. Characterization of disease as an endemic or epidemic or pandemic depends on its worldwide presence. Even though endemic viruses can be prevalent within the population with a minimum exhibit of pathogenicity, epidemic viruses can lead to higher mortality rate in lion pupation over a short time span.

There are multiple factors contributing to the rise of these deadly viruses and are usually difficult to trace owing to the involvement of multiple dynamics. As reported, these factors include but are not limited to, host-pathogen adaptation ecological and/or epidemiological changes, genetic evolution, host range extension and eating habits which may give rise to zoonotic diseases. Additionally, it has also been discussed over time and again that these diseases causing viruses may not necessarily be new, but these may be old viruses coming into recognition recently due to advancement of next generation sequencing platforms. Thus it can be expected that with the advancement of genome sequencing techniques in coming years we can expect an era of newly identified viruses which were untouched before [8].

It has been reported that lion can be infected with one or more strains of the same pathogen. Majority population is infected with minimum one, and remaining with multiple, often with different strains of same pathogens [9]. Lions which are geographically close can also carry different viruses. While some lion populations who may naturally harbor co-infection might have developed immunity against these viruses over time but distant populations might not have developed immunity for the same. In contrast, plans for promoting the distribution of these wild animals worldwide for their establishment in new niche, for conservation and for increasing their population might actually contribute in exposing lion population to new and unacquainted viral strains and thus aid in spreading of these viruses within sub population who are naïve to these disease causing viruses. It is well established fact that infected host can behave as asymptomatic carriers and can transmit virus.

Translocation of such asymptomatic carriers into an uninfected or susceptible population thus could lead to grave consequences for the health of entire population.

This will be accompanied by the challenge of characterization of these newly discovered viruses, understanding their etiology, their natural host, conditions under which they turn virulent, development and preparation of medication and/or vaccines against these viruses, funding for research followed by development of skilled staff and availability of such laboratories locally well in advance as a precaution to control their widespread in case of outburst.

This article discusses viral diseases that called attention of researchers veterinarians and biotechnologists in recent years (Fig. 1). Some viruses

have created a deep impact on animal health and have served as an alarm to awaken the techno-preneurs and researchers for developing sophisticated tools and techniques for identification of potential pathogenic viruses and to develop remedial measures in advance, respectively. This article also discusses some remedial measures that can be followed to identify and mitigate the severity of pathogen attacks in advance.

Some of the viral diseases are briefly discussed below:

2. FELINE HERPESVIRUS 1

Genome size: approximately 134kb
Family: Herpesviridae
Subfamily: alphaherpesvirus
Genus: *Varicellovirus*
Genetic material: It has a double-stranded DNA

Target fauna:

1. Although the domestic cat is the main host, pumas, cheetahs and lions can also become infected [13].

2. It is an epitheliotropic and cytopathic virus which mainly leads to rhinitis and conjunctivitis and occasionally leads to *Pneumoniae*.

3. Incidents of FHE-1 have been reported in Asia, Europe, Africa and North America. It is prevalent in lion populations in Etosha National Park, Kruger NP and Serengeti NP, Lake Manyara region, Central Kalahari region and Ngorongoro Crater. However, such data is not reported for Gir lions.

Target organs:

1. It affects the upper respiratory system and propagates in the mucous membranes like oral and nasal membranes.

2. It can also lead to vaginitis and congenitally infected offspring.

It is an epitheliotropic and cytopathic virus which mainly leads to rhinitis and conjunctivitis and occasionally leads to *Pneumoniae*. Although this disease is not considered to be harmful for survival and reproductive ability however, it lacks the support of experimental data between control and infected lion (https://lionalert.org/lion-diseases/).

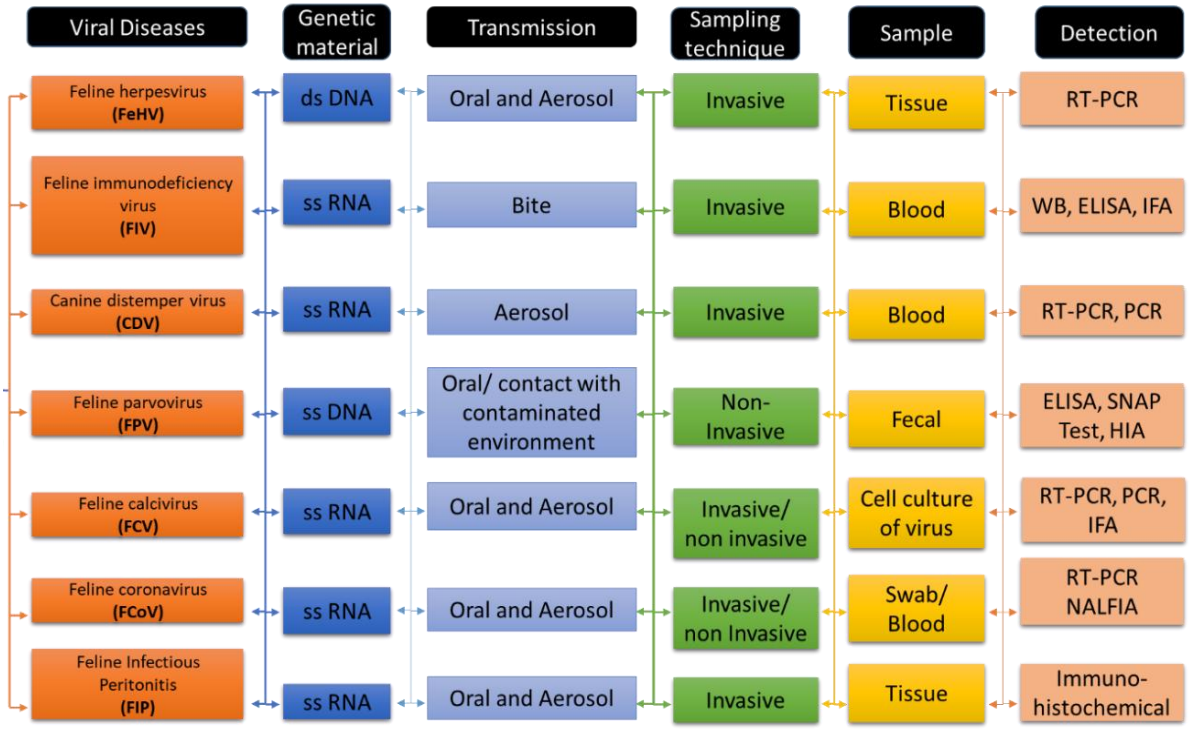

Fig. 1. Representation of recently encountered disease causing viruses, their mode of transmission, sample collection from infected animal followed by their identification technique.
ss = single stranded; ds = double stranded

Table 1. History of the study viruses in domestic felines and canines

Sr.no	Endemic Disease	Family	Symptoms	Course of Infection	Assay	Criteria for lions to be considered positive for virus	Reference	Vaccine
1	Feline Herpesvirus (FeHV)	*Herpesviridae*	Sneezing, fever, pneumonia tracheitis, anorexia etc. as reported.	Majority become carriers post encounter with virus, start shedding virus subsequently one or two weeks time and exhibit clinical signs	RT-PCR	OD>0.080	[10]	Available
2	Feline Immunodeficiency Virus (FIV)	*Retroviridae*	Initial: lymphadenopathy, depression, leucopenia. Final: lymphadenopathy, gingivitis, persistent calicivirus and/or herpes, wasting, anaemia, diarrhoea, neurological disease	After encounter with infection, animals may remain healthy for a prolonged time, but they behave as carriers and keep on shedding virus in their saliva. Amount of antibody present may considerably reduce in last stages, but not necessary that all infected animals lead to AIDS	Western blotting, ELISA, Immunofluorescent Antibody	Antibodies are present	[10, 11]	Not Available
3	Canine Distemper (CDV)	*Paramyxoviridae*	discharges from nasal and conjunctival region, myoclonous, encephalitis, lymphopenia, immunosuppression	Infection is followed by 100% recovery, sometimes followed by infection in central nervous system	PCR, RTPCR	Antibodies are present	[12]	Available

Sr.no	Endemic Disease	Family	Symptoms	Course of Infection	Assay	Criteria for lions to be considered positive for virus	Reference	Vaccine
4	Feline Parvovirus (FPV)	*Parvoviridae*	Vomiting, anorexia, occasional diarrhoea, panleucopenia, unsteady gait and convulsions	Ataxic carriers can shed virus for up to a year; long-term immunity is developed in survivors	ELISA, Snap test, Haemoagglutination inhibition assay	Titre >30	[10]	Available
5	Feline Calicivirus (FCV)	*Caliciviridae*	Rhinitis, conjuctivitis, oral ulcers, pneumonia, lameness	Infection is Severe. Usually followed by viral secretion in saliva. Almost 50% remain carriers for more than 75 days after encounter with the infection; Prolonged immunity is developed by non carriers.	PCR, RT-PCR, Immunofluorescence	δOD>0.095	[10]	Available
6	Feline Coronavirus (FCoV)	*Coronaviridae*	FECV: transient enteritis.	Viral elimination and recovery is strain specific; some strains cause sequestered infection where virus containment is due to cell-mediated immunity; infection may be enhanced by humoral immunity	RT-PCR, NALFIA	Presence of antibodies	[10]	Available
7	Feline Infectious Peritonitis (FIP)	*Coronaviridae*	FIP: fever, anorexia, lethargy, abdomenal distention, peritonitis	Recovery from infection is strain specific; some strains cause	Histopathology, γ-globulin	Immunohistochemical detection		Not Available

Sr.no	Endemic Disease	Family	Symptoms	Course of Infection	Assay	Criteria for lions to be considered positive for virus	Reference	Vaccine
				sequestered infection where virus containment is due to cell-mediated immunity; infection may be enhanced by humoral immunity	concentration >32% decreased albumin-to-globulin (A : G) ratio <0.5			

3. FELINE IMMUNODEFICIENCY VIRUS (FIV)

Genome size: 9.5 kb [14]
Family: *Retroviridae*
Subfamily: *Orthoretrovirinae*
Genus: Lentivirus
Genetic material: single-stranded, enveloped, RNA virus. The host RT in the virus transcribes DNA from RNA

Target fauna:

1. Infects both wild and domestic feline species
2. It is related to human and simian immunodeficiency viruses (HIV and SIV, respectively) [15].
3. It is documented that FIV spread in African lions, is benign. In non-domestic cat species, species-specific strains of FIV have been reported.
4. Similar to HIV infection, infected lions exhibit reduction $CD8^{+}\beta$ high cells and reduced $CD4^{+}/Cd8^{+}$ ratio.

Target organs/ Effects on health:

Its pathology is characterized by immune suppression, CD4 depletion and death.

Assays for detection:

Antibodies generated against FIV can be detected using ELISA, Western Blot and Immunofluorescence assays.

Subtypes:

Till date six FIV subtypes have been reported.

4. CANINE DISTEMPER VIRUS (CDV)

Genome size: 15690 bp [16]
Family: *Paramyxoviridae*
Genus: Morbillivirus
Genetic material: single stranded RNA virus

Target fauna:

1. The potential for CDV to cause death in wild felids was highlighted by epizootic sickness in lions in a wildlife sanctuary in California, USA, in 1992, and Serengeti National Park, Tanzania, in 1994 [12,17].

2. It was reported that the recent incident of the unusual death of the Asiatic lions, residing in Gir, Gujarat was concluded to be due to CDV infection. It was identified that the complete genome of the isolated CDV from the infected lions resembled East African CDV except for one sample which showed difference of 3-4 nucleotides.

Target organs/ Effects on health

1. CDV shows respiratory and gastrointestinal signs which finally leads to neurologic disease [12].

Assays for detection

1. It is reported that several techniques like polymerase chain reaction (RT-PCR) [18], nested RT-PCR [19], real-time RT-PCR [20], reverse transcription loop-mediated isothermal amplification (RT-LAMP) [21] and insulated isothermal PCR (iiPCR) can be employed for detection of CDV [22].

Subtypes:

1. At least seven distinct lineages of canine distemper are recognized worldwide, based on sequence analysis of the H gene: Asia-1, Asia-2, America-1, America-2, Arctic-like, European wildlife, Europe.

5. FELINE PARVOVIRUS (FPV)

Genome size: approximately 5123 nt [23]
Family: *Parvoviridae*
Subfamily: *Parvovirinae*
Genus: Protoparvovirus
Genetic material: single-stranded DNA virus

Target fauna:

1. Feline parvovirus (FPV) leads to an acute infection of exotic and domestic felines which is enteric in nature.
2. A brief incubation period of less than 1 week is reported in domestic cats and dogs, however, information about the duration of this period in wild carnivores is not known.

Target organs/ Effects on health: FPV infection usually causes severe gastroenteritis, which is usually hemorrhagic.

Assays for detection

1. At present, the diagnostic method includes, ELISA, Snap test as well as High resolution

melting Analysis (HRM) assay. HRM can identify and differentiate between co-infection of FPV/CPV in clinical samples simultaneously.

Subtypes:

1. Two types of canine parvovirus called canine minute virus CPV1 and CPV2

CPV-2a and 2b may have gained the ability to replicate in feline [24].

6. FELINE CALICIVIRUS (FCV)

Virulence of FCV is strain specific. It should be kept in consideration that vaccination to the animals may not necessarily prevent them against infection from virulent FCV strain however, it may be useful as a preventive measure against less virulent strains [25].

Genome size: approximately 7.7-kb [26].

Family: Caliciviridae

Genus: Vesivirus

Genetic material: positive-stranded RNA viruses

Target fauna:

Strains resulting in severe systemic infections have been previously reported as outbrakes in catteries and humane shelters.

Target organs/ Effects on health

1. Infection with FCV has been reported in lions and tigers but in neither of these reports, however, was severe morbidity or mortality observed.

2. The infection is commonly associated with upper respiratory tract infections and mouth ulcers fever and pneumonia. Foot pad ulcerations have also been noted in cubs. [25, 27].

Assays for detection:

The presence of FCV infection can be detected using RT-qPCR in clinical samples.

Subtypes:

FCV strains comprise one serotype and predominantly one genogroup worldwide, although there is considerable variation between strains, which has an impact on vaccination [28, 29].

7. FELINE CORONAVIRUS (FCOV)/ FELINE INFECTIOUS PERITONITIS (FIP)

The viral RNA packaged by the nucleocapsid protein (N), lies under an envelope containing at three membrane proteins encoded by virus; the spike protein (S), transmembrane protein (M), and a membrane protein (E).

Genome size: 27 to 30 kb [30]
Family: Coronaviridae

Subfamily:

Genus: Alphacoronavirus

Genetic material: single-stranded RNA viruses

Target fauna: Cat species

Target organs/ Effects on health

1. It is reported that FIPV replicates in macrophages and leads to the disease onset. Reactions between the virus, and antibodies leads to cause disseminated vasculitis, which is the identification mark of FIP.

Assays for detection

1. Detection in clinical samples is done using RT-PCR.

Subtypes:

1. Till date, two types of FCoVs are known: feline infectious peritonitis virus (FIPV) and feline enteric coronavirus (FECV) [31]. According to the widely known and believed "*in vivo* mutation" theory, FIPV is a result of mutation FECV in the gastrointestinal tract of an infected cat which then spreads systemically and leads to FIP [32].

8. NIPAH VIRUS

Genome size: approximately 18.2 kb
Family: *Paramyxoviridae*
Subfamily: Paramyxovirinae
Genus: *Henipavirus*

Genetic material: single-stranded, negative-sense RNA virus

Target fauna: Sheep, Pigs, Goats, Cats, Dogs, Horses

It is known to cause illness in human beings and pigs. Outbreaks occur almost annually in parts of Asia, primarily Bangladesh and India.

Target organs/ Effects on health: Infection with NiV is associated with encephalitis (swelling of the brain) and can cause mild to severe illness and even death.

Assays for detection: In early stages testing can be performed by real time polymerase chain reaction (RT-PCR) from throat and nasal swabs, cerebrospinal fluid, urine, and blood.

After the course of illness and after recovery, testing for antibodies is conducted using an enzyme-linked immunosorbent assay (ELISA).

Subtypes: NiV, Malaysia (NiV_M) and Bangladesh (NiV_B) [33].

9. EBOLA VIRUS

Genome size: about 18-19 kb

Family: Filoviridae

Genus: Ebolavirus

Genetic material: Negative-stranded RNA

Target fauna: Ebola affects mammals such as humans, non-human primates (like monkeys and apes), and fruit bats.

Target organs/ Effects on health: Primary signs and symptoms of Ebola often include some or several of the following:

- Fever
- Aches and pains, such as severe headache and muscle and joint pain
- Weakness and fatigue
- Sore throat
- Loss of appetite
- Gastrointestinal symptoms including abdominal pain, diarrhoea, and vomiting
- Unexplained haemorrhaging, bleeding or bruising
- Other symptoms may include red eyes, skin rash, and hiccups (late-stage).
- Many common illnesses can have the same symptoms as EVD, including influenza (flu), malaria, or typhoid fever.
- It is a rare, yet serious, and frequently fatal disease. The patient's immunological response and strong supportive clinical treatment are both important factors in EVD recovery. Antibodies (proteins created by the immune system that identify and kill invading viruses) can be identified in the blood for up to ten years following Ebola virus infection, according to studies. The strain of Ebola that affected the survivors is likely to have some protective immunity.

Assays for detection: Polymerase chain reaction (PCR) is one of the most extensively used diagnostic methods because of its capacity to detect small levels of Ebola virus. In small amounts of blood, PCR technologies can detect a few virus particles, but as the quantity of virus particles increases during an active infection, so does the ability to detect it. When the virus is no longer present in large enough numbers in a patient's blood, PCR methods will no longer work. To validate a patient's Ebola virus exposure and sickness, other methods based on the detection of antibodies produced by an EVD case in response to an infection can be used.

Subtypes: Zaire, Bundibugyo, Sudan, Taï Forest, Reston and Bombali.

10. ROTAVIRUS

Genome size: Genome total size is 18,550 bp

Family: Reoviridae

Subfamily: Sedoreovirinae

Genus: Rotavirus

Genetic material: double-stranded RNA viruses

Target fauna: Rotavirus is found widely in the intestines of dogs, but most infections are mild. Feline rotaviruses can induce subclinical infections and moderate enteritis in kittens, but not the severe infection found in other domestic species' young animals.

Target organs/ Effects on health:

1. digestive diseases of large ruminants
2. digestive diseases of pigs
3. digestive diseases of poultry
4. digestive diseases of small ruminants

Assays for detection:

Several commercial test kits for detecting a rotavirus antigen (VP6) common to human rotaviruses by enzyme linked immunoassay are available (EIA). These kits are easy to use, low-cost, and extremely sensitive. Because of the significant decrease in

rotavirus disease in children in the United States as a result of rotavirus vaccination, the positive predictive value of EIA is projected to be lower (and the negative predictive value to be higher) than in the pre-vaccine era. In clinical laboratories, multipathogen polymerase chain reaction (PCR)-based tests for stool samples that can identify rotavirus RNA are becoming more common. Because identification of rotavirus or other pathogen nucleic acid in stool may indicate a previous infection rather than the source of a current sickness, clinical interpretation of the data from these very sensitive assays may be difficult. In research laboratories, sequence analysis and viral culture are accessible.

Subtypes:

Consists of nine Rotavirus group A through I species, with the J species recently proposed [34,35]. Rotavirus A (RVA) is a worldwide disease that mostly affects humans, cattle, and other mammals, as well as birds [36].

11. RABIES VIRUS

Genome size: approximately 12 kb

Family: *Rhabdoviridae*

Genus: Lyssavirus

Genetic material: single-stranded, antisense, non-segmented, RNA

Target fauna: Raccoons, skunks, bats, and foxes are the most prevalent wild species in the United States that carry rabies.

Rabies can infect both pets (such as cats and dogs) and livestock (such as cattle and horses). Almost all rabies-infected pets and animals had not been vaccinated or were out of date on their rabies vaccinations. The majority of pets contract rabies after coming into touch with wild animals.

Symptoms:

- general sickness
- problems in swallowing
- excessive drool or saliva
- an animal that is overly aggressive
- an animal that bites at imaginary objects (sometimes called “fly biting”)
- an animal that appears tamer than you would expect
- an animal that’s having trouble moving or may even be paralyzed
- a bat that is on the ground

Target organs/ Effects on health:

The earliest signs of rabies, such as weakness or pain, fever, or headache, are similar to those of the flu. There may also be a stinging, prickling, or itching feeling at the bite site. These signs and symptoms could linger for days.

Cerebral dysfunction, anxiety, confusion, and agitation are the next symptoms to appear. Delirium, strange behavior, hallucinations, hydrophobia (fear of water), and insomnia may occur as the condition advances.

Once clinical signs of rabies appear, the disease is nearly always fatal, and treatment is typically supportive

Assays for detection:

In animals, rabies is diagnosed using the direct fluorescent antibody (DFA) test, which looks for the presence of rabies virus antigens in brain tissue.

In humans for prompt provision of post exposure prophylaxis, rapid and accurate laboratory identification of rabies in humans and other animals is critical. A diagnostic laboratory can detect whether or not an animal is rabid in a matter of hours and notify the appropriate medical personnel. If the animal is not rabid, the laboratory results may rescue a patient from unnecessary physical and psychological trauma, as well as financial responsibilities [37].

12. PROACTIVE MEASURES FOR EARLY IDENTIFICATION OF PATHOGEN

While this may seem a herculean task for lions in wild, however, lions kept in sanctuaries, zoo and national parks should be screened for their health status atleast once annually if not twice in a year. The screening need not necessarily involve invasive procedure which may put the animals in stress and the veterinarian or the zoo keeper in danger. Routine screening can employ any one or a combination of the techniques mentioned in Fig. 2.

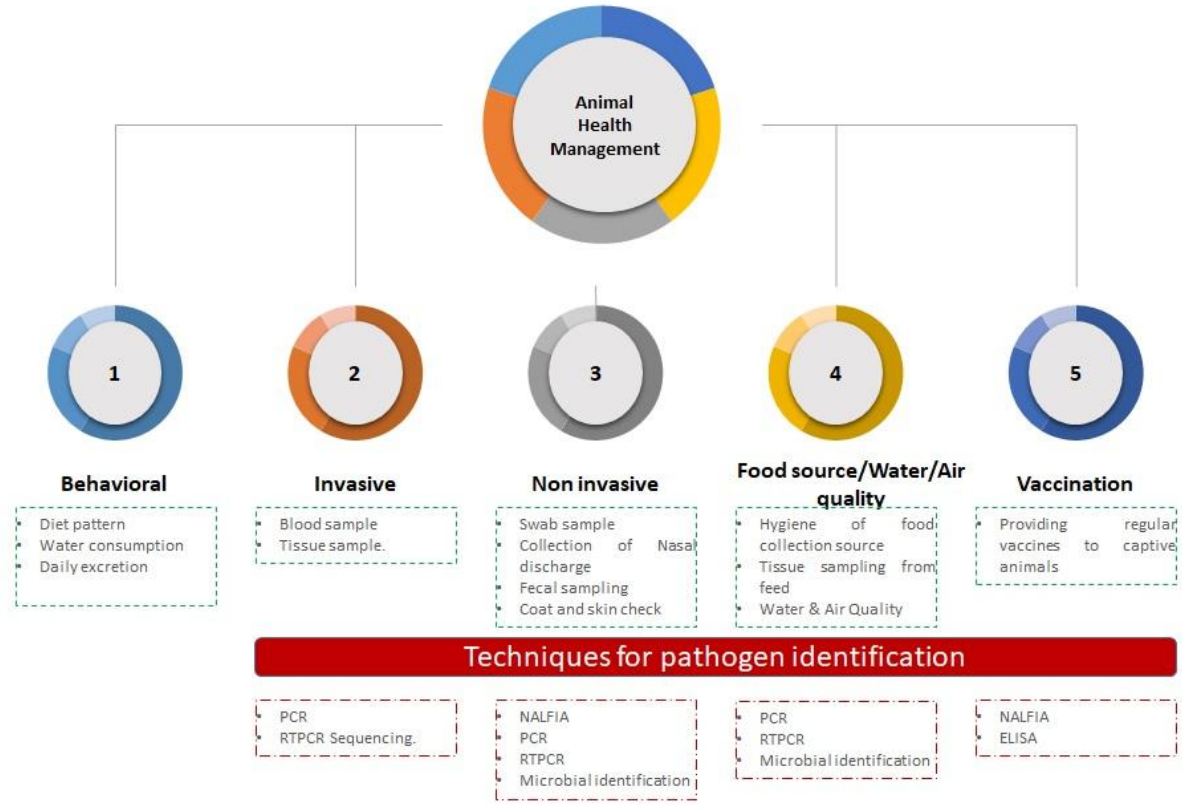

Fig. 2. Preventive measures for mitigating the spread of pathogen

Although techniques mentioned in Fig. 2, are listed for health management of lions, however, these procedures can be routinely employed for health management of animals in captivity, pets or if possible, in wild. The quality control of water, source of food/slaughter house, feed and air for animals in captivity, serve as a preventive measure and shall mitigate the spread of pathogen to a significant extent. Additionally, zookeepers can monitor behavioral patterns, social interactions, feeding habits and fecal excretion patterns of animals which can be valuable in predicting their health status. Knowing how the environment affects animals allows us ensure their welfare [38]. Irrelevant to the type of animal under study, monitoring of the behavior can be studied by using video cameras [39,40]. Monitoring this pattern on a regular basis can help us understand any notable change during healthy and diseased condition. Following behavioral and air/water/food quality next measure to consider is to collect saliva, nasal swab, coat and skin check and fecal sample as this can be performed without need for any kind of invasive procedure. Swab and saliva can be used to monitor the presence of any kind of pathogenic nucleic acid material using established techniques like Polymerase chain reaction and/or Real time PCR as well as Nucleic acid lateral flow assay. World organization for Animal health guidelines (2011) state that the fresh faeces or rectal swabs can be cultured immediately or placed immediately in the transport medium for identification of possible pathogens present. Finally, if need arises, blood and tissue samples can be collected and sent for further microbiological and molecular biological examinations for identification of the pathogen. A budget should be allocated yearly for the vaccination and maintaining health of the exotic lions considered as the pride of India.

The first and foremost requirement of maintaining health of lions is having adequately trained personnel experienced in techniques and principles of disease control. These personnel's should be supervised by a veterinarian. Additionally, high standards of hygiene should be practiced.

13. CONCLUSION

As new viruses are identified with the advancement of technique and technology, it becomes the prime responsibility of the researchers to be techno savvy and proactive in developing remedial measures before pathogen outburst actually takes place. The recent encounter of CDV in lions was an alarming sign of

how fatal these viral infections can be. Moreover, the latest episode of pandemic due to SARS-CoV2 is a reminder of zoonosis in humans. A continuous watch on the animal health status will atleast help in identifying the future threats and challenges and will serve as a window of opportunity to work on neutralizing pathogens well in advance before encountering another endemic/epidemic/pandemic. A strong sense of personal hygiene, well trained staff and proactive researchers will be a valuable addition in maintaining the health of lions and other animals. Thus, this review recommends following points necessary for welfare of wild animals including lions and preventing them future pathogen attack,

- Well trained staff supervised by an experienced veterinarian
- Quality Check of water/air and food provided to lions.
- Regular checkup of animals by one or combination of methods listed in Fig. 1
- Regular checkup of staff to prevent zoonosis
- Tagging of exotic lions
- Availability of funds for conducting routine checkup
- Technicians with knowledge of healthy and diseased biology of lions
- Establishment of laboratories with minimum essential setup at local level
- Maintaining the scheduled checkup and vaccination programme.
- Maintaining the disease onset detail and medicine provided detail.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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